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BIOCHEMICAL ASPECTS OF THE VISUAL PROCESS

XXXII. MOVEMENT OF SODIUM IONS THROUGH BILAYERS COMPOSED OF RETINAL AND ROD OUTER SEGMENT LIPIDS

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SUMMARY

The leakage of Na^+ from sonicated liposomes, composed of rod outer segment lipids, retinal lipids and a 4 : 1 phosphatidylcholine/phosphatidylserine mixture, has been studied. Both retinal and rod outer segment lipid liposomes lose Na^+ faster than Ca^{2+} which indicates that the observed leakage occurs from closed liposomal structures.

Liposomes from rod outer segment lipids are extremely leaky, losing sodium about 10 times as fast as retinal lipid liposomes and twice as fast as the phosphatidylcholine/phosphatidylserine liposomes.

This high permeability of rod outer segment lipid liposomes, as compared to retinal lipid liposomes, is probably due to both the higher degree of unsaturation of the fatty acid chains and their lower cholesterol content. In the rod outer segment lipid extract 48 % of the fatty acid chains consists of docosahexaenoic acid ($C_{22:6}$) against only 24 % in retinal lipid extract. Rod outer segment lipids contain 4.0 % cholesterol against 12.3 % in retinal lipids.

The sodium leakage from rod outer segment lipid liposomes is little affected by the presence of 5 mM calcium in the external dialysis medium, but with the two other types of liposomes significant decreases in permeability of about 20 % are observed.

The results are discussed in connection with the role of cations in visual excitation.

INTRODUCTION

The diffusion of ions through membranes of the photoreceptor cell is apparently an important aspect of the process of visual excitation. Na^+ are thought to be the charge carriers of the current which enters the outer segments in the dark [1, 2]. Illumination

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid.

decreases this sodium influx, presumably by the release from the rod sacs of Ca^{2+} which close the sodium channels in the outer membrane [3, 4].

In order to obtain information about the diffusion of these ions through photoreceptor membranes, we have studied their movements through model membranes. Phospholipid bilayers are generally considered to play an important part in the structure and function of biological membranes [5, 6], presumably functioning primarily as a diffusion barrier and as a matrix for the membrane proteins [7]. Therefore, we have constituted lipid vesicles (liposomes) from the lipid part of these membranes, as a first step to the reconstitution of photoreceptor membranes. In a previous study we have investigated the movement of Ca^{2+} through liposome membranes [12].

In order to assess whether a bilayer formed from rod outer segment lipids shows exceptional behaviour with respect to its ion permeability, we have now compared the sodium leakage from liposomes composed of rod outer segment lipids, retinal lipids, phosphatidylcholine and phosphatidylcholine/phosphatidylserine (4 : 1) mixtures. We have also investigated whether the presence of Ca^{2+} can influence the leakage of Na^{+} through the liposome membrane.

METHODS

Lipid extraction. Retinal lipids are extracted from whole cattle retina by means of chloroform/methanol. Two methods have been used which are found to be equally effective. In the first method the material is extracted by three consecutive treatments with a chloroform/methanol/water mixture (20 : 10 : 1.2, by vol.). The extraction (1 ml solvent per 10 mg material) is performed by continuous shaking on a Griffin shaker for 30 min under nitrogen, followed by centrifugation (10 min at $5900 \times g$) and isolation of the chloroform layer. In the second method the membrane material is first homogenized in chloroform/methanol/water (5 : 10 : 4, by vol.). The suspension is then vigorously shaken for 5 min and chloroform is added, raising the chloroform/methanol ratio to 1 : 1. After shaking the mixture for 1 min, water is added to a final chloroform/methanol/water ratio of 10 : 10 : 9. This suspension is shaken again for 1 min, centrifuged for 10 min at $5900 \times g$ and the chloroform layer is collected. This procedure is repeated twice. In both procedures the combined chloroform extracts are washed once with 0.2 volume of 0.1 M KCl to remove the remaining protein [8]. All manipulations are carried out under nitrogen. After evaporation of the chloroform the lipids are dissolved in benzene/ethanol (4 : 1, v/v) and stored at $-20°C$ under N_2.

