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STUDIES ON PLASMA MEMBRANES

VIII. THE EFFECTS OF SODIUM DEOXYCHOLATE AND DODECYL SULFATE ON ISOLATED RAT-LIVER PLASMA MEMBRANES

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SUMMARY

1. Plasma membranes were isolated from rat liver and dissolved in sodium deoxycholate (1%) or dodecyl sulfate (0.4 or 0.8%). Differences in the effects of the detergents on the membranes were observed.

2. Analytical ultracentrifugation showed the presence of heterogeneous membrane material. The aggregation and precipitation of the solubilized membrane material by dialysis appeared to depend on the nature of the detergent used for dissolving the membranes, and the pH and ionic composition of the dialysis fluid.

3. The amount of detergent bound to the dissolved membrane material after treatment with excess detergent solution was determined by three independent techniques based on a similar experimental approach, and a reasonable accordance of results was obtained. The extent of binding appeared to be a function of the detergent concentration and to exhibit an optimum. Dialysis virtually removed the detergents from the membrane material.

4. Density-gradient centrifugation in a three-layer system showed a high degree of dissociation between membrane proteins and phospholipids, both in the presence of detergent and after its prior removal by dialysis. Ultrasonic irradiation also resulted in dissociation. These results were corroborated by equilibrium centrifugation in the analytical ultracentrifuge in a medium of d 1.17, and, in the case of membrane solutions from which deoxycholate had been removed, also by the capacity of the membrane material present in the individual layers of the density gradient after centrifugation to precipitate at pH 5.4.

5. The results are discussed in relation to the problem of lipoprotein subunits in membranes, and the conclusion reached is that the present and other experiments with detergents do not yield evidence for the occurrence of such a structure in the plasma membrane. Criteria for judging whether the original membrane structure is restored in 'membranes' reconstructed from their constituting components are presented on account of certain properties of membrane precipitates obtained from deoxycholate solution at pH 5.4.

INTRODUCTION

KORN[1] recently summarized a number of studies which indicated that homogeneous lipoprotein subunits may be formed by dissolving biological membranes in certain detergent solutions. Among the studies mentioned were those of RAZIN *et al.*[2], who found that the surface membrane of *Mycoplasma laidlawii* dissolved in dodecyl sulfate solution to give lipoprotein subunits of ultracentrifugally homogeneous size as evidenced by a single schlieren peak of about 3 S. However, in a later report, ENGELMAN *et al.*[3] showed by the use of density-gradient centrifugation that a high degree of separation of the lipid and protein present occurred in 10 mM dodecyl sulfate-treated membranes. Since these authors again obtained a well-defined schlieren peak, they questioned the feasibility of the schlieren pattern as a criterion for homogeneity of such complex systems.

In contrast to the results of ENGELMAN *et al.*[3], RODWELL *et al.*[4] were unable to separate the lipid and protein components of *M. laidlawii* membranes disintegrated by dodecyl sulfate by density-gradient centrifugation. Most of the membrane protein could, however, be separated from the membrane lipid by dodecyl sulfate together with deoxycholate.

In the present paper the effects of 1% deoxycholate and 0.4 or 0.8% dodecyl sulfate on isolated rat-liver plasma membranes are reported. It is shown, both chemically and by schlieren patterns, that the detergents cause a high degree of separation between the phospholipids and proteins of these membranes. Evidence that this separation persists after the detergents have been removed by dialysis is also presented; this finding contrasts with results obtained by TERRY *et al.*[5], and by ENGELMAN AND MOROWITZ[6,7] on mycoplasm membranes. The presentation of results in Sections 4 and 5 of the present paper is preceeded by that of some physical measurements on deoxycholate- and dodecyl sulfate–membrane solutions before and after dialysis, which show that the detergents may vary in their effects on the membranes.

No indication for the presence of a repeat structure in the plasma membrane, consisting of lipoprotein complexes as minimal building blocks, has become evident. Finally, it is shown that, in the membrane material precipitated from deoxycholate solution by dialysis at pH 5.4, the original membrane structure is not restored.

MATERIALS AND METHODS

General procedures. Plasma membranes were isolated from rat liver as described previously[8], washed and suspended in 1 mM $NaHCO_3$ of pH 7.5. In view of the varied conditions under which the experiments were carried out, particulars are given in RESULTS. In general, the membranes were solubilized by adding 1 ml of a doubly concentrated solution of the sodium salts of deoxycholate (Mann Research Laboratories) or dodecyl sulfate (British Drug Houses) to 1 ml of membrane suspension at room temperature. Dodecyl sulfate was either dissolved in bidistilled water or in 0.01 M ammonium formate buffer (pH 9.0). Dialysis, when performed, was carried out in the cold room at 6–12°.

Chemical determinations. Organic P was measured according to MORRISON[9] and protein by the biuret method (with human serum albumin as standard) except in the

density-gradient experiments (Section 4) in which the Folin phenol method according to LOWRY *et al.*[10] was used. In the experiments of Section 4a, the protein values were corrected for the sucrose present in the samples. In the remainder of the experiments, protein was measured after sucrose had been removed by dialysis against 10 mM $NaHCO_3$ for 48–60 h. Concentrations were measured with a differential refractometer (Brice-Phoenix, model BP-2000-4) or, in the case of membrane material, from the protein content using the dry weight (measured by differential refractometry)/protein (biuret) ratio of 1.5 found for the membranes.

Density-gradient centrifugation. The density gradient used in the experiments of Sections 4b–f was made up of ^2H_2O (99.8%) and the required amounts of sucrose to give three layers of *d* 1.15 (2 ml), *d* 1.17 (1 ml, containing the solubilized membrane material), and *d* 1.19 (2 ml); densities were controlled by pycnometry. The top and bottom layers were fortified as indicated under RESULTS. Centrifugation was carried out in a Spinco SW-50 rotor at 48000 rev./min for 48 h at 15–20°. The layers were carefully separated using a pasteur pipette. The membranes used for these experiments were washed once with water and twice with ^2H_2O containing 1 mM $NaHCO_3$. The washed membranes were suspended in ^2H_2O and solubilized by adding an equal volume of doubly concentrated detergent in ^2H_2O–sucrose of *d* 1.23.

Determination of density of membrane solutions. A density-gradient column according to LINDERSTRØM-LANG AND LANZ[11], in which density can be measured as a function of the height, was used (Section 3) for the determination of the density of the solutions. Since the density of a solution is a linear function of the concentration according to the equation: $d = (1-\bar{v}\rho)\, c + d_0$ (in which d = density of solution, d_0 = density of solvent, c = concentration of solute, $\bar{v}$ = partial specific volume of solute, ρ = density of solvent), the slope of the curve obtained by plotting d of the detergent against its concentration gave $(1-\bar{v}\rho)$ for the detergent. Similarly, $(1-\bar{v}\rho)$ for the membranes was obtained by plotting d against the concentration of the detergent-solubilized membrane material. The difference in density (Δd) between the detergent solution and the membrane solution dialysed against detergent, was measured. Since Δd results from the presence of membrane material and bound detergent, the contribution of the former can be calculated from the above equation by substitution of the measured concentration and $(1-\bar{v}\rho)$ of the membranes. Thus the contribution of membrane-bound detergent to Δd, and, hence, the amount of detergent per unit of membrane material can be calculated.

Analytical ultracentrifugation. All runs were performed in a Spinco model E analytical ultracentrifuge. Measurements (Section 1) were taken at 280 nm by using absorption optics in combination with an automatic scanning device, previously developed in this laborarory[12], either the absorbance or the differentiated absorbance was recorded directly. Schlieren optics were also used (Sections 1 and 5).

