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OSMOMETRIC AND MICROSCOPIC STUDIES ON BILAYERS OF POLAR LIPIDS FROM THE EXTREME HALOPHILE, *HALOBACTERIUM CUTIRUBRUM*

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SUMMARY

The major membrane polar lipid components in *Halobacterium cutirubrum* are the diphytanyl ether analogues of phosphatidylglycerol phosphate, glycolipid sulfate, phosphatidylglycerol and phosphatidylglycerol sulfate. Dispersions of total polar lipids in water formed large birefringent liposomes showing concentric lipid bilayers in the electron microscope; they behaved as ideal osmometers in KCl or NaCl solutions in the concentration range 0.005–0.2 M. At concentrations above 0.2 M KCl the liposomes shrank to spherical particles which were much less birefringent, showed no distinct bilayer structures by electron microscopy, and no longer behaved as ideal osmometers. Dispersions of phosphatidylglycerol phosphate, phosphatidylglycerol or phosphatidylglycerol sulfate alone did not behave as osmometers at any concentration of KCl or NaCl, but glycolipid sulfate alone or mixed with phosphatidylglycerol phosphate or phosphatidylglycerol phosphate + phosphatidylglycerol sulfate showed ideal osmometer behavior in 0.005–0.2 M KCl or NaCl. The highly negatively charged total polar lipids of *H. cutirubrum* thus can form stable lipid bilayers only at low ionic concentrations (0.005–0.2 M), much lower than the salt concentration (4 M) of the growth medium, and the presence of glycolipid sulfate is essential. Stability of the membrane in 4 M salt appears to require direct participation of the protein components.

INTRODUCTION

The extremely halophilic bacteria are a group of unusual organisms that require saturated or nearly saturated salt solution for growth and preservation of their structure [1]. They possess very high internal salt concentrations, as high as that of their external environment. However, these cells are able to actively concentrate K^+ in their cytoplasm at the expense of Na^+; thus, the internal concentrations for Na^+

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and K^+ in *Halobacterium salinarium* are reported to be 1.37 M and 4.57 M, respectively, whereas the external concentrations are 4.0 M Na^+ and 0.03 M K^+ [2].

It is generally assumed that a high charge density exists on the cell envelope of these halophilic bacteria and that a high concentration of the shielding counterions is required to maintain their surface structure [3, 4]. The main polar lipid components of the membrane have been identified [5] as the diphytanyl ether analogues of phosphatidylglycerol phosphate, phosphatidylglycerol, phosphatidylglycerol sulfate [6], and a glycolipid sulfate [7]. These lipids are unusual in that each contains negatively charged polar head groups, as well as highly branched hydrocarbon chains (phytanyl groups) linked by ether groups to the glycerol moieties.

It was of interest to determine whether these unusual lipids can play a role in the stability of the membrane of halophilic bacteria particularly in high salt concentrations. Spin-label studies on the cell envelope [8] and lipid vesicles [9] from *H. cutirubrum* have indicated that the fluid lipid chains are immobilized by interaction with the membrane proteins. We have undertaken a study of the physicochemical properties of aqueous dispersions of the individual membrane lipid components of *H. cutirubrum*, and report here results of osmometric, polarizing-microscopic and electron-microscopic studies, and differential thermal analysis of these unusual lipids.

MATERIAL AND METHODS

Individual polar lipids

Total lipids of *H. cutirubrum* were extracted by the Bligh–Dyer procedure [10] as described previously [11] and separated into polar- and neutral-lipid fractions by acetone precipitation [12]. Squalene, dihydrosqualene and tetrahydrosqualene [12–14], and red pigments (bacterioruberins [14–16]) where isolated from the neutral lipids as described previously.

Total polar lipids of *H. cutirubrum* were fractionated by preparative thin-layer chromatography in chloroform–methanol–conc. NH_4OH (65 : 35 : 5, v/v/v) as described elsewhere [6, 7]. Phosphatidylglycerol phosphate and glycolipid sulfate were sometimes not well resolved in the above ammoniacal solvent system but could be completely separated by preparative thin-layer chromatography in chloroform–methanol–90% acetic acid (30 : 4 : 20, v/v/v) [6, 7]. The polar lipid components were obtained in their "natural-salt" form [6, 7] and were finally purified by acetone precipitation to remove any Rhodamine dye [6].

Free-acid and ammonium-salt forms of lipids

The free-acid form of the total polar lipids and of each component was prepared by the "acidic" Bligh–Dyer procedure and converted to the respective ammonium salt form by titration with 0.2 M methanolic NH_4OH, as described elsewhere [6, 7]. The ammonium salts were purified by acetone precipitation and dried in vacuo over KOH pellets, and then dissolved in chloroform to a known concentration.

Preparation of stock lipid dispersions for osmometry

A chloroform solution containing 15 mg of polar lipids or individual components was brought to dryness in a 15-ml centrifuge tube under a stream of N_2

and the lipid residue was dried in vacuo for at least 2 h. The lipid film was dispersed in 1 ml of distilled water or salt solutions of varying concentration (0.01–4 M NaCl* or KCl*) by shaking the mixture on a Vortex mixer; final concentration of stock lipid dispersion, approx. 1.5%. The dispersions were allowed to equilibrate at room temperature (24±1 °C) for 1–2 h before each experiment; no hydrolytic breakdown of the lipid components in the dispersions was detected after 48 h by thin-layer chromatography.

Osmometric measurement

Osmometric studies of these lipid dispersions were carried out essentially following the procedure of De Gier et al. [17]: 100 μl of the stock lipid dispersion in 0.05 M NaCl or KCl was diluted and mixed with 5.0 ml of the respective salt solution of varying concentration (0.01–4.0 M) or 5.0 ml of sucrose or mannitol solutions of varying concentration, as indicated in the figure legends. After 1–2 h equilibration at room temperature, the absorbance of each suspension was measured at 450 nm in a Cary-15 spectrophotometer in 3-ml cells (1 cm pathway; absorbance values were in the range 0.1–0.5). Some measurements were also done with stock lipid solutions prepared in distilled water or in salt solutions greater than 0.05 M and diluted with various salt solutions as indicated in the figure legends. Results were plotted as double reciprocal plots: 1/absorbance ($1/A_{450\ nm}$) against 1/solute concentration (1/M).