Rod outer segments are isolated using a sucrose density gradient as described elsewhere [9]. The rhodopsin-containing layer is collected and diluted with one volume 0.16 M Tris · HCl (pH 7.4). After centrifugation (10 min at $4600 \times g$) the sediment is washed once with water, and lipid is extracted from the particulate material, as described above.

Phosphatidylcholine is isolated from egg yolk [10] and phosphatidylserine from brain [11]. Both phospholipids are stored under N_2 in benzene/ethanol (4 : 1, v/v) at $-20\,°C$.

Liposome preparation. Liposomes are prepared in the following way. Organic solvent is removed from 30 mg phospholipid in a rotating evaporator. To the resulting

thin film is added 4 ml of a salt solution containing: 135 mM NaCl, 10 mM Tris · HCl (pH 7.4) and 3–6 μCi ^{22}Na (3.5 Ci/mmol, Radiochemical Centre, Amersham, England). In the experiments with ^{45}Ca, 0.1 mCi ^{45}Ca (445 mCi/mol, Radiochemical Centre, Amersham) is added instead of ^{22}Na, which brings the total calcium concentration in the medium to approx. 0.05 mM. Suspension is achieved by vigorous mechanical shaking of the mixture under N_2 for 30 min. The suspension is then sonicated using a Branson B12 sonifier with microtip at half-maximal output. The material is cooled in ice and sonicated under N_2 for ten 1-min intervals. The suspension is then left overnight at 4 °C to equilibrate.

Analysis by gas-liquid chromatography shows that the fatty acid composition, also of the highly unsaturated retinal and rod outer segment lipids (Table II), is virtually identical before and after sonication. This indicates that no oxidative damage occurs during sonication.

The radioactive ions which have not been trapped in the liposomes, are removed by means of gel filtration [12]. Complete separation of liposomal and extraliposomal radioactivity is achieved.

Leakage experiments. Samples of 1 ml suspension are placed in small dialysis bags of Visking tube, previously boiled in a solution containing 2 mM $NaHCO_3$ and 0.2 mM EDTA and thoroughly washed with double-distilled water. The closed bags are placed in test tubes (1.3 × 14.5 cm), containing 12 ml of either 135 mM NaCl, 10 mM Tris · HCl (pH 7.4) or 145 mM Tris · HCl (pH 7.4). The stoppered tubes are attached to a vertically rotating disc (7 rev./min). Over a dialysis period of at least 80 min, 1-ml samples are taken from the outer solution for radioactive counting. At the end of the experiment the radioactivity remaining inside the dialysis bag is also measured. All experiments are carried out at room temperature (approx. 20 °C).

All radioactive samples are dissolved in 10 ml Aquasol (New England Nuclear) and counted in a Philips liquid scintillation analyzer.

A mathematical description of the efflux is given elsewhere [12]. Liposomes with permeability p and volume v_c contain $N-n$ counts. The dialysis bag has a permeability p_1, a volume v_1 and contains $n-n_2$ counts. The external solution has a volume v_2 and contains n_2 counts. The efflux of radioactive ions can then be described with the equation:

$$\frac{v_2}{v_0} - \frac{n_2}{N} = \frac{v_2}{v_0(K-L)} Ke^{-Lt} - Le^{-Kt}$$

where

$$v_0 = v_1 + v_2,\ L = \frac{p}{v_c} \quad \text{and} \quad K = \frac{p_1 v_0}{v_1 v_2}$$

Analytical methods. The lipid phosphorus in the lipid extracts is determined by means of a modified Fiske-SubbaRow method after H_2SO_4-$HClO_4$ digestion [13]. In calculating the phospholipid content the average phosphorus content of the phospholipids is assumed to be 4 %.

Cholesterol is quantitatively determined after saponification of the lipid extract with alcoholic KOH. Aliquots of these extracts, containing 20–180 μg cholesterol, are subjected to analysis by a modified Liebermann-Burchard reagent [14].