^{35}S-labeled sodium dodecyl sulfate. ^{35}S-labeled sodium dodecyl sulfate (9.5 mC/mmole) was obtained from the Radiochemical Centre, Amersham, England. Membranes were solubilized in 0.8 % dodecyl sulfate (0.11 μC/mmole dodecyl sulfate) and dialyzed against the latter dodecyl sulfate concentration, 0.4 % dodecyl sulfate (0.22 μC/mmole dodecyl sulfate) and 0.1% dodecyl sulfate (0.88 μC/mmole dodecyl sulfate). The radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer, model 4312.

RESULTS

(1) Sedimentation coefficients and molecular weights of isolated rat-liver plasma membranes disintegrated by detergents

The isolated membranes were suspended in 1 mM $NaHCO_3$ (pH 7.5) and solubilized in a final concentration of 1 % deoxycholate (sodium salt, pH 8.0) or 0.4 % dodecyl sulfate (in 0.01 M ammonium formate; pH 9.0). The solutions containing 0.6–0.9 mg membrane protein per ml were centrifuged for 15 min at 1500 × *g*, the small pellet obtained in the case of the deoxycholate–membrane solutions was discarded, and the 1500 × *g* supernatants were studied in the analytical ultracentrifuge. Some 10 % of the material (based on the absorbance at 280 nm) solubilized by deoxycholate, and some 5 % of that solubilized by 0.4 % dodecyl sulfate, sedimented during the acceleration of the rotor of the analytical ultracentrifuge. The sedimentation of the remaining bulk of the solubilized membrane material was followed by ultraviolet optics.

The sedimentation coefficients of the following types of membrane preparations, dissolved in 1 % deoxycholate or 0.4 % dodecyl sulfate, were determined: (a) intact membranes, (b) membranes from which the protein soluble in 0.15 M NaCl (the saline-soluble membrane protein) was removed to yield the saline-insoluble membranes, and (c) the saline-soluble membrane protein. As shown in Table I, the sedimentation coefficients of the deoxycholate-solubilized membranes, saline-insoluble membranes and saline-soluble protein were all about 2.7 S. The saline-soluble protein, in the absence of detergent, exhibited a sedimentation coefficient of 3.7 S with traces of heavier components corresponding to 4.9 or 7.3 S sometimes present. The sedimentation coefficient of the dodecyl sulfate-solubilized membranes did not differ materially from that obtained with deoxycholate–membrane solutions. In a number of experiments, schlieren optics were used together with absorption optics so as to obtain additional information about the sedimenting material. The schlieren profiles were similar to the derivative curves obtained by absorption optics, and the sedimentation coefficient of the maximum of the peaks was the same in both cases. It follows that the non-protein material of the membranes (*i.e.*, phospholipids which do not absorb

TABLE I

SEDIMENTATION COEFFICIENTS OF RAT-LIVER PLASMA MEMBRANES DISSOLVED BY DEOXYCHOLATE AND DODECYL SULFATE

Measured at 20° and presented without correction. Entries marked* obtained by the schlieren method, otherwise absorbance at 280 nm was used

Material	*Detergent*	$s_{w,20}$
Membranes	1 % deoxycholate	2.4 (2.3)*, 2.6, 2.7, 3.0
Membranes	0.4 % dodecyl sulfate	2.3, 2.4, 2.5
0.15 M NaCl-insoluble membranes	1 % deoxycholate	2.7 (2.8)*
0.15 M NaCl-soluble membrane fraction	—	3.7, 3.7 (4.9, 7.3)**
	1 % deoxycholate	2.7, 2.8
	0.4 % dodecyl sulfate	2.5, 2.5
	5.5 M urea	2.0

** In traces.

at 280 nm) sediments together with the ultraviolet-absorbing material. However, from the broadening of the sedimenting boundary as observed from the derivative curve (Fig. 1) it is evident that the membrane material dissolved in 0.4% dodecyl sulfate is heterogeneous.

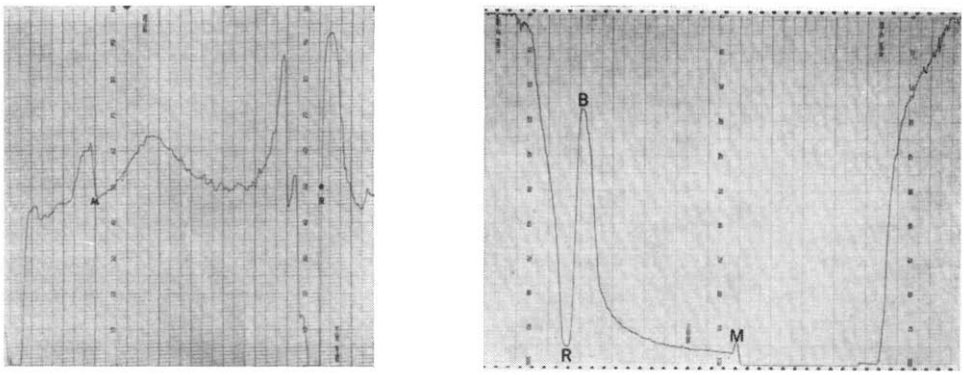


Fig 1 Sedimentation profile of membranes dissolved in 0.4 % dodecyl sulfate. The derivative curve was traced at 59780 rev /min, 64 min after reaching maximum speed R = reference point (7.30 cm), M = meniscus.

Fig. 2. Absorbance of a solution of plasma membranes in 0.4 % dodecyl sulfate after sedimentation–diffusion equilibrium was obtained. The experiment was performed with 0.5 ml of a membrane solution with an absorbance of 0.2. The sample was centrifuged for 5 h at 25000 rev./min and subsequently for 20 h at 8225 rev./min. R = reference point (7.30 cm); B = bottom; M = meniscus.

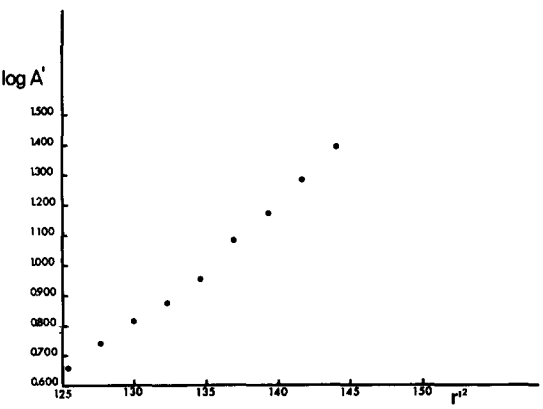


Fig. 3. Curve of log A' plotted against r'^2 (see Fig 2). No straight line is obtained. This indicates that the material is polydisperse. The molecular weights were calculated from the slope of this curve at the meniscus and at the bottom, respectively (for details see text). A' is absorbance in mm measured on the tracing. r' is the radial distance in cm measured on the tracing.

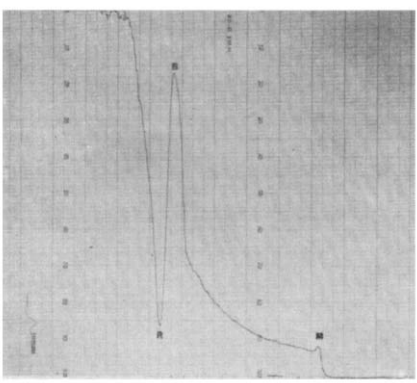
In order to obtain information about the size of the particles in solution, sedimentation–diffusion equilibrium experiments were performed. The distribution of the absorbance as a function of the radial distance is shown in Fig. 2 for a 0.4% dodecyl sulfate solution of the membranes. No straight line resulted when log A'

was plotted against r'^2 (Fig. 3), indicating that the material was polydisperse. Applying the equation of SVEDBERG AND PEDERSON[13]:

$$M = \frac{2\,RT}{(1-\bar{v}\rho)\omega^2} \cdot \frac{d \ln A}{dr^2}$$

(R = gas constant; T = absolute temperature; $\bar{v}$ = partial specific volume; ρ = density of the solution; ω = angular velocity; A = absorbance at a radial distance r), to the data at the meniscus and bottom, respectively, the molecular weight of the membrane material dissolved in 0.4 % dodecyl sulfate was found to change from 54000 to 74000 (not corrected for the amount of detergent attached to the particles, *cf.* Section 3). Thus, disaggregation of the plasma membranes by dodecyl sulfate yields heterogeneous material.

