# THE INTERACTION OF BACTERIAL LIPOPOLYSACCHARIDE WITH PHOSPHOLIPID BILAYERS AND MONOLAYERS

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## SUMMARY

The association of bacterial lipopolysaccharide with artificial membranes was studied in an attempt to understand the mechanism of binding of lipopolysaccharide to cell surfaces and to look for an effect on membrane stability. The membrane models used were phospholipid bilayers and monolayers. As measured by survival time, lipopolysaccharide was found to decrease the stability of bilayers at a concentration of 300 $\mu$g/ml. When assayed by dielectric breakdown, an effect of lipopolysaccharide was noticeable at concentrations of 50 $\mu$g/ml. In studies involving the penetration of monomolecular films of various phospholipids, native and alkali-treated lipopolysaccharide both caused increases in surface pressure, and therefore penetrated the films. However, alkali-treated lipopolysaccharide was at least ten times more efficient than the native product in penetration. Alkali-treated lipopolysaccharide had a greater degree of surface activity than native lipopolysaccharide, since alkali-treated lipopolysaccharide formed monomolecular films by itself, whereas native lipopolysaccharide did not. The changes in the surface pressure and surface potential of phospholipid films produced by lipopolysaccharide in the subsolution suggested that the interaction of lipopolysaccharide with phospholipid monolayers was by a combination of penetration and adsorption to the undersurface.

## INTRODUCTION

Bacterial lipopolysaccharide is a macromolecular complex composed of amphipathic subunits. In its extracted form it is believed to exist in aqueous solution as a bilayer (or biomolecular leaflet) with the lipid groups of each monolayer in opposition and the polysaccharide exposed to the surrounding water[1–5]. Mild alkali treatment of lipopolysaccharide disaggregates large complexes and results in structures with the morphological appearance of monolayers[6]. This result of alkali treatment is postulated to be due to the diminution of the hydrophobic interaction between monolayer halves by the cleavage of ester-linked fatty acids from the lipid A of lipopolysaccharide. The remaining amide-bound fatty acids presumably are inadequate to hold the bilayer together.

Alkali treatment of lipopolysaccharide has also been shown to enhance its binding to erythrocytes 15-fold[7,8]. The dependence on a lipid moiety in the binding

of lipopolysaccharide to red cells has been demonstrated[9] and one might explain the increased binding of alkali-treated lipopolysaccharide to membranes by an increase in the accessibility of its amide-linked fatty acid.

The binding of lipopolysaccharide to red cells can be inhibited by prior incubation of lipopolysaccharide with lecithin and cholesterol[10], major components of cell membranes, suggesting that these compounds may have a role in the binding of lipopolysaccharide to cell membranes. These compounds can also be used in model membrane systems, such as phospholipid bilayers and monolayers, to study the interaction between lipopolysaccharide and membrane-like structures. The ability of various lipopolysaccharide preparations to decrease the stability of phospholipid bilayers has been noted by Schuster and co-workers[11], and the association of lipopolysaccharide with phospholipid monolayers has been studied extensively by Rothfield and co-workers[12–15]. Recently, Romeo *et al.*[16] have characterized the factors promoting maximal interaction of lipopolysaccharide with phospholipid monolayers in connection with the reconstruction of an active enzyme system for the biosynthesis of lipopolysaccharide core polysaccharide. They proposed that the association of lipopolysaccharide with monolayers was similar to a mixed monomolecular film of lipopolysaccharide and phospholipid.

The purpose of this study was to investigate the binding of lipopolysaccharide to model membrane systems and to look for an unstabilizing effect, since it is our belief that some of the biological effects of lipopolysaccharide are mediated by interaction with mammalian membranes and the production of some alteration in their structure–function.

## MATERIALS AND METHODS

### Chemicals

All chemicals used were reagent grade and were obtained from Allied Chemicals, Morristown, N.J. Egg phosphatidylcholine was prepared chromatographically pure by the method of Pangborn[17]. Phosphatidylserine and phosphatidylethanolamine were obtained chromatographically pure from General Biochemicals, Chagrin Falls, Ohio. Cholesterol was also obtained from General Biochemicals. The molecular weight for phosphatidylserine and for phosphatidylethanolamine, obtained from the manufacturer, was 750. Reported molecular weights used for cholesterol and phosphatidylcholine were 386.6 and 790[18], respectively.

The water used in all experiments was passed through two deionizing columns and then double distilled. Its conductance was less than $1.2 \, \mu\Omega^{-1} \cdot cm^{-2}$.