Preparation of fatty acid methyl esters for gas-liquid chromatography is carried out with BF_3 by the method of Morrison and Smith [15]. Gas chromatographic separations are performed on an ethylene glycol succinate column at 200 °C [16]. For identification purposes a silicone column (0.125 inch × 6 ft) is used, packed with 3 % SE-30 on Gaschrom Q (100–120 mesh). The carrier gas is nitrogen (15 ml/min), the temperature is isothermal at 200 °C or is linearly programmed from 160 to 200 °C (2.5 °C/min).

RESULTS

Liposome formation

Treatment of phospholipids as described under Methods yields a suspension of lipid vesicles, which sequester part of the radioactive ions present in the medium. The capture of Na^+ in the various types of liposomes is presented in Table I. Rod outer segment lipid liposomes capture less ^{22}Na (0.7 %) than liposomes derived from retinal lipids (1.9 %). In both types of liposomes the capture of calcium is considerably higher than that of sodium. For example, the calcium capture in rod outer segment lipid liposomes is 1.8 vs. 0.7 % for sodium.

TABLE I

CAPTURE OF Na^+ BY VARIOUS TYPES OF LIPOSOMES

The liposomes are prepared as described under Methods and separated from the medium over a Sephadex G-50 column. Results are expressed as percent of total radioactivity eluted from the column, present in the liposomal fraction (with standard error of the mean for at least three experiments).

Liposome composition	Capture (%)
Phosphatidylcholine	0.6 ± 0.2
Phosphatidylcholine/phosphatidylserine (4:1)	1.2 ± 0.1
Retinal lipids	1.9 ± 0.3
Rod outer segment lipids	0.7 ± 0.3

Sodium efflux from liposomes

The sodium efflux from the four types of liposomes is given in Fig. 1. Log $(v_2/v_0 - n_2/N)$ is plotted against time (see Methods). Two dialysis media have been used, 135 mM NaCl, 10 mM Tris · HCl, pH 7.4 (Fig. 1A) and a medium without sodium, containing only 145 mM Tris · HCl, pH 7.4 (Fig. 1B). The behaviour is the same in both media. All efflux curves are reasonably straight lines with the exception of those of the phosphatidylcholine/phosphatidylserine liposomes, which are clearly discontinuous.

Phosphatidylcholine liposomes show a relatively low efflux. In the first 30 min of dialysis 0.9 % of the total sequestered sodium leaks out. The efflux from retinal lipids liposomes, measured after 30 min dialysis, is 2–3 times higher. Liposomes composed of a 4 : 1 phosphatidylcholine/phosphatidylserine mixture, which is the same phospholipid ratio as is found in retina [17] and rod outer segments (cf. ref. 18), lose sodium 7–11 times as fast as the phosphatidylcholine liposomes. Liposomes