The results of similar experiments carried out with the saline-soluble membrane protein in the presence of 0.4% dodecyl sulfate are shown in Figs. 4 and 5. In this case also, the dissolved material was heterogeneous. The saline-soluble membrane protein has previously been found[8,14,15] to contain haemoglobin, various antigens and enzymes, and to be electrophoretically inhomogeneous. It has been shown that the saline-soluble protein is taken up from the cytoplasm following homogenization of liver[14,15].



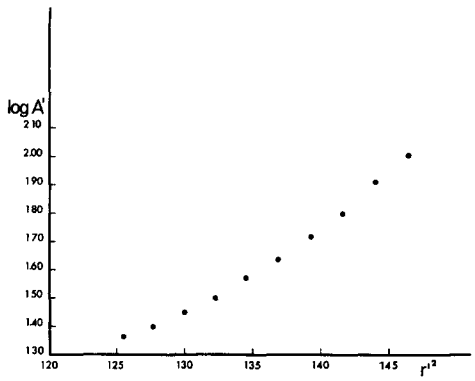


Fig 4 Absorbance of a solution of the saline-soluble part of plasma membranes in 0.4 % dodecyl sulfate after sedimentation–diffusion equilibrium was obtained. The experiment was performed with 0 5 ml of a protein solution with an absorbance of 0.3. The sample was first centrifuged for 6 h at 25 000 rev /min and then for 40 h at 8225 rev./min. R = reference point (7.30 cm), B = bottom, M = meniscus.

Fig 5 Curve of log A' plotted against r'^2 (see Fig. 4). No linear curve is obtained indicating that the material is polydisperse. Molecular weights of 31 000 and 54 000 were calculated from the slope of this curve at the meniscus and at the bottom, respectively. A' is absorbance in mm measured on the tracing, r' is the radial distance in cm measured on the tracing.

(2) *Aggregation and precipitation of solubilized membrane material following removal of detergent by dialysis*

The detergent-membrane solutions used in the following experiments were obtained as described in the previous section; for the precipitation experiments more concentrated membrane solutions were used.

(*a*) *Sodium deoxycholate.* Dialysis of the membrane material solubilized by 1 % deoxycholate against 0.01 M Tris buffer of pH 7.4 at 6–12° for 24 h led to aggregation,

as shown by the polydisperse peak with a maximum corresponding to 10–12 S (Table II), exhibited by the dialysed material on analytical ultracentrifugation. The aggregation size appeared to be inversely related to the pH of the dialysis fluid since the sedimentation coefficients increased when the pH of the dialysis medium was lowered from 9.4 to 7.4 (Table II). After dialysis for 24 h against Tris of pH 6.4 or lower, at least 93% of the dissolved membrane material (on a protein basis) became insoluble and precipitated. Precipitation occurred irrespective of the composition of the dialysis medium which was either Tris, phosphate buffer (pH 6.4, 5.4) or bidistilled water. When the deoxycholate–membrane solutions had first been dialysed against Tris of pH 7.4 to remove detergent, subsequent dialysis against the above media of lower pH also caused the membrane material to precipitate almost completely. Similar results were obtained with saline-insoluble membranes.

TABLE II

SEDIMENTATION COEFFICIENTS OF DETERGENT–MEMBRANE SOLUTIONS DIALYSED AGAINST 0.01 M TRIS BUFFER OF VARYING pH

Dialysis	$s_{w,20}$ (*uncorrected*)	
	1% deoxycholate–membrane solutions	*0.4% dodecyl sulfate–membrane solutions*
None	3.0, 2.7*, 2.6	2.3, 2.4
Tris buffer (pH 9.4)	5.5	2.4
Tris buffer (pH 8.4)	6.3	5.0
Tris buffer (pH 7.4)	12.5, 11.2*, 9.7	5.5, 5.6
Tris buffer (pH 6.4)	Precipitated	5.8
Tris buffer (pH 5.4)	Precipitated	8.9, 9.0

* 0.15 M NaCl-insoluble membranes.

(*b*) *Sodium dodecyl sulfate.* Solubilization of the membranes in 0.1% dodecyl sulfate yielded a sedimentation coefficient of 3.2 S. Dialysis of this solution against ammonium formate of pH 9.0 for 24 h yielded a peak with a maximum corresponding to 5.1 S. Dialysis of a 0.4% dodecyl sulfate–membrane solution against ammonium formate yielded material of 5.9 S. Dialysis of 0.4% dodecyl sulfate–membrane solutions against 0.01 M Tris buffers of various pH's led to much less aggregation than that observed for the deoxycholate–membrane solutions (Table II). This difference became most noticeable after dialysis of the deoxycholate– and dodecyl sulfate–membrane preparations at pH $\leqslant$ 6.4. Whereas under the latter conditions virtually all of the deoxycholate-solubilized membrane material precipitated, only a (very) slight precipitate was found when 0.4 or 0.8% dodecyl sulfate–membrane solutions were dialysed against Tris of pH 6.4 or 5.4, or water.

The influence of the ionic composition of the dialysis medium on the aggregation of the membrane material is shown by the following results. (i) Aggregates of 17.2 and 34 S were formed by dialysis of 0.1 or 0.4% dodecyl sulfate–membrane solutions against bidistilled water for 24 h as compared with 9 S after dialysis against Tris buffer of pH 5.4 (Table II); still higher aggregates, *i.e.*, of 41.2, 49.2, 65.8 and 87.8 S, but only a slight precipitate, were observed by prolonging the dialysis for 48 h. (ii)

Precipitation occurred when 1% deoxycholate–membrane solutions were dialysed against 1 mM $NaHCO_3$ for 70 h, whereas in the presence of 10 mM $NaHCO_3$ no visible precipitation was observed (Section 4c).

From these experiments it follows that the size of the membrane particles, arising by dialysis of detergent–membrane solutions, is governed by at least three parameters: the pH and the ionic strength of the dialysis fluid, and the nature of the detergent used for dissolving the membranes.