### Erythrocyte lipid extraction

This extract was prepared by the method of Andreoli *et al.*[19] which is a revision of the method of Rose and Oklander[20] with additions by Ways and Hanahan[21] and Reed *et al.*[22].

### Bilayer preparation

The formation of bilayers was essentially the same as the procedure of Mueller *et al.*[23]. "Delrin" cups were prepared from a solid rod of DuPont "Delrin" (polyformaldehyde). One side was thinned to less than 0.5 mm and a 1-mm diameter hole was drilled in that side. The cup was placed in a glass dish and a solution of 0.05 M

Tris–HCl, pH 7.0, or various dilutions of lipopolysaccharide in 0.05 M Tris were added to the cup and dish. The height of the liquid was adjusted to approximately 3 mm above the hole. Films were formed across the hole in the cup by a sable brush previously dipped into a solution of 1% phosphatidylcholine and cholesterol (1:1 molar ratios) in *n*-decane. The thinning of the lipid droplet to bilayer dimensions was observed by reflecting light from a microscope lamp from the surface of the film into a low power microscope. Interference fringes were initially observed, and as the film thinned to a bimolecular thickness, black spots began to appear which eventually covered the entire hole, indicating a bimolecular thickness of the entire film. Black films formed in this manner were found by others to have a thickness of $50 \pm 5$ Å[24]. The yield of successful bilayer membranes was > 50%. The conductance of the bilayer was measured by Ag–AgCl electroplated electrodes on either side of bilayer which were connected to a Keithley picoammeter. Measurements were made with a 25 mV potential supplied by a 1.5-V battery connected to a rheostat which was in parallel with a Keithley 610C electrometer. Data were plotted by a Hewlett–Packard X–Y recorder. Plots for voltage *versus* current for bilayers formed with this system were ohmic for voltages up to 50 mV after which the line curved in the direction of the Y axis. Resistance in all cases was consistently $10^{10}$ $\Omega \cdot cm^{-2}$ which is common for systems in which a neutral hydrocarbon such as cholesterol is used[25]. All bilayer experiments were performed at 22 °C.

*Monolayer preparation*

Monolayer films were formed on the surface of a lucite trough of 50-ml capacity by using an Alga microsyringe to spread 0.01 ml of a solution of phospholipid (1 mg/ml, dissolved in chloroform–methanol–hexane, 1:1:3, v/v/v) on 0.05 M Tris buffer (pH 7.0). Films were allowed to stabilize 15 min before measurements were taken in all cases.

For surface pressure measurements, the Wilhelmy plate method[26] was used with slight modifications. The surface pressure is defined as: $\pi = \gamma_0 - \gamma_f$ where $\gamma_0$ is the interfacial tension without a film on the surface of the liquid and $\gamma_f$ is the interfacial tension with the film. The surface pressure was measured with a sand-blasted rectangular platinum plate (5-cm perimeter) suspended from a Roller-Smith torsion balance which was mounted on an adjustable stand. Positive deviations in surface pressure were interpreted to indicate more molecules per unit area for the same state of compression assuming there was no major change in the orientation of the molecules in the monolayer. This indicates that molecules from the subsolution (lipopolysaccharide) have penetrated the film.

Surface potential measurements were made with an ionizing (α radiation) air electrode, the tip of which was covered with a 6 mm × 6 mm gold sandwich containing $^{226}$Ra (5 $\mu$Ci). The ionizing electrode was held 5 mm above the surface. A Ag–AgCl electroplated electrode was dipped into the trough and used as a reference electrode. The electrodes were connected to a Keithley 610C electrometer. The surface potential $\Delta V$ is defined as: $\Delta V = V_f - V_0$ where $V_0$ is the interfacial potential without the film and $V_f$ is the interfacial potential with a film. Experiments were performed at 22 °C.

The curves represented in each graph in the results are a composite of three trials. Variations in measurements of $\pi$ were less than 1 dyne/cm and variations in $\Delta V$ were $\pm 5$ mV within one series of experiments.

*Bacteria*

   *Salmonella typhimurium* 7 was obtained from M. Herzberg (Department of Microbiology, University of Hawaii) and grown in batch culture with aeration at 37 °C in M9 medium[27] supplemented with 0.1% Casamino acids (Difco).