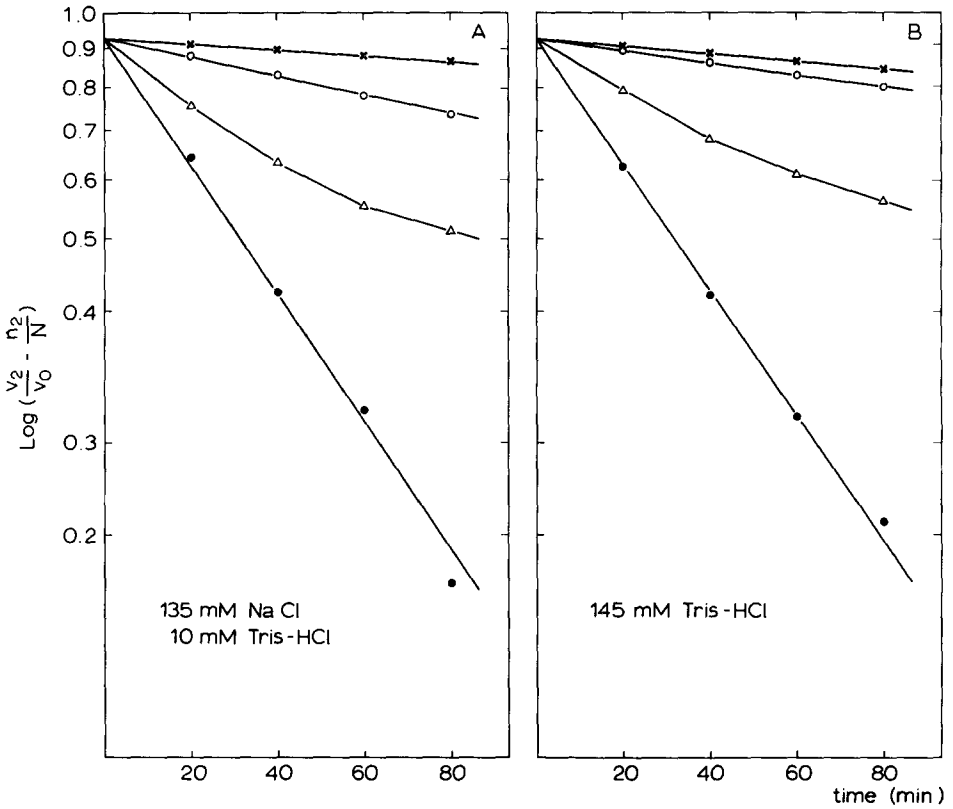

Fig. 1. Efflux of ^{22}Na from different types of liposomes. Log $(v_2/v_0 - n_2/N)$ is plotted against time (in min). 1 ml liposome suspension (in 135 mM NaCl, 10 mM Tris · HCl, pH 7.4) is dialyzed against 12 ml 135 mM NaCl, 10 mM Tris—HCl, pH 7.4 (A) or 12 ml 145 mM Tris · HCl, pH 7.4 (B). The liposomes are prepared from phosphatidylcholine (-×-), retinal lipids (-○-), a 4:1 phosphatidylcholine phosphatidylserine mixture (-△-) or rod outer segment lipids (-●-).

composed of rod outer segment lipids are even more leaky, losing sodium 18–22 times as fast as phosphatidylcholine liposomes and 7–13 times as fast as the retinal lipid liposomes. This latter difference is particularly interesting, since the phospholipid composition of whole retina and rod outer segment is rather similar.

The high sodium leakage rate for rod outer segment lipid liposomes raises the question whether this lipid mixture might form incompletely sealed particles, with ions diffusing through large openings between lamellae rather than across continuous bilayers. This phenomenon is known to occur with liposomes made of pure phosphatidylethanolamine [19]. If this should be the case, no discrimination between the leakage rates of different ions is expected. Fig. 2, however, shows that there exists a clear difference between the leakage rates of sodium and calcium from rod outer segment lipid liposomes, the leakage rate for ^{22}Na being about four times as fast. A similar discrimination exists in the case of retinal lipid liposomes which lose ^{22}Na twice as fast as ^{45}Ca. Addition of 1 mM of the calcium-chelating agent ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA) to the dialysis medium does not enhance the calcium efflux from these liposomes. In "open" struc-

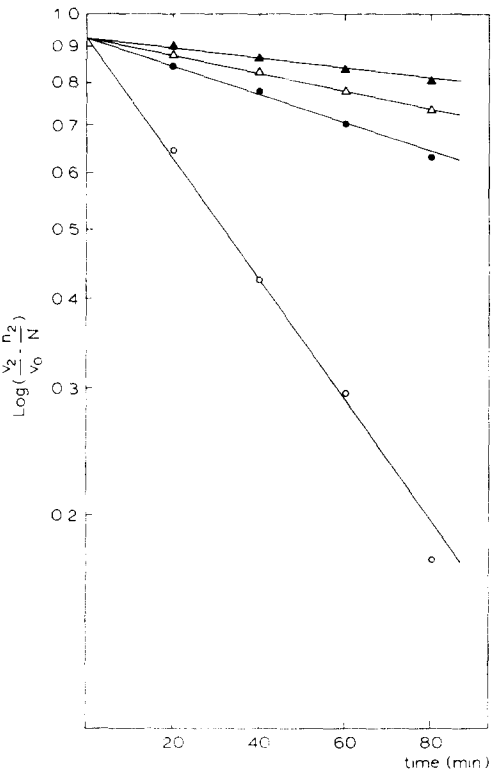

Fig. 2. Efflux of ^{22}Na and ^{45}Ca from liposomes, composed of retinal and rod outer segment lipids. 1 ml liposome suspension is dialyzed against 12 ml 135 mM NaCl, 10 mM Tris · HCl, pH 7.4. The leakage rates from retinal lipid liposomes (–△–, ^{22}Na; –▲–, ^{45}Ca) and rod outer segment lipid liposomes (–○–, ^{22}Na; –●–, ^{45}Ca) are plotted as log $(v_2/v_0 - n_2/N)$ vs. time (in min).