(3) The amount of detergent bound to dissolved membrane material

The amount of detergent bound to the dissolved membrane material (2–3 mg protein/ml) was measured in a density-gradient column according to LINDERSTRØM-LANG AND LANZ[11] as described under MATERIALS AND METHODS. The principle of this method is that the amount of free detergent withdrawn from the solution by binding to the membranes can be reintroduced into the membrane-detergent solution by dialysing the latter against excess of detergent solution. The resulting increase in the specific density of the dialysed, as compared with the non-dialysed preparations, was calculated and used to determine the amount of detergent bound per mg of membrane material. In some experiments, membranes were dissolved with ^{35}S-labelled dodecyl sulfate and the amount of bound dodecyl sulfate was calculated from the difference in radioactivity before and after dialysis of membrane solutions against various concentrations of ^{35}S-labelled dodecyl sulfate. In another series of experiments, based

TABLE III

AMOUNT OF DETERGENTS BOUND TO DISSOLVED MEMBRANES

3–4 mg membrane material (on dry wt. see MATERIALS AND METHODS) were dissolved per ml detergent solution (1% deoxycholate, 0.4 or 0.8% dodecyl sulfate). In some of the experiments dodecyl sulfate was used without the ammonium formate buffer of pH 9.0. Dialysis against the various detergent solutions, as indicated, was for 60–64 h. For principle of method see text and MATERIALS AND METHODS. Number of experiments indicated in parentheses

Detergent used for membrane solubilization (% final concn.)	*Detergent in dialysis fluid (%)*	*mg detergent bound per mg membrane material as measured by*		
		Density	*Differential refractometry*	*^{35}S-labelled dodecyl sulfate*
Deoxycholate, 1	1	1.05 ± 0.15 (4)		
	0*	0.0 (4)		
1.5	1.5		0.53 ± 0.03 (3)	
	1		0.91 ± 0.05 (2)	
Dodecyl sulfate, 0.4	0.4	1.20 ± 0.08 (4)		
	0	0.0 (2)		
0.8	0.8	0.75 ± 0.02 (2)	0.77 ± 0.15 (2)	0.78 ± 0.16 (2)
	0.4	1.25 ± 0.17 (2)	1.44 ± 0.11 (2)	1.45 ± 0.19 (2)
	0.1	0.31 ± 0.01 (2)	0.47 (1)	0.36 ± 0.10 (2)
	0*	0.0 (2)		0.022 ± 0.004 (2), 0.049**

* Dialysis against 1 mM Tris buffer (pH 7.4).

** Dialysis for 40 h; corresponding value for 60-h dialysis period of the same sample was 0.026 mg dodecyl sulfate bound per mg membrane material. A control in which 0.8% dodecyl sulfate (*minus* membrane) was dialysed for 46 h showed that no radioactivity was left in the dialysis bag).

on the same principle, the calculation was made from measurements made with a differential refractometer. The various techniques yielded a reasonable accordance of results. From the data collected in Table III, the following conclusions can be drawn. (i) The amount of detergent bound to the membrane material from a detergent solution of a given concentration depends on the nature of the detergent. (ii) Binding is a function of the concentration of detergent in the dialysis fluid. (iii) An optimal binding is found at a certain detergent concentration of the dialysis fluid, *i.e.*, 1% in the case of deoxycholate and 0.4% in that of dodecyl sulfate, since at higher detergent concentrations (1.5% deoxycholate and 0.8% dodecyl sulfate) less detergent is bound. However, given the limited number of detergent concentrations in this present study, the quantitative relation between the amount of bound detergent and the detergent concentration should be considered as approximate, since the binding optimum might well have been shifted to either higher or lower detergent concentrations had these been included in the measurements. (iv) Dialysis for 60 h against 0.01 M Tris buffer of pH 7.4 (containing no detergent) removed all of the detergent from the membrane material, as measured in the density gradient column. By the more sensitive radio method it was shown that only a very small amount of ^{35}S-labelled dodecyl sulfate was retained after dialysis in the absence of dodecyl sulfate.

It should be pointed out that quantitatively the present results apply only to detergent–membrane solutions which have been dialysed against excess of detergent. When membranes are dissolved in a detergent solution of certain concentration, the amount of bound detergent will depend on the effective detergent concentration, which is lowered by binding and, thus, the amount of bound detergent wil differ from that obtained after dialysis against excess of that detergent concentration. The curve relating the amounts of bound detergent with the effective concentrations of detergent can in principle be constructed from the data obtained in the dialysis experiments. Providing enough detergent concentrations are included, this curve could be used to determine the amount of bound detergent when a given quantity of membranes is dissolved in detergent solution of known concentration, and not dialysed. We have not pursued this application in view of the fact that only a few detergent concentrations were studied in the above experiments.

(4) Detergent-induced dissociation of membrane proteins and phospholipids as demonstrated by density-gradient centrifugation

Isolated rat-liver plasma membranes were solubilized by deoxycholate or dodecyl sulfate (3–4.5 mg membrane protein per ml final detergent solution) and subjected to density-gradient centrifugation under various experimental conditions. In assesssing the following data it should be noted that about 25% of the protein of the freshly isolated rat-liver membranes is soluble in 0.9% NaCl; 0.15% NaCl, which yields a Na^+ concentration approximately similar to that of the sodium salts of deoxycholate (1%) and dodecyl sulfate (0.8%) used in the present experiments, released 80–90% of the former amount of saline-soluble membrane protein. As a result, about 20% of the membrane protein will always, due to its specific density, be present in the gradient layer of the highest density on centrifugation.

(a) Dissociation produced by sodium dodecyl sulfate. In two experiments the isolated membranes were solubilized by 0.8% dodecyl sulfate (no buffer) in ^2H_2O supplemented with sucrose to a density of 1.17. A discontinuous gradient of eight

layers was set up consisting of ^2H_2O, 0.8% dodecyl sulfate and varying amounts of sucrose to yield densities from *d* 1.25 (bottom) to *d* 1.11 (top) (Δd per layer = 0.02; 5 ml total volume), containing the membrane solution in the *d* 1.17 layer (1 ml). After centrifugation for 48 h at 48000 rev./min at 15° in the Spinco SW-50 rotor, the gradient was separated into two parts at the *d* 1.17–1.19 interface and these parts were analyzed for protein and organic P. Since more than 90% of the membrane-bound P consists[8] of phospholipid P, the values reported for organic P can be applied to the phospholipid-P content of the membrane preparations. The upper part of the gradient (*d* 1.11–1.17) contained 13.0 ± 1.7% of the total protein and 80.3 ± 0.3% of the phosphorus, whereas 87.0 ± 1.7% of the protein and 19.7 ± 0.3% of the phosphorus was recovered in the lower part (*d* 1.19–1.25).

(*b*) *Differences in dissociation produced by sodium deoxycholate and sodium dodecyl sulfate.* That the membrane phospholipids were separated to an appreciable extent from the membrane proteins by the action of detergent was also demonstrated for deoxycholate. The membranes were dissolved in the above manner with either 1% deoxycholate or 0.8% dodecyl sulfate. The ^2H_2O–sucrose solution of *d* 1.17 containing the dissolved membranes was 'sandwiched' (*cf.* ref. 3), as the middle layer (1 ml), between two layers of *d* 1.15 and 1.19 (respectively the top and the bottom layer, 2 ml each) made up of ^2H_2O, detergent in the concentration indicated, and the required

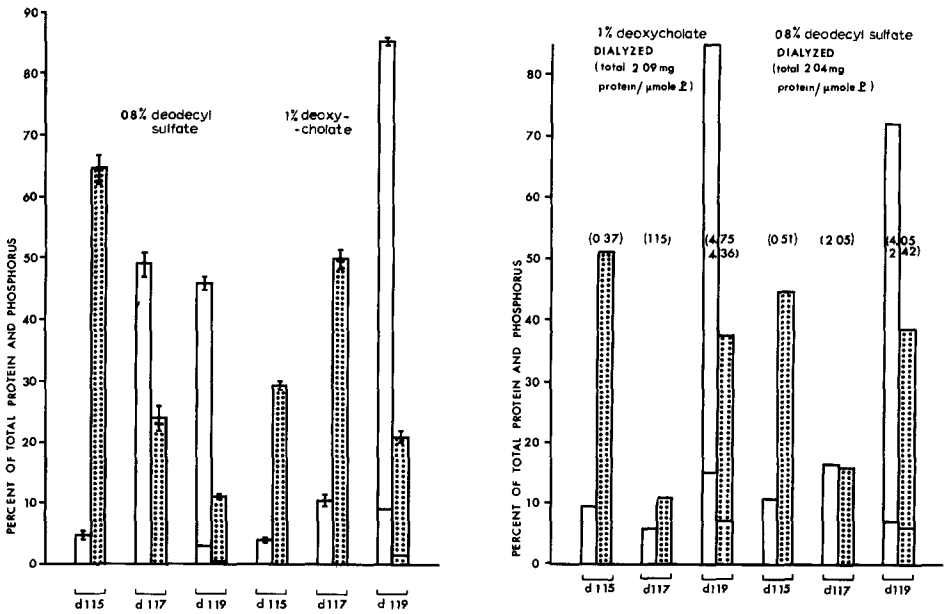


Fig. 6. Distribution of membrane protein and phospholipid following density-gradient centrifugation of detergent–membrane solutions. The three layers of the gradient were analyzed for protein and organic P and the sum contents were taken as 100. Open bars represent protein and dotted bars organic P. The *d* 1.19 layer contained some insoluble material, its protein and P contents are indicated.