*Lipopolysaccharides*

   Lipopolysaccharide was prepared by phenol–water extraction of whole formalin-fixed cells. Except for the formalin fixation, the procedure was essentially that of Westphal *et al.*[28]. The absence of contaminating RNA was confirmed by ultraviolet absorption spectra. Lipopolysaccharide to be treated with alkali (alkali lipopolysaccharide) was first suspended in aqueous solution (5 mg/ml). NaOH (0.75 M) was added to a final concentration of 0.25 equiv/l and the mixture was incubated at 37 °C for 3 h with frequent agitation. The solution was then neutralized with the appropriate amount of 0.75 M HCl and refrigerated overnight. A small amount of sediment appearing upon refrigeration was removed by centrifugation. The supernatant was recovered and extracted 4 times with 3 vol. of chloroform. The lipopolysaccharide solution was then precipitated in the cold (0 °C overnight) with absolute ethanol (10 vol.). The precipitate was washed once with ethanol and resuspended in distilled water, allowed to dialyze against 2 l of distilled water, for 8 h with 2 changes, and then lyophilized.

   All other lipopolysaccharide preparations used were first extracted with chloroform–methanol (2:1, v/v) two times, centrifuged, and lyophilized. All lipopolysaccharide preparations used in experiments were resuspended in 0.05 M Tris buffer (pH 7.0; 5 mg/ml) and dilutions were made from this preparation.

RESULTS

*Bilayer studies*

   When formed in the presence of native lipopolysaccharide, the survival time of phospholipid bilayers composed of egg phosphatidylcholine and cholesterol was
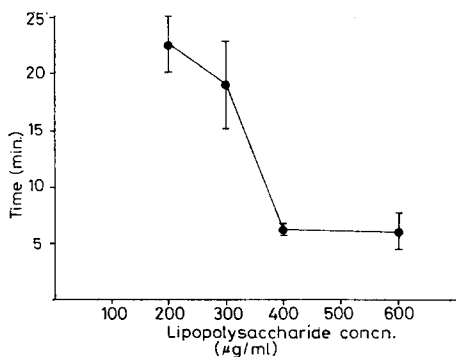


Fig. 1. Survival time of lecithin–cholesterol bilayers formed in the presence of lipopolysaccharide. Bars represent standard error (S.E.). Each point represents the mean of 7–10 determinations.
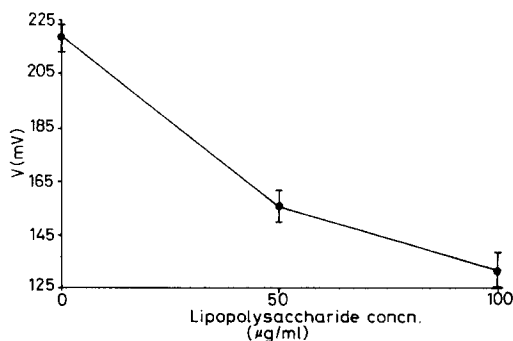
Fig. 2. The dielectric breakdown of lecithin–cholesterol bilayers formed in the presence of lipopolysaccharide. Bars represent standard error (S.E.). Each point represents the mean of 7–10 determinations.

greatly reduced (Fig. 1). All bilayers formed in the absence of lipopolysaccharide were stable for 4 h or longer. In the presence of 300 $\mu$g/ml native lipopolysaccharide, the average survival time was reduced to 18 min. At concentrations of 400 $\mu$g/ml and above, the survival time was reduced to 6 min or less.

An alternative way of measuring bilayer stability is to progressively increase the potential across the bilayer over a 5-min period until dielectric breakdown occurs. The results of such experiments are shown in Fig. 2. In the absence of lipopoly-saccharide, dielectric breakdown occurred at 218 mV which was consistent with the results of other experimenters using similar films[29]. In the presence of 50 $\mu$g/ml of lipopolysaccharide, there was a considerable reduction in the breakdown potential to 156 mV and a further reduction to 131 mV in the presence of 100 $\mu$g/ml. It appears that at low concentrations of 50 $\mu$g/ml there was enough adsorption of lipopoly-saccharide onto (or penetration into) the bilayer to cause a noticeable effect when an increasing potential was applied. Similar results using both techniques were obtained with alkali lipopolysaccharide.

*Monolayer studies*

Fig. 3 shows the surface pressure ($\pi$) produced by compressing a film of lipo-polysaccharide formed by allowing alkali-treated lipopolysaccharide in the sub-solution (50 $\mu$g/ml) to rise to the surface over a 60-min period. The $\pi$–area curve shows a very gradual slope as compression proceeds. Similar films could be formed when alkali lipopolysaccharide was spread on the surface of the trough by a micro-syringe (0.2 ml of 1 mg/ml solution of alkali lipopolysaccharide in 0.4% amyl alcohol). Such films could not be formed with native lipopolysaccharide at concentrations of 200 $\mu$g/ml in the subsolution nor upon spreading native lipopolysaccharide on the surface in a variety of solvents including 0.4% amyl alcohol and 67% isopropanol.