tures, where calcium can be reached by a relatively large molecule as EGTA which is unable to pass a lipid bilayer, an effect would have been expected. Thus it appears that both types of liposomes do indeed form closed structures.

Fatty acid and cholesterol effects on leakage rates

The strikingly higher sodium leakage rate for liposomes composed of rod outer segment lipids than for those composed of retinal lipids raises the question which factors are responsible for this phenomenon. Since the phospholipid composition of whole retina is about the same as that of isolated rod outer segments, it appears unlikely that variation in phospholipid composition is the cause of the difference in leakage rate.

However, another important factor which influences the permeability properties of liposomes is the fatty acid composition of the lipids [20]. Rod outer segments are known to contain a high proportion of poly-unsaturated fatty acids (cf. ref. 18). Therefore, we have compared the fatty acid composition of the lipids which constitute the different types of liposomes (Table II). There does indeed exist a large difference in degree of unsaturation of the fatty acid chains between the lipid extracts from retina and rod outer segments. Particularly noteworthy is the difference in

TABLE II

FATTY ACID COMPOSITION OF LIPIDS USED FOR LIPOSOME PREPARATION

The major fatty acids are expressed as percentage of the total fatty acid content.

Fatty acid (C atoms: double bonds)	Phosphatidyl-choline	Phosphatidyl-serine	Cattle retina extract	Cattle rod outer segment extract
16:0	52.0	1.2	20.2±0.5	13.0±0.9
16:1	0.8	0.1	1.0	0.4
18:0	10.1	37.2	19.9±0.9	19.3±0.2
18:1	19.0	31.2	10.7±0.2	3.2±0.3
18:2	11.5	—	1.4	0.7±0.1
20:4	2.9	1.6	9.7±0.7	5.4±0.7
22:4/22:5	0.4	5.2	7.4±2.2	8.2±3.4
22:6	2.5	7.2	24.1±3.5	48.2±4.4

docosahexaenoic ($C_{22:6}$) acid content which in rod outer segment lipids is twice as high as in whole retina lipid extracts. The percentage of this long-chain, highly unsaturated fatty acid species is even higher than previously reported for rod outer segment extracts (cf. ref. 18). Calculation shows that the average number of double bonds per fatty acid molecule is 2.3 for retinal lipids and 3.5 for rod outer segment lipids.

The cholesterol content of the lipid extracts has also been reported to be an important factor in determining the permeability of liposomes [20, 21]. Therefore, we have determined the amount of cholesterol in our lipid extracts. When expressed as weight-percent of the phospholipid content, the retinal lipid extracts contain 12.3 % (S.E.: 1.2, three determinations) and the rod outer segment lipid extracts 4.0 % (S.E.: 0.8, four determinations) cholesterol.

In order to test the influence of cholesterol on the sodium efflux, liposomes have been prepared from the 4 : 1 phosphatidylcholine/phosphatidylserine mixture and from rod outer segment lipids, to each of which 10 % (w/w) of cholesterol is added. As compared to the controls, a decrease in ^{22}Na leakage of 39 and 43 % is observed in the phosphatidylcholine/phosphatidylserine and rod outer segment lipid liposomes, respectively, during the first 30 min of dialysis. It should be noted, that the addition of 10 % cholesterol to the rod outer segment lipids interfered with the preparation of liposomes insofar as only about half of the lipid at the wall of the vessel became suspended during the usual shaking procedure.

Thus it appears that both the degree of unsaturation of the fatty acids and the cholesterol content play a role in causing the high sodium leakage rate in rod outer segment lipid liposomes.