Fig. 7. Distribution of membrane protein and phospholipid following density-gradient centrifugation of membrane solutions from which detergent had been removed by dialysis. See Fig. 6. The protein (mg Folin)–P (μmole) ratio calculated from the sum contents of the three gradient layers and the ratios for the individual layers are indicated in parentheses.

amount of sucrose. The tubes were then centrifuged at 18–20° as described and the three layers separated and analyzed for protein and organic P. The results of two experiments are shown in Fig. 6. In the experiments with dodecyl sulfate, the phosphorus content of the layers decreased in the order: top (64.7 ± 2.3%) > middle (23.9 ± 2.5%) > bottom layer (11.3 ± 0.3%), whereas the protein was almost equally divided between the middle and the bottom layers (49.1 ± 2.0 and 46.1 ± 1.8%, respectively), with a lesser amount (4.7 ± 1.0%) present in the top layer. The results of a similar experiment with 0.4% dodecyl sulfate, run concurrently with one using 0.8% dodecyl sulfate, showed close similarity except that in the former experiment the middle layer contained some 15% more and the bottom layer some 15% less protein than the corresponding layers in the 0.8% dodecyl sulfate experiment. In the experiments with deoxycholate, a general shift of material to layers of a higher density, as compared with the former results, was observed. The protein content of the three layers increased in the order: top (4.1 ± 0.1%) < middle (10.8 ± 0.5%) < bottom layer (85.1 ± 0.6%). The latter layer again contained the lowest percentage of the total phosphorus (20.8 ± 1.2%, this being, however, higher than that found in the corresponding layer of the 0.8% dodecyl sulfate experiments), while the highest amount of phosphorus was now present in the middle layer (49.7 ± 1.7%) with the top layer containing an intermediate amount (29.5 ± 0.5%).

The distribution of materials over the sandwich-gradient layers following prolonged centrifugation may be affected by diffusion. The effect of diffusion during 48 and 72 h, in the absence of centrifugal force, is shown in Table IV. Protein and P diffused in a constant ratio but the diffusion of 0.8% dodecyl sulfate-solubilized membrane material was asymmetric in that more material reached the *d* 1.15 than the *d* 1.19 layer. The difference between the distribution profiles illustrated here and in Fig. 6 gives further weight to the significance of the latter results. The differences between the layer profiles of protein and phospholipid for the two detergents (Fig. 6) might be due in part to the differences in the specific densities of deoxycholate (1.36) and dodecyl sulfate (1.25) which are bound to the membrane material.

TABLE IV

DIFFUSION OF PHOSPHOLIPID P AND PROTEIN OF DETERGENT-DISSOLVED MEMBRANES FROM MIDDLE LAYERS (*d* 1.17) TO TOP (*d* 1.15) AND BOTTOM LAYERS (*d* 1.19) OF SANDWICH GRADIENT IN THE ABSENCE OF CENTRIFUGAL FORCE

Membranes were dissolved with detergent as described in the text, included as middle layers in the sandwich gradient and kept for 48 h at room temperature in the tubes without centrifugation. In a separate experiment, indicated in parentheses, the dodecyl sulfate–membrane material was allowed to diffuse for 72 h. Distribution of organic P and protein as % of total amounts in the three layers.

Detergent	*Layer*	*Percentage distribution*	
		Organic P	*Protein*
Dodecyl sulfate, 0.8%	*d* 1.15	23.5 (21.0)	21.7 (19.5)
	d 1.17	65.8 (68.5)	66.5 (68.2)
	d 1.19	10.7 (10.5)	11.8 (12.3)
Deoxycholate, 1%	*d* 1.15	21.6	18 2
	d 1 17	60.6	66.8
	d 1.19	17 8	15.0

(*c*) *Persisting dissociation after removal of detergent.* Support for the latter conclusion was derived from the results obtained by the same sandwich-gradient technique but now using membrane-detergent solutions from which the detergent had been removed prior to centrifugation. To this end membrane-detergent solutions prepared in ^2H_2O, sucrose and 10 mM $NaHCO_3$ of *d* 1.17 were dialysed for at least 48 h against the latter medium. Centrifugation of the dialysed material in the sandwich gradient, lacking detergent but containing 10 mM $NaHCO_3$, led to the results of Fig. 7. Comparison of the latter with Fig. 6 shows that prior dialysis of the detergent-membrane solutions had in the case of the dodecyl sulfate experiments led to a shift of P from layers *d* 1.17 and especially *d* 1.15 to that of *d* 1.19, and of protein principally from *d* 1.17 to 1.19, whereas in the case of the deoxycholate experiments 35 % of the total P had been shifted from *d* 1.17 to *d* 1.15 and 1.19. As a result the distribution profiles of P and protein over the 3 layers were now (*i.e.*, after prior dialysis) quite similar for the deoxycholate- and dodecyl sulfate-dissolved membrane materials. 10 mM $NaHCO_3$ was added in these experiments to prevent precipitiation of the membrane material during dialysis. When 1 mM $NaHCO_3$ was used instead, appreciable precipitation occurred during dialysis (against ^2H_2O–sucrose–1 mM $NaHCO_3$, final pH after dialysis was 7.8) of the deoxycholate–membrane but less of the dodecyl sulfate–membrane solutions. Centrifugation of the dialysed material, including the precipitate, in the sandwich gradient and chemical analysis of the three layers yielded results (Table V) similar to those of Fig. 7. The precipitated material exhibited a much higher protein–P ratio than that of intact membranes or the precipitate obtained from deoxycholate–membrane solution by dialysis at pH 5.4 (Section 4d).