The penetration of various lipid films formed from cholesterol, phosphatidyl-choline, phosphatidylethanolamine, phosphatidylserine and erythrocyte lipid, respec-tively, by either native lipopolysaccharide (200 $\mu$g/ml) or alkali lipopolysaccharide (50 $\mu$g/ml), is compared in Fig. 4. The lipids were spread on the surface of the trough containing Tris buffer and native lipopolysaccharide (200 $\mu$g/ml) or in experiments
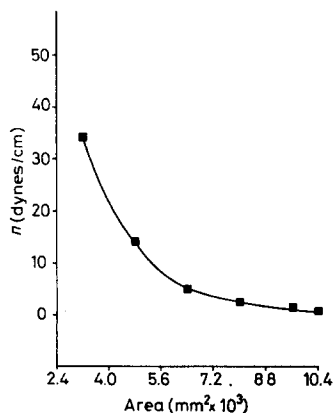


Fig. 3. The surface pressure (dynes/cm) *versus* area (mm$^2$ $\times$ 10$^3$) curve of a surface film formed from alkali lipopolysaccharide.

with alkali lipopolysaccharide, Tris buffer alone. In the latter case, immediately after spreading the lipids, alkali lipopolysaccharide in 1 ml of Tris buffer was injected into the subsolution in 10 places such that the final concentration of lipopolysaccharide in the subsolution was 50 $\mu$g/ml. The results were the same in stirred and unstirred systems. Unlike native lipopolysaccharide, alkali lipopolysaccharide could not be mixed into the subsolution prior to spreading the lipids, since its surface activity would change the interfacial potential of the clean surface. The initial $\pi$ was
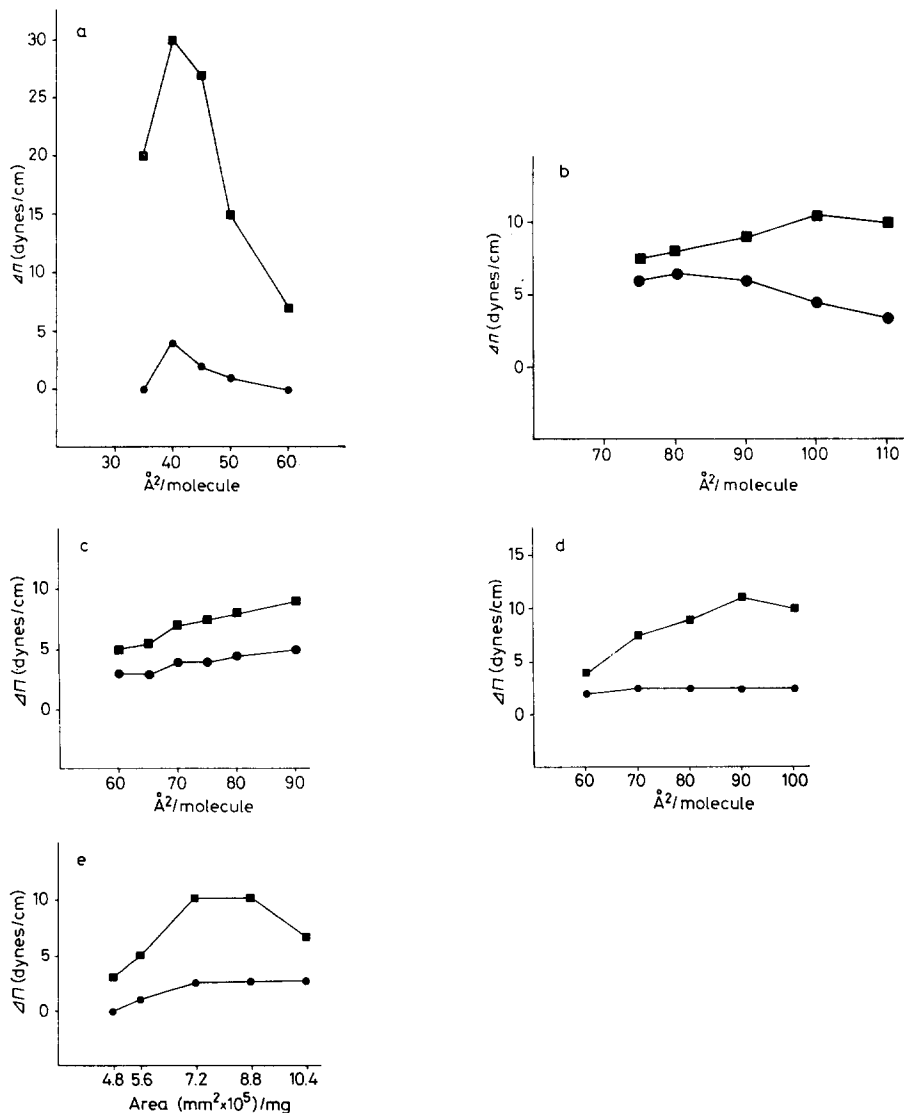