Effect of calcium on sodium leakage from liposomes

It has been proposed that calcium acts in visual excitation by closing the sodium channels in the rod outer segment outer membrane. Therefore, we have investigated whether addition of Ca^{2+} to the dialysis medium affects the leakage rate of Na^+ from the various types of liposomes. Table III represents the results of our experiments. Addition of 5 mM calcium to a dialysis medium, containing 135 mM

TABLE III

EFFECT OF CALCIUM ON ^{22}Na LEAKAGE FROM VARIOUS TYPES OF SONICATED LIPOSOMES

1 ml liposome suspension is dialysed against 12 ml buffer, with or without 5 mM calcium. The results in columns 2 and 3 are expressed as the percentage of captured ^{22}Na which is lost during 30 min dialysis. *P* value for differences in ^{22}Na leakage with and without 5 mM calcium: double-sided values determined by the combined Wilcoxon test for *n* experiments, each carried out in triplicate.

Liposome composition	^{22}Na leakage in 30 min (%)		Ratio +Ca/−Ca	No. of experiments	*P* value
	−Ca	+Ca			
Dialysis medium: 135 mM NaCl, 10 mM Tris · HCl (pH 7.4), 0 or 5 mM Ca^{2+}					
Phosphatidylcholine/phosphatidylserine (4:1)	25.8	22.6	0.88	2	0.02
Retinal lipids	7.0	5.2	0.74	4	0.03
Rod outer segment lipids	32.4	31.5	0.97	2	0.80
Dialysis medium: 145 mM Tris · HCl (pH 7.4), 0 or 5 mM Ca^{2+}					
Phosphatidylcholine/phosphatidylserine (4:1)	20.5	17.0	0.83	2	0.01
Retinal lipids	4.6	3.6	0.87	7	0.001
Rod outer segment lipids	33.9	31.1	0.92	4	0.02

NaCl, 10 mM Tris · HCl (pH 7.4), decreases the leakage rate of ^{22}Na from phosphatidylcholine/phosphatidylserine and retinal lipid liposomes significantly by 26 and 12 %, respectively. With rod outer segment lipid liposomes the effect, if any, is very small. In addition, flocculation repeatedly occurs during the dialysis. The latter difficulty is overcome by using 145 mM Tris · HCl (pH 7.4) instead of 135 mM NaCl, 10 mM Tris · HCl. Now addition of 5 mM calcium to the dialysis medium causes significant decreases in all three leakage rates of 17, 23 and 8 % in phosphatidylcholine/phosphatidylserine, retinal lipid and rod outer segment lipid liposomes, respectively.

In an attempt to increase the relatively low effect in rod outer segment lipid liposomes, we have also tried to sequester calcium inside this type of liposome to see whether this would influence the sodium leakage. However, we are unable to prepare liposomes from rod outer segment lipids in the presence of 5 mM calcium. Even when only 0.5 mM calcium is added to the medium in which the liposomes are usually formed, no clear lipid suspension can be obtained. This is the more intriguing because addition of calcium, up to a concentration of 10 mM, does not prevent the formation of liposomes from retinal lipids.

DISCUSSION

The phospholipid mixtures used in these experiments do indeed appear to form closed lamellar structures. While this has already been shown for liposomes composed of pure phosphatidylcholine [22], the phosphatidylcholine/phosphatidylserine mixture and the lipid mixtures extracted from rod outer segments and from whole retina appear to behave similarly. This is strongly indicated by the fact that the sodium leakage rates from the last two types of liposomes are significantly higher than the calcium leakage rates (Fig. 2). Another indication is given by the fact that

the presence of EGTA in the dialysis medium does not affect the efflux of calcium. Finally, electron micrographs of negatively stained liposomes seem to confirm the presence of closed structures.