Differential thermal analysis

Samples (5–6 mg) for differential thermal analysis measurements were prepared by sealing weighed amounts of lipids and water or salt solution (weight ratio 1 : 1 or 3 : 1, respectively) into glass capillary tubes (2 mm diameter). The samples were mixed by alternately centrifuging the material in the tube from one end to the other at room temperature. Differential thermal analysis was carried out with a DuPont 900 Differential Thermal Analyzer. Measurements were made in the range −100 to +80 °C at a heating rate of approx. 8 °C/min. Thermograms were corrected for the non-linearity of the alumel–chromel thermocouples.

Polarizing microscopy

Lipid samples were observed with a Zeiss Standard RA microscope, fitted with polarizer and analyzer and with camera attachment. Dry films of lipid were deposited on the microscope slide by adding a chloroform solution of the lipid dropwise and allowing the solvent to evaporate; a few drops of the appropriate aqueous solution were added and a cover slip positioned on top during equilibration for several hours. Samples were then viewed under polarized and non-polarized light.

Electron microscopy

Lipids (0.3–0.4 mg) were dispersed in 0.5 ml of distilled water or 0.5 ml of salt solution of varying concentrations as described above; after 1 h at room temperature, 0.5 ml of 1% phosphotungstic acid (pH 7.0) was added and one or two drops of this mixture were then applied to a Formvar–carbon-coated grid. The grid edge was

* Baker 'Analyzed' Reagent, containing approx. 0.003 % di- and trivalent metal ions.

touched with a filter paper to remove any excess solution. After drying for 2 h at room temperature the grids were examined in an AEI 6B electron microscope.

RESULTS

Differential thermal analysis

Phase-transition measurements of total *H. cutirubrum* lipids (polar+non-polar lipids) in 25% water showed a broad transition in the region −55 to −35 °C with the main endothermic peak at −45 °C (Table I). Total polar lipids alone in 25% or 50% water also showed a broad transition in the same region but with a peak at −49 °C. However, no phase transition was detected in the range −60 to +80 °C for the total polar lipids when mixed with 50% 2 M NaCl (Table I). The individual polar lipid components showed only very weak or no transition in the range −80 to +80 °C. Phosphatidylglycerol phosphate (the major component) showed no transition in 50% water. The glycolipid sulfate in 50% water showed a very weak peak at −45 °C. Anhydrous phosphatidylglycerol showed a somewhat broader transition between −35 to −10 °C, and a very weak peak at −49 °C in 50% water. No transition was detected for anhydrous phosphatidylglycerol sulfate in the range −100 to +70 °C, but a very weak peak was observed at −49 °C in 50% water. The absence of clearly defined transitions above −35 °C probably indicates that the total lipid and total polar-lipid mixtures are in a liquid–crystalline state at ambient temperatures.

TABLE I

DIFFERENTIAL THERMAL ANALYSIS OF LIPIDS OF *HALOBACTERIUM CUTIRUBRUM*

Lipid*	Dispersing medium**	Transition-temperature range (°C)
Total cellular lipids	Water (25%)	Broad, centered at −45
Total polar lipids	Water (25%)	Broad, centered at −49
	Water (50%)	Broad, centered at −49
	2 M NaCl (> 50% water)	Not detected in range −60 to +80
Phosphatidylglycerol phosphate	Water (50%)	Not detected in range −80 to +80
Glycolipid sulfate	Water (50%)	Broad and weak centered at −45
Phosphatidylglycerol	None (anhydrous)	Broad and weak in range −10 to −35
	Water (50%)	Broad and weak centered at −49
Phosphatidylglycerol sulfate	None (anhydrous)	Not detected in range −100 to +70
	Water (50%)	Not detected in range −100 to +70

* All polar lipids are derivatives of 2,3-diphytanyl-*sn*-glycerol; they were used in their natural-salt forms [6, 7].

** Proportion of dispersing medium given in parentheses as percent by weight.

Osmotic properties of lipids of H. cutirubrum

Dispersions of total polar lipids (natural-salt form [6, 7]) prepared in distilled water and subsequently equilibrated in NaCl or KCl solution (0.001–4 M) showed ideal osmometric behaviour in the salt-concentration range 0.0016–0.2 M (Figs 1A, 1B); at higher salt concentrations (0.2–4 M), they no longer behaved as ideal osmometers the double reciprocal plot showing a reversal in slope (Fig. 1C). When the stock total polar-lipid dispersions were prepared in 0.5, 1 or 4 M NaCl and equilibrated with NaCl solutions in the final concentration range 0–4 M NaCl, all dispersions showed ideal osmometric behaviour only in the range 0.2–1 M; at lower concentrations (0–0.2 M) the curve had zero slope indicating the liposomes were leaky, while

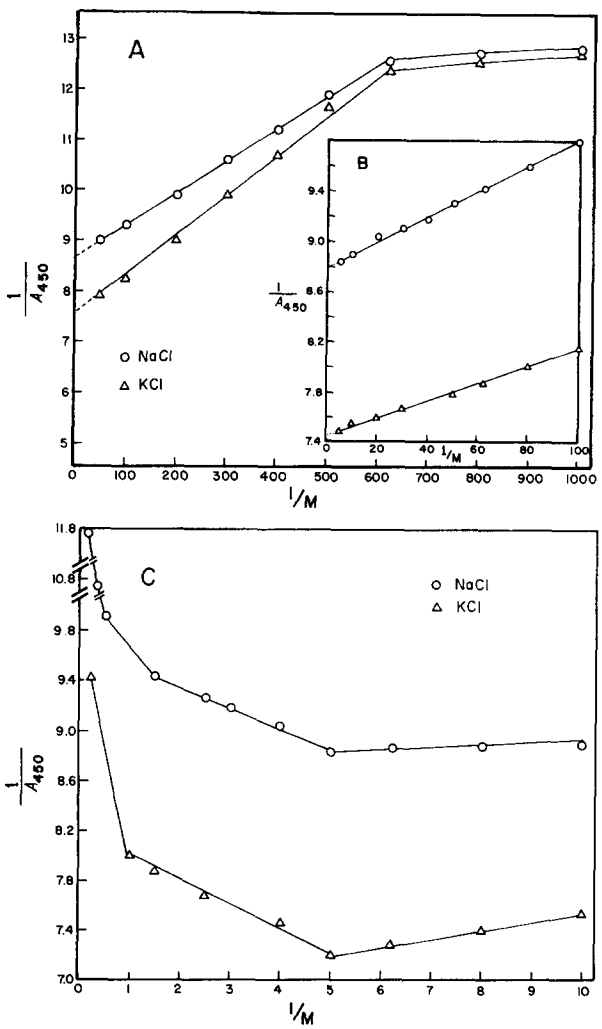


Fig. 1. Double reciprocal plots of absorbance at 450 nm vs final salt concentration for dispersions of total polar lipids (natural-salt forms [6, 7]) of *H. cutirubrum* prepared in distilled water and equilibrated in NaCl or KCl solutions in the range: (A) 0.001–0.02 M; (B) 0.01–0.2 M; and (C) 0.1–4 M.