Fig. 4. $\Delta\pi$ *versus* area curves for lipopolysaccharide and alkali lipopolysaccharide with: (a) cholesterol; (b) phosphatidylcholine; (c) phosphatidylethanolamine; (d) phosphatidylserine; (e) erythrocyte lipid. ●—●, native lipopolysaccharide (200 $\mu$g/ml); ■—■, alkali lipopolysaccharide (50 $\mu$g/ml).

zero when alkali lipopolysaccharide was injected beneath the film. It was also zero with native lipopolysaccharide in the subsolution prior to compression of the film.

Native lipopolysaccharide caused changes in surface pressure of 2–6 dynes/cm depending upon the type of lipid film and its state of compression, whereas alkali lipopolysaccharide caused changes of 8–10 dynes/cm. The results indicated a lesser amount of penetration by native than alkali lipopolysaccharide. Assuming a linear relationship to exist between the concentration of lipopolysaccharide in the subsolution and the surface pressure, alkali lipopolysaccharide was about 10–15 times more surface active in its ability to penetrate surface monolayers.
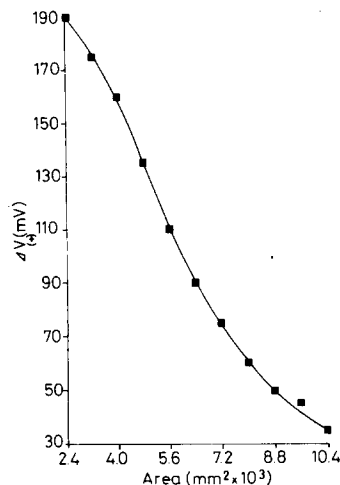


Fig. 5. $\Delta V$ versus area curve for a film of alkali lipopolysaccharide.

The $\Delta V$ for a surface monolayer of alkali lipopolysaccharide alone was positive (Fig. 5). This indicated an overall vertical dipole for lipopolysaccharide with the positive pole on top. Therefore, the incorporation of alkali lipopolysaccharide into a phospholipid monolayer should contribute positively to the potential assuming that alkali lipopolysaccharide incorporates with its lipid toward the surface. Although, other possible orientations of alkali lipopolysaccharide could contribute a positive $\Delta V$, its most likely orientation while penetrating the surface film is with its lipid chains up and perpendicular to the surface.

When alkali lipopolysaccharide was injected beneath various phospholipid films, significant changes in $\Delta V$ occurred. Fig. 6 illustrates the effects on monolayers of cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and erythrocyte lipid. Four of these compression curves show inflections in $\Delta V$. When alkali lipopolysaccharide interacted with cholesterol, phosphatidylcholine, phosphatidylethanolamine, and erythrocyte lipid there was a sudden drop in $\Delta V$ during the compression cycle. In contrast, when alkali lipopolysaccharide interacted with phosphatidylserine there was no sudden drop. Instead, the curve stayed consistently above the control throughout the compression cycle.