It appears likely that the four types of liposomes differ in size and shape. This must be taken into account in comparing the sodium efflux rates as a function of the phospholipid composition, because differences in size and shape of the liposomes may affect the efflux rates. However, it appears that these variations in size do not change the efflux rates very much. Papahadjopoulos and Watkins [19] have compared the leakage rates of some ions from various types of liposomes which had been prepared by manual shaking, mechanical shaking or sonication. Sonication produces small vesicles with one or two concentric bilayers, while shaking yields much larger structures surrounded by many bilayers. While a higher efflux rate might be expected for smaller liposomes, due to a high surface to volume ratio, the opposite is true. The small vesicles produced by sonication generally show about half as high an efflux rate as the liposomes produced by mechanical shaking. This finding is confirmed in our experiments with phosphatidylcholine/phosphatidylserine liposomes. The sonicated liposomes lose 33 % of their ^{22}Na during 1 h of dialysis, while those prepared by mechanical shaking lose 45 %. On the other hand, the sodium efflux from sonicated retinal lipid liposomes is 13 % per h which is twice as high as the efflux rate from a non-sonicated preparation (6 % per h). Thus it appears that, while changes in size and structure may affect the efflux rates, the differences are not as large as e.g. those encountered between retinal lipid and rod outer segment lipid liposomes (see Fig. 1).

The difference in sodium leakage rates for retinal lipid liposomes and liposomes composed of rod outer segment lipids is striking indeed. Rod outer segment lipid liposomes in NaCl/Tris lose sodium seven times and those in Tris thirteen times as fast as retinal lipid liposomes do. In view of the foregoing discussion, it appears that this difference must be explained by the composition rather than by the configuration of the liposomes. Since the phospholipid composition of both preparations is about the same [17, 18], there must be other factors responsible for this difference.

The degree of unsaturation of the fatty acid chains is an important factor in determining the permeability of liposomes [20, 23, 25, 26]. The fatty acids of the rod outer segment membranes are highly unsaturated, much more so than those of the total retinal lipids. This is mainly caused by the extremely high docosahexaenoic acid content (48 %) in the outer segments. This percentage is significantly higher than previously reported for rod outer segment membrane extracts, which range from 23 to 37 % (cf. ref. 18). This may well be due to the improved isolation method for outer segments used in our experiments.

If unsaturation of the phospholipids would be the only factor in determining the permeability of the liposomes used in this study, one should expect the leakage rate to increase in the order phosphatidylcholine/phosphatidylserine, retinal lipid, rod outer segment lipid liposomes (Table II), taking into consideration the approximately equal surface charge of the constituting phospholipids. However, the order is actually reversed for the first two types of liposomes (Fig. 1).

This points to a possible role of the cholesterol content of the lipids, since it is known that the presence of large amounts of cholesterol decreases the permeability of liposomes for glycerol [20, 21] and monovalent cations [23, 24]. There is indeed a difference in cholesterol content between our lipids: in the rod outer segment extract

cholesterol constitutes 4.0 % of the total lipid weight, in the retinal extract 12.3 %, while it is absent in the phosphatidylcholine/phosphatidylserine mixture. Incorporation of 10 % cholesterol in phosphatidylcholine/phosphatidylserine and rod outer segment lipid liposomes does indeed considerably reduce the permeability of these liposomes. Thus the cholesterol content appears to be another important factor in determining the order of increasing leakage rates in our experiments (Fig. 1). Together with the effect of fatty acid unsaturation it satisfactorily explains our results. It is noteworthy to mention that addition of small amounts of cholesterol (10 %) to synthetic lecithins hardly decreases their permeability [20, 21]. This difference with our observations is probably due to the fact that we have used mixtures of phospholipids with a high content of polyunsaturated fatty acid chains [21].

Finally, we have investigated whether the presence of 5 mM calcium in the dialysis medium has an effect on the sodium leakage rate. With phosphatidylcholine/phosphatidylserine and retinal lipid liposomes a decrease of about 20 % is observed, but with rod outer segment lipid liposomes the effect is smaller (less than 10 %) and is only statistically significant in a Tris · HCl medium (Table III). Although the direction of the effect is in accordance with the proposed calcium-induced decrease in sodium permeability of intact rod outer segments, we feel that the small size of the effect at this high calcium concentration (5 mM) suggests that the presence of membrane proteins in the rod outer segment membranes is crucial for the calcium-induced decrease in sodium permeability.

