in the membrane or a fundamental weakness in the membrane model. It should be noted that the use of Eqn. 2 to describe the membrane resistance implies that the total membrane conductance is simply the sum of the K^+ and Na^+ conductances. This, however, need not be the case since recent experiments with *Nitella clavata*[6] suggests that it is the H^+ conductance which provides the major contribution to the total conductance. If this is also the case in *Nitella translucens* then it follows that the values of P_{K^+} and P_{Na^+} estimated from electrical experiments such as are described herein are grossly overestimated.

Biophysics Section,
Department of Natural Philosophy,
University of Edinburgh (Great Britain)

E. J. WILLIAMS
J. HOGG

1 A. B. HOPE AND N. A. WALKER, *Australian J. Biol. Sci.*, 14 (1961) 26.
2 R. M. SPANSWICK, J. STOLAREK AND E. J. WILLIAMS, *J. Exptl. Botany*, 18 (1967) 1.
3 J. HOGG, E. J. WILLIAMS AND R. J. JOHNSTON, *Biochim. Biophys. Acta*, 150 (1968) 640.
4 G. P. FINDLAY AND A. B. HOPE, *Australian J. Biol. Sci.*, 17 (1964) 62.
5 R. M. SPANSWICK AND J. W. F. COSTERTON, *J. Cell Sci.*, 2 (1967) 451.
6 H. KITASATO, *J. Gen. Physiol.*, 52 (1968) 60.

Received October 16th, 1969

Biochim. Biophys. Acta, 203 (1970) 170–172

BBA 73095

Separation of membrane components produced by anionic detergents and maintained after the latter's removal

In a previous paper[1] it was reported that sodium deoxycholate and sodium dodecyl sulfate produced a high degree of separation of the proteins and the phospholipids of isolated rat-liver plasma membranes that persisted after removal of the detergent by dialysis. This permanent separation was at variance with the results obtained by TERRY *et al.*[2] and by ENGELMAN AND MOROWITZ[3] on surface membranes of *Mycoplasma laidlawii*. According to these authors, the proteins and phospholipids of the mycoplasma membranes, dissociated by sodium dodecyl sulfate, reaggregated to form a homogeneous particle by dialyzing the detergent out.

Rat-liver plasma membranes are rich in cholesterol, whereas the mycoplasma membranes are not; their molar ratios of cholesterol to phospholipid-P amount to 0.65 and 0.1, respectively. In order to explain the aforementioned difference between the two membrane species, it was suggested[1] that by dialyzing detergent-solubilized liver membranes phospholipid–cholesterol complexes might be formed in preference to phospholipid–protein complexes, whereas homogeneous complexes of the latter type might arise from detergent-solubilized membranes containing but little cholesterol.

In the present experiments, the first possibility was studied by comparing the equilibrium distributions of cholesterol and phospholipid-P of isolated rat-liver mem-

branes, solubilized by sodium deoxycholate (1%) or sodium dodecyl sulfate (0.8%), in a density gradient consisting of three layers (*d* 1.15, 1.17, 1.19), both in the presence of detergent and after its prior removal by prolonged dialysis of the preparations. The results are illustrated in Table I. As was previously observed for the membrane proteins and phospholipids (ref. 1 and Table I), the distribution of cholesterol over the three gradient layers after centrifugation was different for the deoxycholate- and dodecyl sulfate-solubilized membrane preparations. However, after prior removal of the detergents by dialysis, these differences were much less pronounced. Under all conditions, the gradient layer of lowest density (*d* 1.15) contained relatively less phospholipid-P than cholesterol, whereas the opposite was true for the two other layers (*cf.* also the molar ratios in Table I). However, the percentage distribution of cholesterol over the three gradient layers resembled much more closely that of the

TABLE I

PERCENTAGE DISTRIBUTION OF CHOLESTEROL AND ORGANIC P IN A THREE-LAYER GRADIENT FOLLOWING DENSITY-GRADIENT CENTRIFUGATION OF ISOLATED RAT-LIVER PLASMA MEMBRANES SOLUBILIZED BY SODIUM DEOXYCHOLATE OR SODIUM DODECYL SULFATE, BEFORE AND AFTER DIALYSIS