Table I is the result of experiments in which films were formed at a high state of compression (26 dynes/cm) after which changes in $\pi$ and $\Delta V$ were recorded 15 min
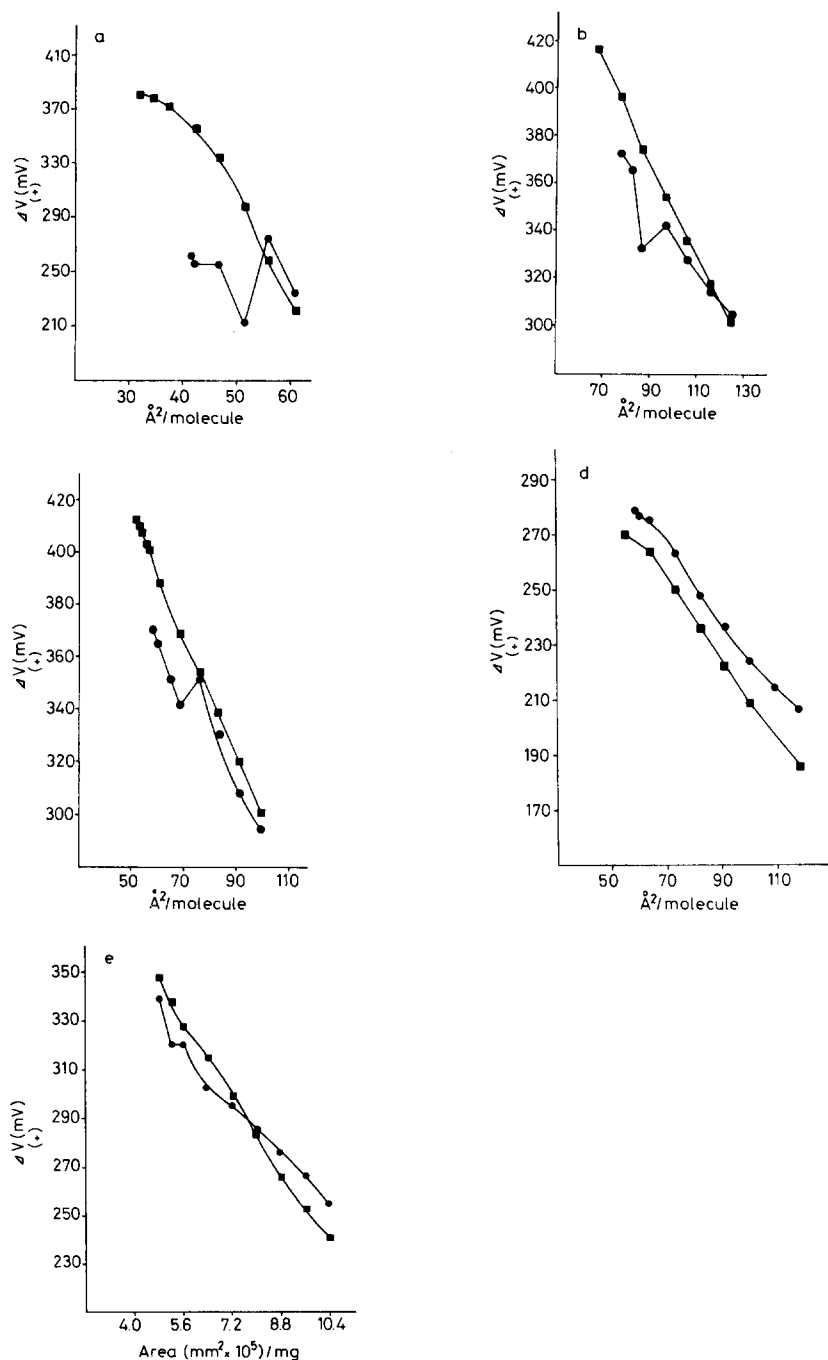
Fig. 6. *ΔV versus* area curves for the interaction of alkali lipopolysaccharide (50 μg/ml) with: (a) cholesterol; (b) phosphatidylcholine; (c) phosphatidylethanolamine; (d) phosphatidylserine; (e) erythrocyte lipid. ●——●, film *plus* alkali lipopolysaccharide; ■——■, control (film without lipopolysaccharide).

after alkali lipopolysaccharide (1 ml of a 1 mg/ml solution) was injected into the subsolution directly under the air electrode. There were changes in surface pressure in all films varying from 1.2 dynes/cm for phosphatidylserine to 8.8 dynes/cm for cholesterol. The surface potential of all phospholipid films decreased (from − 12 mV for erythrocyte lipid to − 33 mV for phosphatidylcholine) except that of phosphatidylserine which did not change.

TABLE I

CHANGES IN SURFACE PRESSURE AND POTENTIAL CAUSED BY ALKALI-LIPO-POLYSACCHARIDE IN LIPID FILMS AT HIGH COMPRESSION

| Film | $\Delta$ Surface pressure (dynes/cm) | $\Delta$ Surface potential (mV) |
|---|---|---|
| Phosphatidylcholine | 3.8 | − 33 |
| Phosphatidylethanolamine | 2.4 | − 25 |
| Phosphatidylserine | 1.2 | 0 |
| Erythrocyte lipid | 3.0 | − 12 |
| Cholesterol | 8.8 | − 15 |

When native lipopolysaccharide (200 $\mu$g/ml) was added to the subsolution, and $\Delta V$–area curves were plotted for the same lipids (Fig. 7), the responses were significantly different from those of alkali lipopolysaccharide. Native lipopolysaccharide did not produce sudden decreases in $\Delta V$ as did alkali lipopolysaccharide (Fig. 6). Instead, the curves were parallel and either above or below the controls (cholesterol below, phosphatidylethanolamine below, erythrocyte lipid below, phosphatidylcholine above, and phosphatidylserine above). The noticeable variations in the control curves of Figs 6 and 7 are probably due to slight oxidation and degradation of the phospholipid since these experiments were performed months apart.