A permeability-decreasing effect of calcium would appear to have to be closely related to the degree of calcium binding by the phospholipids in the liposomes. Since outer segment liposomes have about the same phospholipid composition as retinal lipid liposomes, one would expect the rod outer segment lipid liposomes to be affected in a similar fashion as the retinal lipid liposomes. The smaller effect on rod outer segment lipid liposomes may mean that less calcium is bound to the phospholipids in this case. This again could be caused by the high percentage of unsaturation in the fatty acid chains, since Shah and Schulman [27] report that introduction of unsaturated fatty acids in lecithins decreases their affinity for divalent cations.

In conclusion it can be stated that the presence of an exceptionally high degree of unsaturation in the fatty acid moiety and the lower cholesterol content, introduces a high sodium permeability in liposomes composed of rod outer segment lipid extracts, as compared to retinal lipid liposomes. This property of the lipid core of the rod outer segment membranes may be largely responsible for the high sodium permeability of these membranes in vivo. It would be interesting to observe whether incorporation of rhodopsin in rod outer segment lipid liposomes would further increase their sodium permeability and possibly even confer a larger "calcium sensitivity" to the sodium leakage.

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REFERENCES

1 Sillman, A. J., Ito, H. and Tomita, T. (1969) Vision Res. 9, 1443–1451
2 Yoshikami, S. and Hagins, W. A. (1973) in Biochemistry and Physiology of visual pigments (Langer, H., ed.), pp. 245–255. Springer Verlag, Heidelberg
3 Hagins, W. A. (1972) Ann. Rev. Biophys. Bioenerg. 1, 131–158
4 Hendriks, Th., Daemen, F. J. M. and Bonting, S. L. (1974) Biochim. Biophys. Acta 345, 468–473
5 Danielli, J. F. and Davson, H. (1953) J. Cell. Comp. Physiol. 5, 495–508
6 Hendler, R. W. (1971) Physiol. Rev. 51, 66–97
7 Singer, S. J. (1971) in Structure and function of biological memebranes (Rothfield, L. I., ed.), pp. 145–222, Academic Press, New York
8 Folch, J., Lees, M. and Stanley, G. H. S. (1957) J. Biol. Chem. 226, 497–509
9 de Grip, W. J., Daemen, F. J. M. and Bonting, S. L. (1972) Vision Res. 12, 1697–1707
10 Pangborn, M. C. (1951) J. Biol. Chem. 188, 471–476
11 Sanders, H. (1967) Biochim. Biophys. Acta 144, 485–487
12 Hyono, A., Hendriks, Th., Daemen, F. J. M. and Bonting, S. L. (1975) Biochim. Biophys. Acta 389, 34–46
13 Broekhuyse, R. M. (1968) Biochim. Biophys. Acta 152, 307–315
14 Abell, L. L., Levy, B. B., Brodie, B. B. and Kendall, F. E. (1952) J. Biol. Chem. 195, 357–366
15 Morrison, W. and Smith, L. M. (1964) J. Lipid Res. 5, 600–608
16 Broekhuyse, R. M. (1972) Biochim. Biophys. Acta 280, 637–645
17 Anderson, R. E. (1970) Exp. Eye Res. 10, 339–344
18 Daemen, F. J. M. (1973) Biochim. Biophys. Acta 300, 255–288
19 Papahadjopoulos, D. and Watkins, J. C. (1967) Biochim. Biophys. Acta 135, 639–652
20 de Gier, J., Mandersloot, J. G. and van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666–675
21 Demel, R. A., Geurts van Kessel, W. S. M. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 266, 26–40
22 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
23 Scarpa, A. and de Gier, J. (1971) Biochim. Biophys. Acta 241, 789–797
24 Papahadjopoulos, D., Nir, S. and Ohki, A. (1972) Biochim. Biophys. Acta 266, 561–583
25 Moore, J. L., Richardson, T. and Deluca, H. F. (1969) Chem. Phys. Lipids 3, 39–58
26 Chen, L., Lund, D. B. and Richardson, T. (1971) Biochim. Biophys. Acta 225, 89–95
27 Shah, D. O. and Schulman, J. H. (1967) J. Lipid Res. 8, 227–233