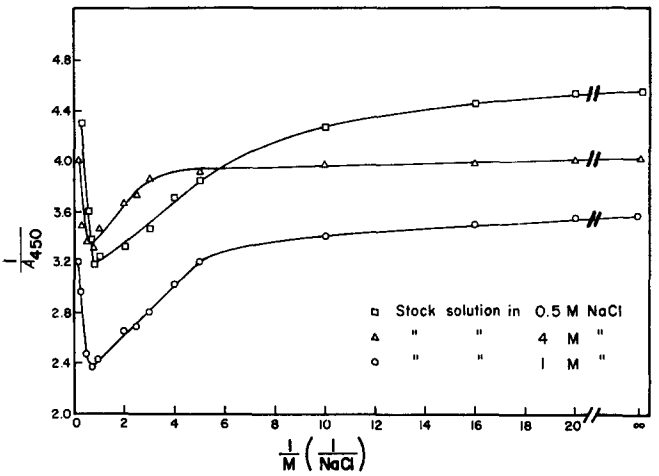


Fig. 2. Double reciprocal plots of absorbance at 450 nm vs NaCl molarity for dispersions of total polar lipids (natural-salt forms [6, 7]) prepared in 0.5, 1 or 4 M NaCl and equilibrated with NaCl solutions in final concentration range 0–4 M.

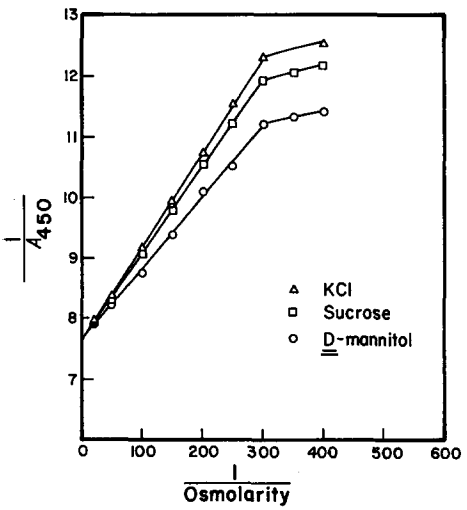


Fig. 3. Double reciprocal plots of absorbance at 450 nm vs final osmolarity of dispersing medium for dispersions of total polar lipids (natural-salt form) prepared in distilled water and equilibrated in KCl, sucrose or D-mannitol solutions in the range 0.0025–0.04 osmolar.

at higher ionic strength (1 M–4 M) the double reciprocal plot showed a complete reversal in slope (Fig. 2), that may be due to precipitation of the lipids at the high ionic concentrations*.

* The polar lipids may precipitate at high ionic concentrations as salts of divalent metal ions present in their natural-salt forms [6, 7] or as salts of di- and trivalent metal impurities in the NaCl or KCl. Under these conditions the proportionality between reciprocal absorbance and volume of the liposomes which is valid at low ionic concentrations, could not be expected to hold at high salt concentrations.

To determine the relative contributions of water penetration and ion penetration to the osmometric behaviour of lipid dispersions in salt solutions, the experiment described in Fig. 1A was repeated using non-ionic, non-penetrating solutes such as sucrose or mannitol. The dispersions in these non-penetrating solutes were found to behave osmometrically almost identically to those in KCl solution (Fig. 3). The double reciprocal plots for these dispersions showed linearity in the range of 0.003–0.04 osmolarity; the straight lines differed somewhat in slope with each solute, but extrapolated to the same value for the osmometric "dead space" (Fig. 3). These results indicate that the osmometric behaviour of the total polar-lipid dispersions is largely accounted for by water penetration.

All studies described above were done with the natural-salt form [6, 7] of the

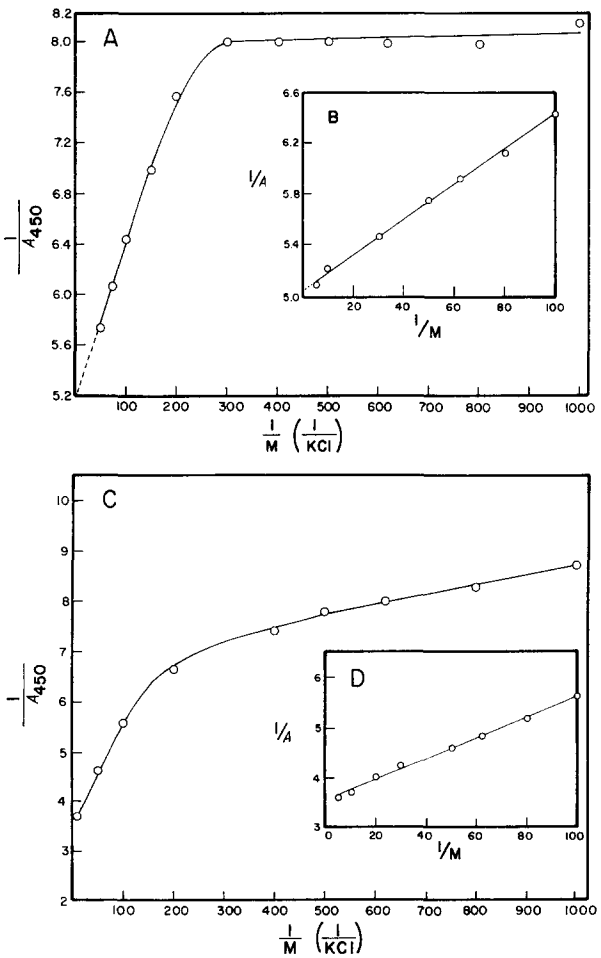


Fig. 4. Double reciprocal plots of absorbance at 450 nm vs final KCl concentration for dispersions of: (A) and (B) total polar lipids, free-acid forms; (C) and (D) total polar lipids ammonium-salt forms, prepared in water and equilibrated in KCl solutions in range: (A), (C) 0.001–0.02 M; and (B), (D) 0.01–0.2 M.