DISCUSSION

The effect of lipopolysaccharide on phospholipid bilayers has been reported previously by Schuster and co-workers[11] who noted a decreased stability of bilayers in the presence of lipopolysaccharide from S. typhimurium and from Escherichia coli. The results that are reported in this study confirm the observations that lipopolysaccharide reduces the stability of phospholipid bilayers. The results of the dielectric breakdown study show it to be a more sensitive method for detecting the effect of lipopolysaccharide on bilayers. The lowest concentration used (50 $\mu$g/ml) decreased considerably the potential necessary for disruption of bilayers. An effect by this concentration of lipopolysaccharide would be difficult to detect by survival time studies.

Evidence for the proposed bilayer structure of lipopolysaccharide has come mainly from studies involving electron microscopy and X-ray diffraction[1-5]. The formation of a monomolecular film of alkali lipopolysaccharide developing a significant surface pressure (34 dynes/cm) is evidence consistent with the proposal that alkali
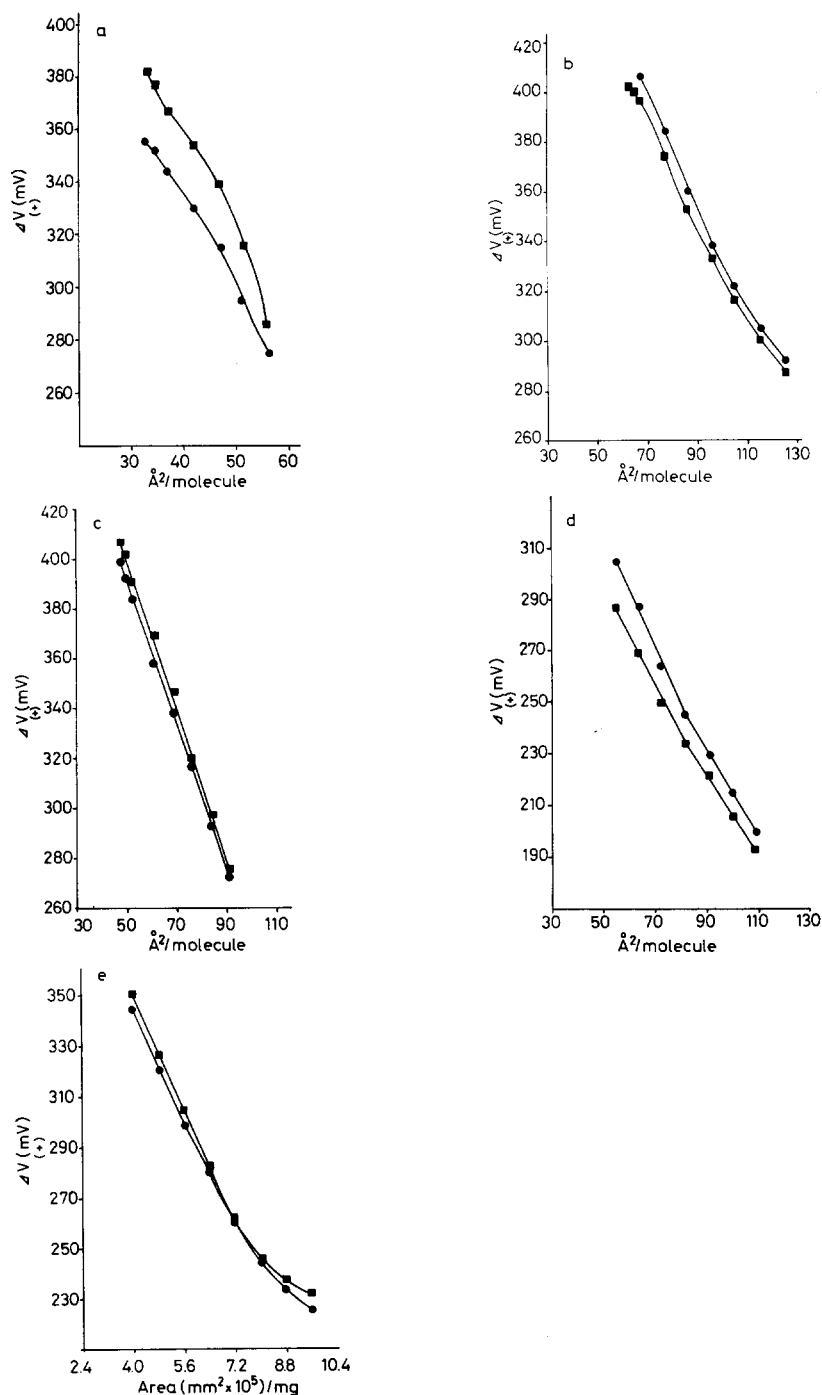
Fig. 7. $\Delta V$ *versus* area curves for the interaction of native lipopolysaccharide (200 $\mu$g/ml) with: (a) cholesterol; (b) phosphatidylcholine; (c) phosphatidylethanolamine; (d) phosphatidylserine; (e) erythrocyte lipid. ●——●, film *plus* lipopolysaccharide; ■——■, control (film without lipopolysaccharide).