The former experiments were repeated with the following modifications: (i) Freshly prepared detergent-membrane solutions in ^2H_2O, containing 1 mM $NaHCO_3$, were centrifuged for 1 h at 105000 × *g* to remove sedimentable material amounting to 20–25 % and 7–10 % of the membrane protein of 1 % deoxycholate and 0.8 % dodecyl sulfate solutions, respectively. The supernatants of the deoxycholate and dodecyl sulfate–membrane preparations were each divided into two equal parts. (ii) One part of each preparation was dialysed for 24 h against ^2H_2O and sucrose of *d* 1.17 containing the corresponding detergent concentration (for rationale see Section 3) and subsequently subjected to density-gradient centrifugation in the sandwich system containing detergent. The percentage distribution of P and protein over the three layers, listed in Table V, resembled the corresponding values obtained in the experiments of Fig 6 (in which the present dialysis step had been omitted) except for some differences in the protein distribution over the *d* 1.17 and *d* 1.19 layers in the dodecyl sulfate experiments. (iii) The remaining parts of the detergent-membrane solutions were dialysed for 48 h against ^2H_2O, sucrose and 10 mM $NaHCO_3$ of *d* 1.17 to remove detergent. The results of the chemical analyses after density-gradient centrifugation were identical with those of Fig. 7 and Table V, first experiment. The presence in the top layer (*d* 1.15) of the sandwich gradient of some 50 % of the phospholipids as against at the most 14 % of the protein, while 72–89 % of the protein was recovered in the bottom layer (*d* 1.19) after density-gradient centrifugation in the various experiments, indicates that a substantial dissociation of membrane protein and phospholipids had persisted following the removal of the detergents. From Figs 6 and 7 and Table V, the minimal percentage phospholipid P dissociated from protein (that present in excess to the percentage ratio of 1:1 of a hypothetical homogeneous

TABLE V

PERCENTAGE DISTRIBUTION OF PROTEIN AND PHOSPHOLIPID P IN A THREE-LAYER GRADIENT FOLLOWING DENSITY-GRADIENT CENTRIFUGATION

Two experiments are illustrated. In the first experiment the detergent-membrane solutions, containing 1% deoxycholate or 0.8% dodecyl sulfate and ^2H_2O–sucrose of *d* 1.17, were dialysed against ^2H_2O–sucrose of *d* 1.17 containing 1 mM $NaHCO_3$ (indicated in table dialysed). In the second experiment the detergent-membrane solutions had first been centrifuged at 105000 × *g* and the supernatants were either dialysed against ^2H_2O–sucrose and the corresponding detergent (indicated in table deoxycholate, dodecyl sulfate present), or against ^2H_2O–sucrose of *d* 1.17 containing 10 mM $NaHCO_3$ (indicated in table dialysed). Each preparation was layered between ^2H_2O–sucrose solutions of *d* 1.15 and 1.19 and centrifuged.

Conditions	*Protein and organic P in layers* (%)			
	d 1.15	*d* 1.17	*d* 1.19	
			soluble	*precipitated*
Deoxycholate (dialysed)				
Protein	10.7	2.9	27.0	59.4
P	50.3	5.2	13.1	31.4
(Protein/P*	0.44	1.18	4.42	4.01)
Dodecyl sulfate (dialysed)				
Protein	12.9	5.1	67.8	14.2
P	55.2	10.7	25.2	8.9
(Protein/P*	0.46	1.09	5.04	3.15)
Deoxycholate (present)				
Protein	2.5	12.5	85.0	
P	16.8	56.2	27.0	
Deoxycholate (dialysed)				
Protein	14.0	7.3	78.7	
P	52.4	15.0	32.6	
Dodecyl sulfate (present)				
Protein	3.9	24.5	71.6	
P	63.2	23.3	13.5	
Dodecyl sulfate (dialysed)				
Protein	1.3	9.6	89.1	
P	44.4	12.2	43.4	

* The protein (mg)–P(μmole) ratio is listed; that of the three layers combined, as calculated from the sum contents, was 2.13 for the deoxycholate experiment and 1.93 for the dodecyl sulfate experiment.

subunit) can be obtained by subtracting the percentages of protein present in the *d* 1.15 and *d* 1.17 layers from those of the P. In round figures, with dodecyl sulfate present 59–60%, and after removal of dodecyl sulfate 34–48% of the phospholipid P was free, while the corresponding values for the deoxycholate experiments amounted to 55–58% and 42–48%, respectively. These estimates are conservative since there is no indication that the remainder of the phospholipids (including that present in the *d* 1.19 layer) is attached to protein.

(*d*) *Supporting evidence for dissociation after removal of deoxycholate.* (i) Membranes were dissolved in 1% deoxycholate, dialysed first against 0.01 M Tris (pH 8.4) for 24 h and then against 0.01 M Tris (pH 5.4) for 80 h. More than 99% of the phosphorus together with 96.9% of the protein precipitated during the second dialysis. A preliminary experiment showed the membrane cholesterol also to be present in the precipitate.

Thus, the protein (mg, Folin)–P (μmole) ratio of the precipitated material was similar to that of the soluble material in the dialysis bag, *i.e.*, about 2.1 (Fig. 7, Table V). The corresponding ratio for fresh membranes was 2.1–2.3 in the present experiments.

(ii) When the membrane material, solubilized by deoxycholate, was first fractionated in the sandwich gradient as described above, the resulting layers separated and each layer dialysed at pH 8.4 and 5.4 as indicated in the previous paragraph, the following results were obtained. After the second dialysis step and centrifugation for 10 min at 13000 rev./min, all the phosphorus present in the bottom (*d* 1.19) layer, comprising about 30% of the total P present in the three gradient layers, had precipitated together with most of the protein present in the *d* 1.19 layer; the latter precipitate contained about 80% of the total protein present in the three gradient layers, whereas an amount corresponding to 5% of the total protein remained in solution in the *d* 1.19 layer. However, little precipitate was discernible in the top and middle gradient layers after they had been dialysed at pH's 8.4 and 5.4 and centrifuged. Virtually all of the material present in these fractions (their P content corresponding to about 70% of the total P) remained soluble. These results give additional support to the conclusion arrived at from the previous experiments (4c), and show that for precipitation of the deoxycholate-less proteins and phospholipids present in the top and middle layers to occur, the presence of the material in the bottom layer is necessary.

(*e*) *Some properties of the membrane precipitate.* The precipitated membrane material of the bottom layer, obtained in the latter experiments (4d, ii), was taken up in sucrose–H_2O of *d* 1.15 and the resulting suspension was layered on sucrose solutions of *d* 1.16, 1.18, 1.20 and 1.22. After sedimentation for 3 h at 38000 rev./min and 20° in the SW-39 rotor, the vast majority of the material gathered visually at the *d* 1.20/1.22 interface, a minor amount being present in the *d* 1.20 layer In contrast, when the membrane material precipitated *in toto* in experiments of type 4d (i), was suspended in sucrose–water of *d* 1.14, layered on sucrose solutions of *d* 1.15–1.20 ($\Delta d = 0.01$) and centrifuged to equilibrium, only a trace amount gathered at the *d* 1.19–1.20 interface while virtually all of the material had now accumulated at the interfaces or in the layers of *d* 1.15–1.19. Thus the buoyant density of the membrane material precipitated *in toto*, although more heterogeneous, was closer to that of intact liver plasma membranes (which gathered at the *d* 1.16/1.17/1.18 interfaces following flotation in a sucrose gradient at 2°) than was the buoyant density of the material precipitated from the *d* 1.19 layer of the sandwich gradient. This difference between the two types of precipitates is most likely related to their relative contents of protein and phospholipid (*cf.* Section 4d). The heterogeneous buoyant density of the material precipitated *in toto* suggests that by dialysing at pH 5.4, various complexes differing in individual protein–phospholipid ratios, but with an overall ratio resembling that of intact membranes, had been formed. In earlier experiments, in which the second dialysis step at pH 5.4 had been confined to a 24-h duration (and the first dialysis step at pH 7.4 had sometimes been omitted), both the buoyant density and the protein–P ratio of the precipitated material closely resembled that of intact membranes. The morphology of the latter membrane precipitate will be considered in DISCUSSION. Table VI shows that the ATPase (EC 3.6.1.3) and (Na^+-K^+)-ATPase—enzymes which are dependent on plasma membrane lipid[16] in different ways—are not increased in activity when the membrane material is precipitated from solution from which deoxycholate had

TABLE VI

ATPASE, (Na^+-K^+)-ATPASE AND 5'-NUCLEOTIDASE ACTIVITIES OF DIALYSED DEOXYCHOLATE–MEMBRANE SOLUTIONS AND MEMBRANE MATERIAL PRECIPITATED AT pH 5.4

Membranes solubilized in 1 % deoxycholate were dialysed for 22 h against 0.01 M Tris buffer of pH 7 4 (a), and subsequently for 22 h against 0.01 M Tris buffer of pH 5.4 to yield a precipitate (b). Mean of three experiments with standard deviation. For controls see ref. 16.