total polar lipids (mixtures of sodium, potassium, magnesium and ammonium salts). It was of interest to study the osmometric behaviour of the free-acid form of the polar lipids to eliminate the effect of different cationic groups. When the free-acid form of the total polar lipids was dispersed in distilled water and subsequently equilibrated in KCl solution of varying concentrations, these dispersions did not show

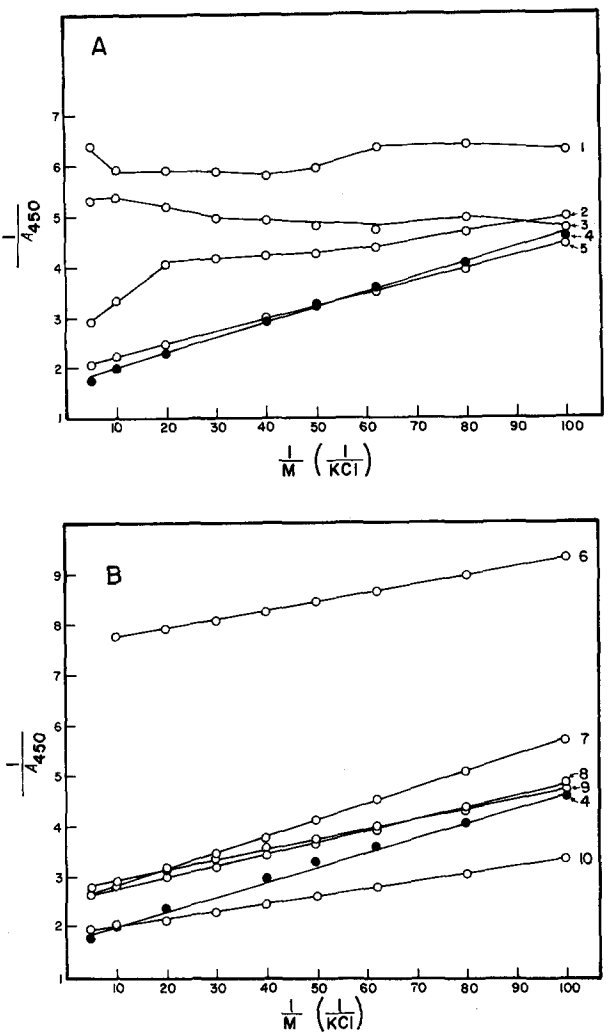


Fig. 5. Double reciprocal plots of absorbance at 450 nm vs final KCl concentration for dispersions of: (A) individual polar lipids (ammonium-salt forms) and (B) mixtures thereof, prepared in 0.05 M KCl and equilibrated with KCl solutions in the range 0.01–0.2 M; 1, phosphatidylglycerol phosphate; 2, phosphatidylglycerol sulfate; 3, phosphatidylglycerol; 4, total polar lipids (natural-salt form); 5, glycolipid sulfate; 6, phosphatidylglycerol phosphate+glycolipid sulfate+phosphatidylglycerol sulfate (20 : 10 : 1, by wt); 7, phosphatidylglycerol phosphate+glycolipid sulfate+phosphatidylglycerol sulfate+phosphatidylglycerol (20 : 10 : 1 : 1.3, by wt); 8, total polar lipids+squalenes [13] (90 : 5.5, w/w); 9, phosphatidylglycerol phosphate+glycolipid sulfate (2 : 1, w/w); 10, total polar lipids+squalenes [13]+bacterioruberins [15, 16] (90 : 5.5 : 4.5, by wt).

ideal osmometric behaviour below 0.003 M KCl (Fig. 4A), but behaved ideally in the range of 0.005–0.2 M KCl (Figs 4A, 4B). Thus, in the salt concentration range 0–0.003 M, when the counterion concentration is very low, the highly negatively charged polar head groups (phosphate and sulfate) of the lipids repel each other forming "open" liposome systems which are too permeable to water to behave as ideal osmometers. At higher salt concentration (0.005–0.2 M), the shielding counterions reduce the ionic repulsion, allowing the formation of "closed" liposomes necessary for ideal osmometry.

The effect of using dispersions in water of total polar lipids in a specific salt form (ammonium salt) on their osmometric behaviour was then studied. These dispersions behaved as ideal osmometers in the range of 0.005–0.2 M KCl, but not below 0.005 M (Figs 4C, 4D). The similarity of this behaviour to that of the free-acid form (Figs 4A, 4B) indicated that in water or dilute KCl solutions below 0.005 M the ammonium salts are readily dissociated to the free acids which do not form "closed" osmometer systems. At higher KCl concentrations (above 0.005 M), as observed with the free-acid form (Figs 4A, 4B), the counterion concentration was sufficient to overcome repulsion of the negatively charged ionic groups.

Subsequent experiments with individual lipid components were then carried out using the ammonium-salt forms, since these were prepared most conveniently (see Methods section). Osmometric measurements were made in the KCl concentration range 0.01–0.2 M suitable for "ideal" osmometry (Fig. 4) and the dispersions were prepared in 0.05 M KCl to ensure a sufficiently high initial concentration of counterions. Neither the major phospholipid, phosphatidylglycerol phosphate, nor the minor phospholipids, phosphatidylglycerol sulfate and phosphatidylglycerol, behaved individually as ideal osmometers under the above conditions, but the glycolipid sulfate alone showed the same ideal osmometric behaviour as the total polar lipids (Fig. 5A). Furthermore, various combinations of glycolipid sulfate and the other components in their "natural" proportions (e.g. phosphatidylglycerol phosphate+glycolipid sulfate, 2 : 1, w/w; phosphatidylglycerol phosphate+glycolipid sulfate+phosphatidylglycerol sulfate, 2 : 1 : 0.1, by wt) as well as the reconstituted polar lipids (phosphatidylglycerol phosphate+glycolipid sulfate+phosphatidylglycerol sulfate+phosphatidylglycerol, 2 : 1 : 0.1 : 0.1, by wt) all showed ideal osmometric behaviour (Fig. 5B). Addition of squalenes [13] or squalenes plus red pigments (bacterioruberins [15, 16], which together account for most (95%) of the neutral lipids, did not change the ideal osmometric behaviour of the total polar lipids (Fig. 5B).