The experimental procedures were similar to those described previously[1] and consisted of solubilizing freshly isolated rat-liver plasma membranes with either 1% sodium deoxycholate or 0.8% sodium dodecyl sulfate in ^2H_2O containing sucrose of *d* 1.17. Part of each preparation was dialyzed at 12° for 48–60 h against ^2H_2O, sucrose and 10 mM $NaHCO_3$ of *d* 1.17 and then, concurrently with an undialyzed sample, subjected to density-gradient centrifugation. To this end 1 ml of each preparation, containing 1.2–1.9 μmoles organic P, was "sandwiched" (*cf.* ref. 2) between two layers of *d* 1.15 and *d* 1.19 (2 ml each) made up of ^2H_2O, detergent (present in the case of undialyzed, absent in that of dialyzed samples), and the required amount of sucrose. The tubes were centrifuged for 48 h at 48000 rev./min at 15–18° in the Spinco SW-50 rotor, and the three layers were separated, dialyzed to remove sucrose, and analyzed for cholesterol[4] and organic P[5]. Since more than 90% of the membrane-bound P consists[6] of phospholipid-P, the values reported for organic P illustrate the phospholipid content of the materials. Two experiments are listed. The molar ratios of cholesterol to P present in the combined layers of each experiment were calculated from the sum contents and amounted to 0.72 with deoxycholate present, 0.82 with deoxycholate dialyzed out, 0.68 with dodecyl sulfate present, and 0.80 with the latter dialyzed out, suggesting that some phospholipid had been lost by dialysis.

Conditions	*Cholesterol and organic P in gradient layers* (%)		
	d 1.15	*d* 1.17	*d* 1.19
Deoxycholate (present)			
Cholesterol	31.1 ± 0.8	44.1 ± 3.1	24.8 ± 2.3
P	23.6 ± 1.4	49.9 ± 4.9	26.5 ± 3.4
Cholesterol/P (molar ratio)	1.0	0.62	0.70
Deoxycholate (dialyzed)			
Cholesterol	60.5 ± 1.4	13.9 ± 2.4	25.6 ± 0.9
P	49.3 ± 2.3	12.5 ± 0.1	38.2 ± 1.8
Cholesterol/P (molar ratio)	1.07	0.76	0.55
Dodecyl sulfate (present)			
Cholesterol	72.6 ± 2.2	19.1 ± 2.4	8.3 ± 0.2
P	54.1 ± 2.0	30.7 ± 2.6	15.2 ± 0.6
Cholesterol/P (molar ratio)	0.92	0.44	0.35
Dodecyl sulfate (dialyzed)			
Cholesterol	63.9 ± 6.2	17.9 ± 4.6	18.2 ± 1.7
P	40.1 ± 3.8	21.2 ± 2.5	38.7 ± 1.3
Cholesterol/P (molar ratio)	1.27	0.56	0.35

phospholipid-P than did the distribution of the latter resemble that of the proteins (as illustrated previously[1]). These results suggest that phospholipids and cholesterol may associate mutually in preference to protein and thus are not at variance with the first possibility mentioned above.

The second possibility, *i.e.* that a homogeneous type of phospholipid–protein complex might arise by removal of detergent from solubilized membranes containing but little cholesterol, was studied by the same technique on microsomal membranes stripped of ribosomes. These membranes are poor in cholesterol; the molar ratio of cholesterol to phospholipid-P of liver microsomes amounted to 0.1–0.15. As shown in Table II, the proteins and phospholipid-P of the microsomal membranes were separated to a large extent by deoxycholate (1 %), and separation was maintained, though in altered form, after dialysis. The dissociation, as judged by the equilibrium distributions of the microsomal membrane components over the three gradient layers, was similar in pattern but quantitatively more pronounced than that observed for the plasma membranes.

TABLE II

PERCENTAGE DISTRIBUTION OF PROTEIN AND ORGANIC P (OR PHOSPHOLIPID P) FOLLOWING DENSITY-GRADIENT CENTRIFUGATION OF ISOLATED RAT-LIVER MICROSOMAL MEMBRANES SOLUBILIZED BY SODIUM DEOXYCHOLATE BEFORE AND AFTER DIALYSIS

Liver homogenate was prepared in 1 mM $NaHCO_3$ (w/v as for plasma membrane isolation) and centrifuged for 20 min at 20000 × *g*. The resulting supernatant was subjected to 105000 × *g* for 1 h and the pellet washed with ^2H_2O containing 1 mM $NaHCO_3$. The suspension was treated with 1% sodium deoxycholate in ^2H_2O to a final concentration of 0.2% detergent. Ribosomes were sedimented by centrifugation for 3 h at 105000 × *g*. Detergent in ^2H_2O and sucrose was then added to the supernatant to a final concentration of 1% detergent and *d* 1.17. Further conditions as in Table I. Protein was determined by the biuret method and phospholipid P in chloroform–methanol (2:1, v/v) extracts[5]. RNA-P was calculated from RNA measured according to MUNRO AND FLECK[7], on the basis of a P content of 9.7%.