Membrane preparation	*ATPase*	*(Na^+-K^+)-ATPase*	*5'-Nucleotidase*
Dialysed (a)	30.2 ± 6.6	0.0–1.0	87.2 ± 19.0
Precipitate (b)	29 4 ± 5.4	1.2 ± 0 4	77.0 ± 7.3

been removed by dialysis. Since unpublished experiments have shown that in the deoxycholate–membrane preparations, dialysed at pH 7.4, some 50 % of the ATPase and 90–95 % of the (Na^+-K^+)-ATPase activities were lost as compared with fresh membranes, it follows that none of the lost activity was restored in the membrane precipitate.

(*f*) *Ultrasonic oscillation.* A concentrated membrane suspension in ^2H_2O containing 1 mM $NaHCO_3$ was subjected to ultrasonic waves for 6 min at 0° using an M.S.E. ultrasonic disintegrator (18000–20000 cycles/sec, 60 W), resulting in a slightly opaque solution. The latter was diluted with an equal volume of a ^2H_2O solution containing 19 mM $NaHCO_3$ and sucrose of *d* 1.23 to yield a final density of 1.17, and layered between two layers consisting of ^2H_2O–sucrose–10 mM $NaHCO_3$ of

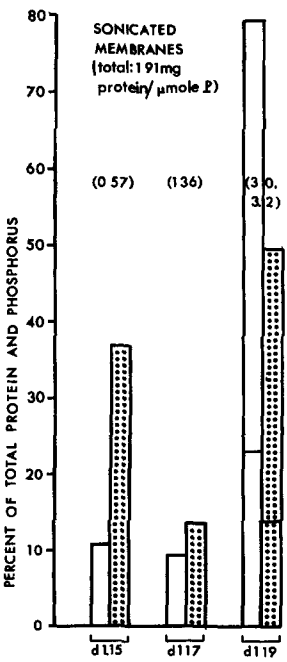


Fig. 8. Distribution of membrane protein and phospholipid following density-gradient centrifugation of membranes dissolved by sonic oscillation. For explanation see legends of Figs 6 and 7.

d 1.15 and *d* 1.19 respectively. After centrifugation at 20° for 26 h at 49000 rev./min in the SW-50 rotor, the resulting layers were somewhat turbid, the surface of the upper layer containing floating material while the bottom layer contained a pellet, as found in the previous experiments (4c). The distribution of P and protein over the three layers is shown in Fig. 8. The varying protein–P ratios of the three layers (indicated by the figures in parentheses in Fig. 8) show that sonication under the conditions of the present experiments leads to at least a partial separation between membrane protein and phospholipid.

(6) *Dissociation of membrane components in the presence and after removal of detergents as shown by analytical ultracentrifugation*

(a) *Sodium dodecyl sulfate.* Membranes were dissolved in ^2H_2O–sucrose of *d* 1.17 containing 0.8% dodecyl sulfate and centrifuged at 50740 rev./min for 24 h in the analytical ultracentrifuge. After reaching equilibrium, schlieren optics showed (Fig. 9b) that a large negative concentration gradient at the meniscus, formed by floating material, was superimposed on the high positive gradient formed by the sucrose solution. The broadening of the meniscus (*cf.* control on dodecyl sulfate in the absence of membranes, Fig. 9a) indicated the accumulation of material at the meniscus. From the accumulation of material near the bottom it is evident that material with higher

Figs 9. a and b. Equilibrium centrifugation in the analytical ultracentrifuge of membrane–dodecyl sulfate solution in *d* 1.17 medium. Membranes were dissolved in a solvent of *d* 1.17 with 0.8% dodecyl sulfate (see text) a. (left) · no membranes b (right) the solution of membranes. The samples were centrifuged for 24 h at 50740 rev /min. Centrifugal force from right to left

Figs 10. a and b. Equilibrium centrifugation in the analytical ultracentrifuge of detergent-free membrane solutions in *d* 1.17 medium Membranes were dissolved in a detergent solution with a *d* 1 17 (see text). a (left): a dialysed solution of membranes originally dissolved in 1% deoxycholate. Two cells containing the solution of membranes and the medium, respectively, were spun together. The solution of membranes corresponds to the broad (centrifugal) meniscus. b. (right) a dialysed solution of membranes originally dissolved in 0.8% dodecyl sulfate. In all experiments centrifugation was for 24 h at 50740 rev./min. Centrifugal force from right to left.

specific density than that causing the negative gradient at the meniscus was also present. Similar results were obtained (Fig. 10a) with membrane solutions from which dodecyl sulfate had previously been removed by dialysis as described in Section 4c. Thus, the present experiments show that at an appropriate density of the medium, analytical ultracentrifugation can demonstrate the dissociation of the membranes, both with dodecyl sulfate present and after its removal, into materials of higher and lower density than *d* 1.17. These results, which must be due to the presence of materials of at least varying lipid and protein contents, are in accordance with the results of the chemical measurements following density-gradient centrifugation, described in Sections 4b and c.

(*b*) *Sodium deoxycholate.* Similar experiments were carried out with 1 % deoxycholate. After centrifuging the deoxycholate–membrane solution to equilibrium in the analytical ultracentrifuge in a medium of *d* 1.17, no negative gradient was observed at the meniscus, which was only slightly broadened (*cf.* Fig. 6 and Table V for the smaller amount of phospholipid arriving on the *d* 1.15 layer in the case of deoxycholate than in that of dodecyl sulfate). After the prior removal of deoxycholate by dialysis, again no negative gradient could be observed (Fig. 10b) but the meniscus had now become broader (*cf.* Fig. 7 and Table IV for the increased amount of phospholipid now arising on the *d* 1.15 layer), though less extensive than in the above experiments in which dodecyl sulfate had been used for membrane solubilization. These results may be explained by assuming that larger micelles or complexes, which were compactly layered (resulting in the limited broadening of the meniscus) allowing no back diffusion at equilibrium (hence no negative gradient), rather than less of the same materials (*cf.* Fig. 7, Table V), had floated to the meniscus in the present experiments than had been the case in the previous ones with dodecyl sulfate. The differences in sedimentation coefficients observed (Section 2) between the dialysed deoxycholate– and dodecyl sulfate–membrane solutions, support this conclusion. The presence of floating and much sedimented material, the latter being demonstrated by the broad bottom (Fig. 10b), visualized by schlieren optics in the membrane solutions from which deoxycholate had been removed, shows that no homogeneous population of lipoprotein particles or lipid–protein complexes had arisen.

DISCUSSION

Dissociation of membrane components by detergents

Analytical ultracentrifugation, as described in Section 1 of this paper, has shown that heterogeneous material is formed when isolated rat-liver plasma membranes are solubilized by deoxycholate or dodecyl sulfate. This result contrasts with that of RAZIN *et al.*[2] and ENGELMAN *et al.*[3] on dodecyl sulfate-solubilized surface membranes obtained from *M. laidlawii*. Considering that the conditions under which the experiments were carried out do not allow one to observe dissociation between membrane components (see below), the above difference between our results and those of the latter authors may be related to the more complex nature of the liver than of the mycoplasm plasma membranes. The liver membranes contain membrane junctions and specialized areas coated with a globular repeat structure[14,15].