The structures of these lipid dispersions were then examined by polarizing-light microscopy and electron microscopy.

Polarizing-light microscopy

Dispersions of total polar lipids (natural salt forms) in water were observed to form large liquid–crystalline myelin figures which swelled rapidly and were strongly birefringent (Figs 6A and 6B). As the salt concentration was increased, the size of the myelin figures decreased drastically and very small focal conic-textured structures (maltese crosses) were seen (Figs 6C, 6D). The shrinkage was quite dramatic beginning at about 0.1–0.2 M NaCl, and no myelin figures were observed at or above 0.5 M NaCl (Fig. 6D). When mixtures of the total polar lipids (ammonium salt) and squalenes plus red pigments were dispersed in distilled water, birefringent myelin

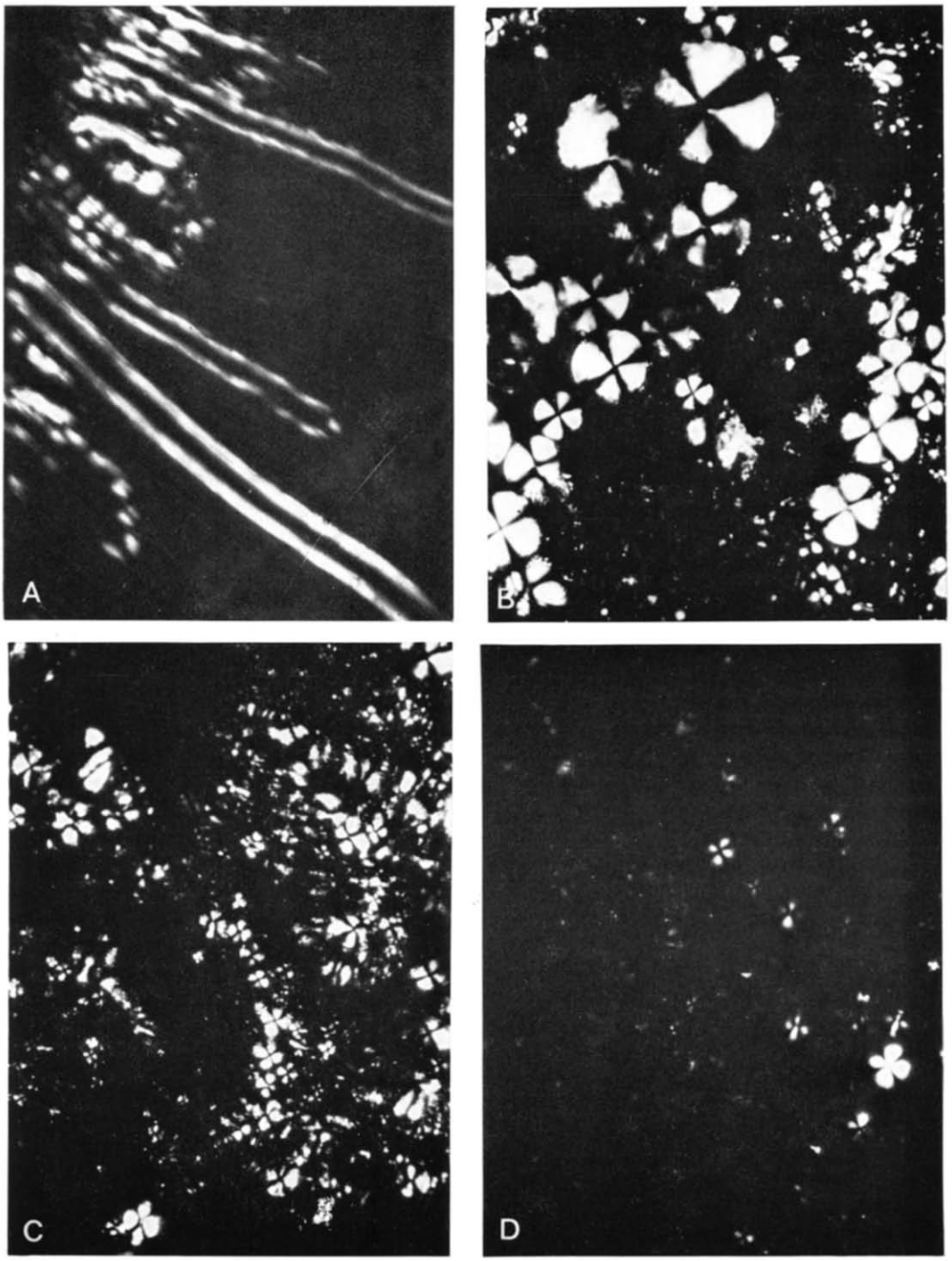
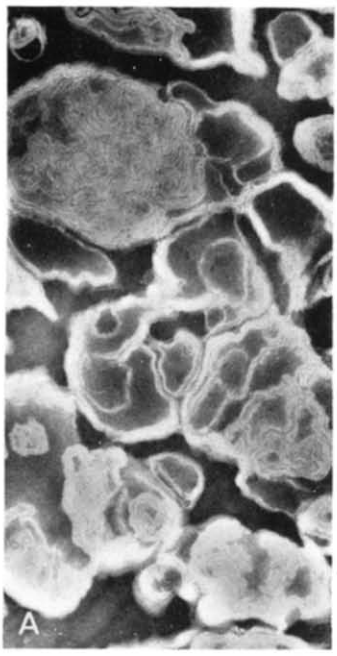