Expt.	*Conditions*	*Protein and P (phospholipid P) in layers (%)*		
		d 1.15	*d* 1.17	*d* 1.19
1a	Deoxycholate (present)			
	Protein	1.2	0.1	92.7
	P	16.2	63.9	19.9
	Protein/P ratio*	0.10	0.14	6.65
1b	Deoxycholate (dialyzed out)			
	Protein	19.2	8.5	72.3
	P	63.0	12.7	24.3
	Protein/P ratio*	0.44	0.97	4.32
2a	Deoxycholate (present)			
	Protein	1.2	5.0	93.8
	Phospholipid-P	8.2	61.2	30.6
	Protein/phospholipid-P ratio*	0.23	0.14	5.1
	Phospholipid-P/RNA-P ratio	0.93	17.6	96.3
2b	Deoxycholate (dialyzed out)			
	Protein	17.1	5.9	77.0 (24.8)**
	Phospholipid-P	76.1	6.5	17.4 (3.1)**
	Protein/phospholipid-P ratio*	0.40	1.60	7.7

* The protein (mg)/P (or phospholipid-P, μmole) ratio is listed; that of the three layers combined, as calculated from the sum contents was 1.43, 1.45, 1.64 and 1.76 for Expts. 1a, 1b, 2a and 2b, respectively.

** Values indicate the percentage of material which became insoluble (*cf.* ref. 1).

Separation of the microsomal membrane components was also obtained with dodecyl sulfate (0.8 %). The separation which persisted after removal of this detergent by dialysis was not changed by addition of cholesterol (finely dispersed by ultrasonic oscillation in detergent solution and added in an amount corresponding to an ultimate cholesterol to phospholipid-P ratio of 0.70) to the membrane preparation before dialysis.

The present and previous[1] results show that detergent-solubilized liver plasma and microsomal membranes both before and after dialysis contain materials of varied buoyant densities, materials which greatly differ in their protein, phospholipid and cholesterol contents as compared with the mean composition of the intact membranes. Since it seems very unlikely that these results reflect a heterogeneously structured organization of the intact membranes, it follows that under our experimental conditions (i) a pronounced dissociation between the membrane components had been induced by the detergents, (ii) dissociation was maintained after removal of detergent, and (iii) no homogeneous particles had arisen irrespective of the cholesterol content of the membranes. It might, however, appear that divalent cations, such as Mg^{2+} or Ca^{2+}, are required for plasma-membrane reconstitution (*cf.* ref. 8). The appearance of sedimentable vesicles upon diluting deoxycholate (0.26 %)-solubilized liver microsomes has been reported by ERNSTER *et al.*[9].

Department of Biochemistry,
Antoni van Leeuwenhoekhuis:
The Netherlands Cancer Institute,
Amsterdam (The Netherlands)

P. EMMELOT
H. VAZ DIAS

1 W. S. BONT, P. EMMELOT AND H. VAZ DIAS, *Biochim. Biophys. Acta*, 173 (1969) 389.
2 T. M. TERRY, D. M. ENGELMAN AND H. J. MOROWITZ, *Biochim. Biophys. Acta*, 135 (1967) 391.
3 D. M. ENGELMAN AND H. J. MOROWITZ, *Biochim. Biophys. Acta*, 150 (1968) 376.
4 T. C. STADTMAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 3, Academic Press, N.Y., 1957, p. 392.
5 W. R. MORRISON, *Anal. Biochem.*, 7 (1964) 218.
6 P. EMMELOT, C. J. BOS, E. L. BENEDETTI AND PH. RÜMKE, *Biochim. Biophys. Acta*, 90 (1964) 126.
7 H. N. MUNRO AND A. FLECK, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 14, Interscience Publ., New York, 1966, p. 113.
8 S. ROTTEM, O. STEIN AND S. RAZIN, *Arch. Biochem. Biophys.*, 125 (1968) 46.
9 L. ERNSTER, PH. SIEKEVITZ AND G. E. PALADE, *J. Cell Biol.*, 15 (1962) 541.

Received October 24th, 1969