Density-gradient centrifugation and chemical analysis (Section 4) demonstrated

that the liver-membrane phospholipids were to an appreciable extent separated from the membrane protein by the action of the detergents. This result is in agreement with that of ENGELMAN *et al.*[3]. Equilibrium centrifugation in the analytical ultracentrifuge, using a medium of d 1.17 (Section 5), corroborated the chemical results by showing the presence of floating and sedimenting material. Obviously then, the schlieren pattern may be used as a criterion for the heterogeneity of the dodecyl sulfate-solubilized membrane material, provided that a medium of proper density is used in which the various components can be separated and do not sediment with equal speed. Hence, the questioning by ENGELMAN *et al.*[3] of the utility of the schlieren pattern for the present problem should be amended.

It was demonstrated by chemical analysis of the density-gradient layers (Section 4c) and corroborated by equilibrium centrifugation in the analytical ultracentrifuge in d 1.17 medium (Section 5) that the dissociation between the two main components of the liver membranes had persisted after removal of the detergents by dialysis. Additional support for the latter conclusion was provided by the finding (Section 4d, ii) that after dialysing deoxycholate out, two-thirds of the membrane phospholipids (present in the gradient layers of d 1.15 and 1.17) required the presence of material of $d \geqslant 1.19$, probably protein, for their precipitation at pH 5.4, whereas the unfractionated membrane material precipitated *in toto* at pH 5.4. The persistent dissociation after detergent removal observed in liver plasma membranes stands in sharp contrast to the results of ENGELMAN AND MOROWITZ[6,7] who reported that a small homogeneous lipoprotein aggregate was formed from mycoplasm membranes after removal of dodecyl sulfate by dialysis. The reason for this discrepancy is less apparent. However, apart from a difference in the dialysis media in the two studies, liver plasma membranes differ from mycoplasm membranes by containing much more cholesterol. The molar ratio of cholesterol to phospholipid P was 0.65 for rat-liver plasma membranes[15] and approx. 0.1 for *M. laidlawii* membranes (calculated from ref. 2). The possibility that cholesterol–phospholipid micelles are formed in preference to phospholipid–protein complexes after the removal of detergent in our experiments is under investigation.

Differences in effects of deoxycholate and dodecyl sulfate on plasma membranes

Although both deoxycholate and dodecyl sulfate caused separation of the membrane components, the following differences between the two detergents in their action on the membranes have been observed.

(i) Membranes dissolved in 0.8 % dodecyl sulfate contained less material sedimenting at 105 000 $\times g$ than did 1 % deoxycholate–membrane solutions (Sections 1 and 4c). (The 105 000 $\times g$ sediment of the deoxycholate–membrane solution contained almost 50 % of the total ATPase activity of the intact plasma membranes whereas only 10 % at most of this activity was accounted for by the deoxycholate-soluble material after removal of the detergent by dialysis. The former sediment contains[17] the tight junctions and other non-identified material.)

(ii) Different amounts of the detergents were bound to the dissolved membrane material and the detergent concentrations needed for optimal binding differed (Section 3). The finding of maximal binding at a certain detergent concentration and of decreased amounts of detergent being bound at higher concentrations might be related to the stability of the detergent micelles formed at different concentrations

relative to that of the complexes (micelles) between membrane components and detergent.

(iii) Aggregation of membrane materials, as shown by the sedimentation coefficients, was much more marked when deoxycholate-membrane solutions were dialysed against media of decreasing pH than in the case of dodecyl sulfate–membrane solutions. Dialysis at pH $\leqslant 6.4$ caused all material present in the dialysis bag containing membrane preparations originally dissolved in deoxycholate, to precipitate, whereas only a tiny precipitate was formed in the experiments with dodecyl sulfate (Section 2).

(iv) Differences in the distribution of materials over the three layers of the sandwich gradient were observed with dodecyl sulfate– and deoxycholate–membrane solutions (Section 4b). Although these differences disappeared for the greater part after prior dialysis of the two types of solutions against a detergent-free medium (Section 4c), the results of equilibrium centrifugation in the analytical ultracentrifuge in a medium of d 1.17 (Section 5) indicated a dissimilarity between the complexicity (micellar size) of the materials present in the two types of solutions (see (iii), above).

The results suggest that the types of complexes (micelles) formed between the membrane components and each of the two detergents may be fundamentally different and that differences, although modified, may remain after removal of detergent. To throw further light on this problem, the cholesterol distribution and the nature of the individual phospholipids present in the three layers after density-gradient centrifugation of membrane solutions in the presence and after removal of detergent, are being studied.

Plasma membrane structure

The structural configuration of biological membranes is still a matter of dispute[15]. The use of deoxycholate and dodecyl sulfate for the study of the plasma membrane structure appears of limited significance in view of their dissociative effect on the membrane components. Even if homogeneous lipid–protein–detergent particles had been formed, the possibility of artifacts induced by the detergents existed. Likewise, the finding by ENGELMAN and co-workers[5–7] that homogeneous lipid–protein particles arise as a result of dodecyl sulfate removal from mycoplasm membrane solutions, may stem from an artificial recombination of the previously separated membrane components to particles which are not present *in situ*. ENGELMAN AND MOROWITZ[6] were unable to demonstrate electron microscopically the soluble lipid–protein particles in negatively-stained preparations. Although we could not find evidence for lipid–protein particles after detergent removal in the present experiments, a morphologically distinct particle (and aggregates thereof), next to randomly and rod-like shaped structures, has previously been demonstrated (Figs. 14 and 15 in ref. 14) after dialysis of dodecyl sulfate– or deoxycholate–membrane solutions against ammonium formate buffer of pH 9.0. The possibility cannot be excluded that these particles of unknown composition are artificially formed when the negatively-stained preparations are drying.

Following removal of deoxycholate by dialysis of the membrane solutions at pH 7.4, and subsequent dialysis at pH 5.4, the precipitated membrane material showed a buoyant density and protein–P ratio resembling those of intact membranes. Electron microscopy of thin sections of the precipitate has demonstrated (Fig. 13 in

ref. 14) the presence of short membraneous sheets and vesicles of globular appearance (resembling that of fresh smooth endoplasmic reticulum membranes and Ca^{2+}-depleted plasma membranes[15]) and less of a triple-layered structure. A similar appearance resulted after dialysis at pH 5.4 in the presence of 0.1 mM $CaCl_2$. By dialysing their dodecyl sulfate–membrane solutions against medium of pH 7.4 containing 20 mM Mg^{2+}, ENGELMAN and co-workers[5,7] also obtained a membrane precipitate that by buoyant density, chemical analysis and morphology resembled intact mycoplasm membranes. Yet these criteria are not sufficient to demonstrate that the original membrane structure has been restored. Special 'markers' of intact plasma membranes, such as the asymmetric location of sialic acid in the plasma membrane element[18] and the lipid dependence of certain membrane enzymes[16], should be studied in the 'reconstructed' membranes. This has been done with the membrane precipitate obtained from deoxycholate solution, and the results, shown in Table VI of the present paper and Figs. 6 and 7 of ref. 14, point to the conclusion that by the above criteria the original membrane configuration was not restored.

Finally, the existence of a small lipid–protein aggregate of plasma membrane origin—as found by ENGELMAN AND MOROWITZ[6,7] in the case of *M. laidlawii*, but for which we have not obtained evidence in the case of rat liver—that is soluble in water and capable of assembling into a membraneous structure, may lead one to assume[6] that such particles are prefabricated somewhere in the cell, and thence transported and assembled at the plasma membrane. In the case of liver this view is difficult to reconcile with the finding of WIDNELL AND SIEKEVITZ[19] that the half-life of the plasma-membrane protein is at least twice that of the phospholipid–glycerol of the membranes.

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