Fig. 6. Photomicrographs of dispersions of total polar lipids (natural-salt form) in: (A) distilled water, viewed in unpolarized light; (B) distilled water, under polarized light; (C) 0.2 M NaCl, under polarized light; (D) 0.5 M NaCl, under polarized light. Magnification in all cases was $\times 200$.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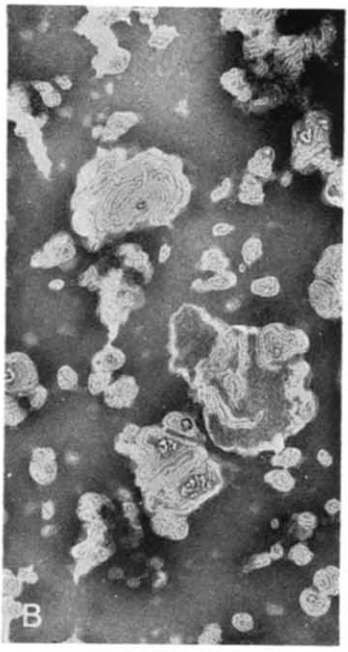
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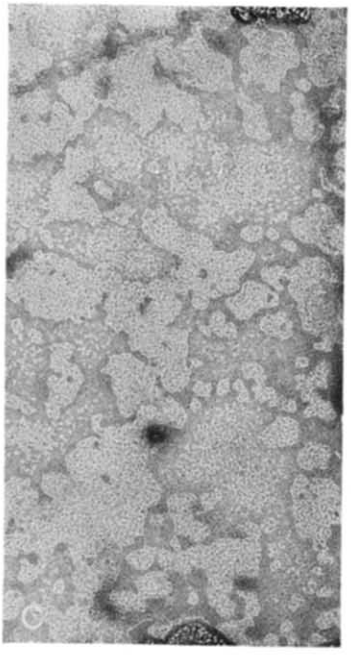
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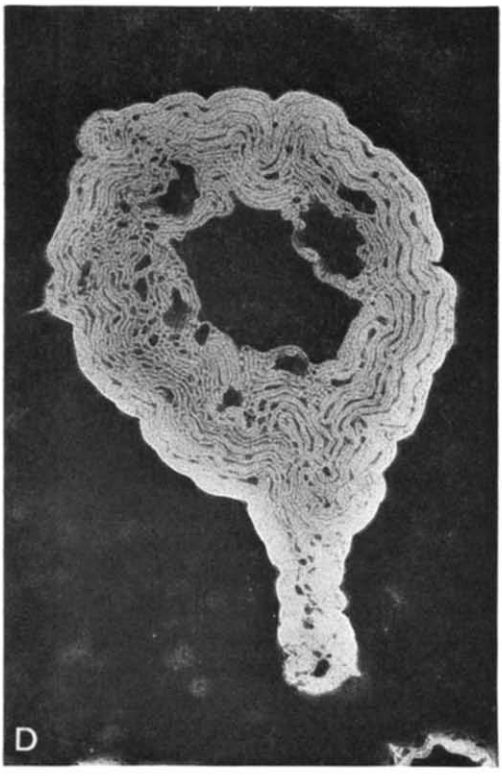
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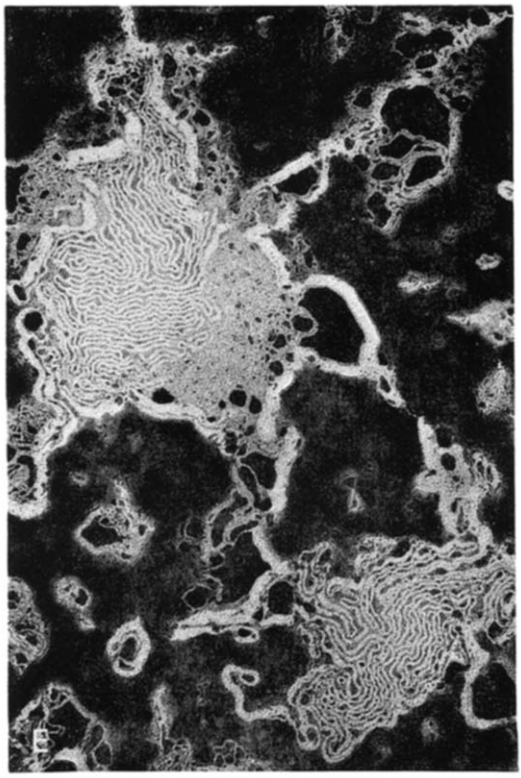


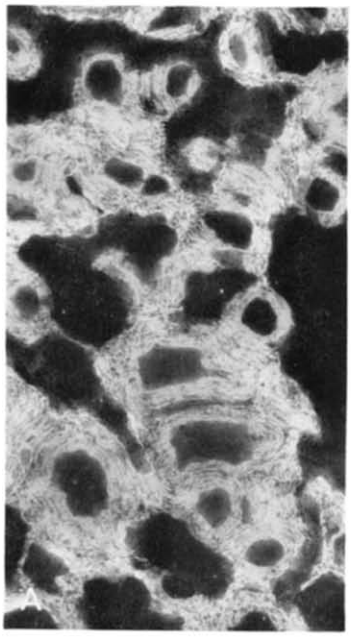
Fig. 7. Electron micrographs of negatively stained dispersions of total polar lipids (natural-salt form) in: (A) distilled water (×60 000); (B) 0.2 M NaCl (×60 000); (C) 0.5 M (×60 000); and of: (D) total polar lipids+squalenes [13] (90 : 5.5, by wt) in distilled water (×100 000) and (E) total polar lipids+squalenes [13] +bacterioruberins [15, 16] (90 : 5.5 : 4.5, by wt) in distilled water (×80 000).

figures were also observed. Dispersions of glycolipid sulfate–phosphatidylglycerol phosphate mixtures (1 : 2, w/w) in water also showed birefringence, but the glycolipid sulfate alone dispersed in water, 0.05 or 0.2 M NaCl formed small vesicular structures which were only weakly birefringent.

Electron microscopy

The polarizing-microscopic observations described above on the various lipid dispersions were confirmed by electron microscopy. Thus, dispersions of the total polar lipids (natural-salt forms) in water showed multibilayer liposome structures which shrank in size and in spacing of the bilayers when the dispersions were made in increasing concentrations of NaCl up to 0.5 M NaCl, at which concentration no bilayer structure was apparent (Figs 7A–C). The liposomal structure of the total polar lipids in water (Fig. 7A) was essentially unchanged on addition of squalenes or squalenes plus red pigments (Figs 7D, 7E).

Dispersions of phosphatidylglycerol phosphate alone in water did show particles with some lamellar structure (Fig. 8A), but these did not appear to be "closed"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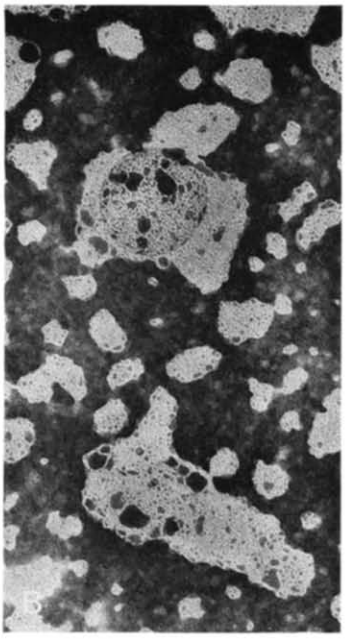
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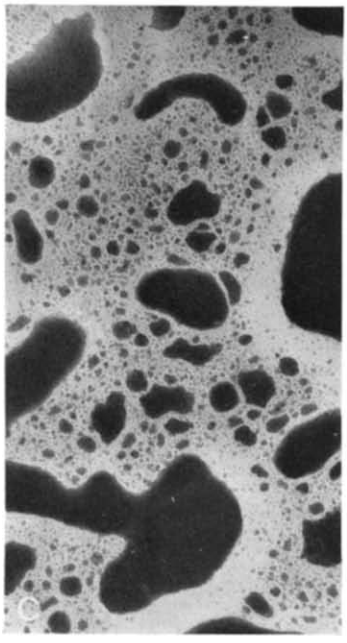
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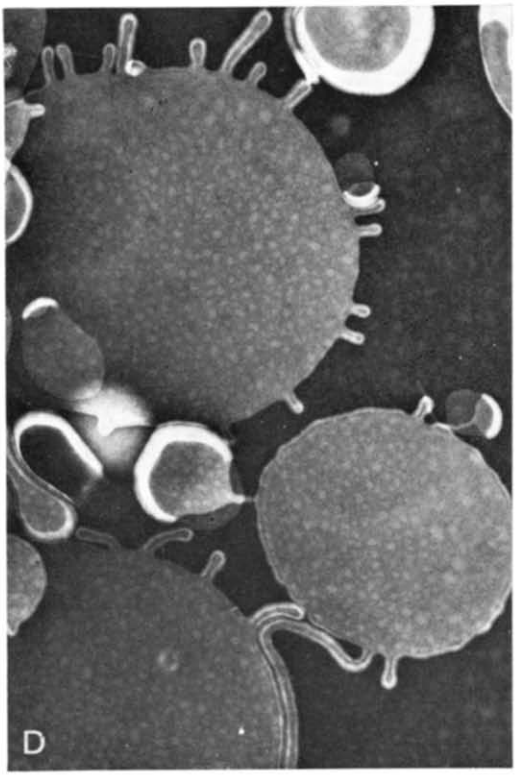
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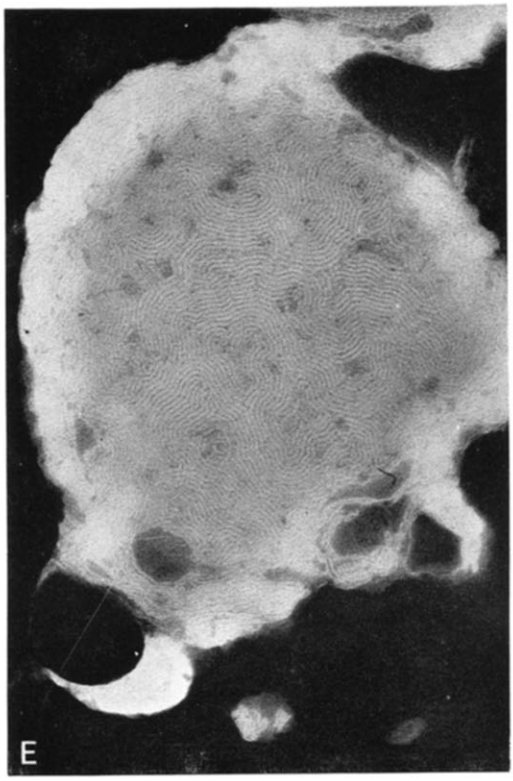

Fig. 8. Electron micrographs of negative stained dispersions of various polar lipids (ammonium-salt forms) in distilled water: (A) phosphatidylglycerol phosphate (×60 000); (B) phosphatidylglycerol (×80 000); (C) phosphatidylglycerol sulfate (×80 000); (D) glycolipid sulfate (×40 000); (E) glycolipid sulfate+phosphatidylglycerol phosphate (1 : 2, w/w) (×110 000).

liposomal systems from their osmometric behaviour (Fig. 5A). Phosphatidylglycerol or phosphatidylglycerol sulfate dispersions in water showed only amorphous structures (Figs 8B, 8C). The glycolipid sulfate dispersion in water showed particles with myelin figures protruding from their surfaces, suggesting that liposomes were in the process of formation (Fig. 8D). Mixtures of phosphatidylglycerol phosphate and glycolipid sulfate (2 : 1, w/w), however, formed liposomes with an internal close-packed bilayer structure (Fig. 8E). All other combinations of glycolipid sulfate and polar lipid components (in their natural proportions) showed similar liposomal structures.

DISCUSSION

Broad thermal transitions centered between −45 and −49 °C were observed for aqueous dispersions of the total membrane lipids or the total polar lipids, respectively (Table I). Such low transition temperatures are to be expected [18] for lipids containing only phytanyl (3,7,11,15-tetramethylhexadecyl) groups, since the four CH_3-branches would sterically interfere with close-packing of the hydrocarbon chains (see refs 8 and 19 for molecular models of phosphatidylglycerol phosphate).

By comparison, thermal transitions centered at +16 °C have been observed for the membrane lipids of *Micrococcus lysodeikticus* [20], which contain largely iso- and anteiso-C_{15} fatty acids [21].

Thus, the total membrane lipids of *H. cutirubrum* dispersed in water at room temperature probably exist in a lamellar liquid–crystalline state, which would be consistent with the swollen birefringent structures observed by polarizing microscopy (Figs 6A, B). Electron microscopy (Fig. 7A) further showed the existence in these lipid dispersions of multibilayer structures which were closed liposomal systems since they behaved as ideal osmometers [17, 22] in the range 0.005–0.2 M NaCl (Fig. 1B).

In contrast, when the individual polar lipid components were examined, only the glycolipid sulfate alone or mixtures of glycolipid sulfate with the other polar lipid components showed ideal osmometric behaviour (Figs 5A, B) and formed liposomes (Figs 8D and E); neither phosphatidylglycerol phosphate, phosphatidylglycerol sulfate nor phosphatidylglycerol alone showed typical multibilayer liposomal structures (Figs 8A–C). The inability of the diphytanyl ether phosphatidylglycerol from *H. cutirubrum* to form bilayer structures in low salt concentrations (< 0.3 M) is in sharp contrast to the reported formation under the same conditions of stable liposomes with the diester phosphatidylglycerol isolated from *Staphylococcus aureus* [23] or from *Acholeplasma laidlawii* B [24], or prepared [25] enzymatically (phospholipase D) from egg lecithin. Recently we have shown that the lack of formation of liposomes by the diphytanyl ether phosphatidylglycerol in low salt concentration is not due to the presence of ether groups but to the highly branched phytanyl chains (Chen, J. S., McElhaney, R. N. and Kates, M., unpublished results).

The present findings tentatively suggest that in the diphytanyl ether phosphatidylglycerol, as well as in the phosphatidylglycerol phosphate and phosphatidylglycerol sulfate components of *H. cutirubrum* the polar head group may be too small relative to the effective cross-sectional area of the phytanyl chains for stable bilayer formation and that the glycolipid sulfate with its relatively large polar head may be needed for proper packing of these polar lipids into a stable bilayer structure. Similar considerations may apply also to the formation of stable lipid bilayers in other systems [22, 26–28].

Of great interest was the finding that the total membrane polar lipids formed stable bilayers only at low ionic concentrations (0.005–0.2 M NaCl or KCl) (Figs 1, 6 and 7) and not at all at the salt concentration (4 M) required for growth of the halophile. The low ionic strength (0.005–0.2 M) is presumably sufficient for neutralization of the repulsive negative charges of the phosphate and sulfate groups on the polar lipids, but higher ionic concentrations would be expected [29] to decrease greatly the surface area of the polar head groups resulting in immobilization or tight packing of the lipid chains, so that stable bilayer structures would not be possible. Stability of the membrane of *H. cutirubrum* in 4 M salt would thus appear to require the direct participation of the membrane protein components, a conclusion reached earlier from studies of delipidated or deproteinated envelopes [30] and from spin-label studies on envelopes and lipid vesicles [8, 9]. It would appear that the membrane proteins probably interact hydrophobically with the phytanyl chains, as well as hydrophilically with the polar head groups of the polar lipids in order to maintain the integrity of the membrane [31].

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REFERENCES

1 Larsen, H. (1962) in The Bacteria (Gunsalus, I. C. and Stanier, R. Y., eds), Vol. 4, pp. 297–342, Academic Press, New York
2 Christian, J. H. B. and Waltho, J. A. (1962) Biochim. Biophys. Acta 65, 506–508
3 Brown, A. D. (1963) Biochim. Biophys. Acta 75, 425–435
4 Kushner, D. J., Bayley, S. T., Boring, J., Kates, M. and Gibbons, N. E. (1964) Can. J. Microbiol. 10, 483–497
5 Kates, M. (1972) in Ether Lipids, Chemistry and Biology (Snyder, F., ed.), pp. 351–397, Academic Press Inc., New York
6 Hancock, A. J. and Kates, M. (1973) J. Lipid Res. 14, 422–429
7 Kates, M. and Deroo, P. W. (1973) J. Lipid Res. 14, 438–445
8 Esser, A. F. and Lanyi, J. K. (1973) Biochemistry 12, 1933–1939
9 Lanyi, J. K. (1974) Biochim. Biophys. Acta, in the press
10 Bligh, E. C. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
11 Joo, C. N., Shier, T. and Kates, M. (1968) J. Lipid Res. 9, 782–788
12 Tornabene, T. G., Kates, M., Gelpi, E. and Oro, J. (1969) J. Lipid Res. 10, 294–303
13 Kramer, J. K. G., Kushwaha, S. C. and Kates, M. (1972) Biochim. Biophys. Acta 270, 103–110
14 Kushwaha, S. C., Pugh, E. L., Kramer, J. K. G. and Kates, M. (1972) Biochim. Biophys. Acta 260, 492–506
15 Gochnauer, M. B., Kushwaha, S. C., Kates, M. and Kushner, D. J. (1972) Arch. Mikrobiol. 84, 339–349
16 Kelley, M., Norgard, S. and Liaaen-Jensen, S. (1970) Acta Chem. Scand. 24, 2169–2182
17 DeGier, J., Mandersloot, J. G. and Van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666
18 Chapman, D. and Wallach, F. H. (1968) in Biological Membranes(Chapman, D., ed.), pp. 125–202, Academic Press, London
19 Vandenheuvel, F. A. (1965) J. Am. Oil Chem. Soc. 42, 481–492
20 Ashe, G. B. and Steim, J. M. (1971) Biochim. Biophys. Acta 233, 810–814
21 Thorne, K. J. I. and Kodicek, E. (1962) Biochim. Biophys. Acta 59, 306
22 Bangham, A. D., DeGier, J. and Greville, G. D. (1967) Chem. Phys. Lipids 1, 225–246
23 Haest, C. W. M., DeGier, J., Op Den Kamp, J. A. F., Bartels, P. and Van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 255, 720–733
24 Romijn, J. C., Van Golde, L. M. G., McElhaney, R. N. and Van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 280, 22–32
25 Papahadjopoulos, D. (1971) Biochim. Biophys. Acta 241, 254–259
26 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
27 Papahadjopoulos, D. and Watkins, J. C. (1967) Biochim. Biophys. Acta 135, 639
28 Szabo, G. (1972) in Membrane Molecular Biology (Fox, C. F. and Keith, A. D., eds), Sinauer Associates Inc., Stamford, Conn., U.S.A.
29 Graham, D. E. and Lea, E. J. A. (1972) Biochim. Biophys. Acta 274, 286–293
30 Kushner, D. J. and Onishi, H. (1966) J. Bacteriol. 91, 653–660
31 Chuang, T. F., Awasthi, Y. C., Funk, L. and Grave, F. L. (1970) Biochim. Biophys. Acta 211, 599–601