treatment breaks down the bilayer structure of lipopolysaccharide to monolayer units. In aqueous solution they probably aggregate into micellar structures. However, at least some of these structures, unlike native lipopolysaccharide, seem to readily dissociate and form monomolecular films when spread. The fact that native lipopolysaccharide will not form a monomolecular film suggests that it is a highly stable bilayer structure and its halves are difficult to separate. Perhaps this dissociation is facilitated by the alkaline cleavage of the ester linked fatty acids.

The $\pi$–area curve for a monolayer of alkali lipopolysaccharide alone had a low slope upon compression, indicating that the film was very expanded with large intermolecular and/or intramolecular cavities. This is understandable, since a large portion of the lipid was cleaved off during hydrolysis. It is highly unlikely that the observed film properties as measured by $\pi$ were the result of low molecular weight, surface active breakdown products of the alkaline hydrolysis, since all surface active molecules like free fatty acids would have been extracted during the preparation (see Materials and Methods). Moreover, the same material was reextracted twice in the dry state with chloroform–methanol (2:1, v/v) and the residue behaved in a similar fashion.

A monolayer of alkali lipopolysaccharide at the interface produced a positive $\Delta V$. This indicated that the overall vertical electrical dipole of alkali lipopolysaccharide was with the positive pole up and the negative pole down, we have interpreted this to correlate with an orientation of alkali lipopolysaccharide with lipid up and polysaccharide down. This proved to be very helpful in the interpretation of other data, since changes in the orientation of lipopolysaccharide at the interface could be detected by changes in $\Delta V$. Changes in $\Delta V$ could also be produced by changes in counter ion binding or the orientation of water dipoles. However, we feel that these changes would be minimal in this system.

Alkali lipopolysaccharide was able to penetrate all of the lipid films tested, and native lipopolysaccharide was also able to penetrate all films except that formed with cholesterol. The inability of native lipopolysaccharide to penetrate a monolayer of cholesterol was probably due to the fact that, in contrast to phospholipids cholesterol forms a tightly packed film with little intermolecular spacing. Data obtained by Romeo and co-workers[16] point out the importance of intermolecular spacing in the penetration of monomolecular films by native lipopolysaccharide. They observed that penetration was increased by unsaturation and by the presence of cyclopropane in the fatty acid chains of phospholipids. These alterations would tend to increase the size of intermolecular cavities.

Comparing the degree of penetration of monolayers by native *versus* alkali lipopolysaccharide, it is evident that alkali lipopolysaccharide per unit weight has approximately a ten times greater ability to penetrate than does native lipopolysaccharide. The increased penetration of surface monolayers by alkali lipopolysaccharide can be explained by its greater surface activity in addition to its smaller size. This correlates well with the enhanced attachment of alkali lipopolysaccharide to red cells[7,8] and its recently described hemolytic effect[30].

The data concerning the influence of native lipopolysaccharide and particularly alkali lipopolysaccharide on the surface potential of lipid monolayers are complex and difficult to interpret. As previously stated, the incorporation of alkali lipopolysaccharide into a lipid film should contribute positively to the $\Delta V$ of lipid monolayers.

In fact, the $\Delta V$–area curves of films formed from lipids (cholesterol, phosphatidyl-
choline, phosphatidylethanolamine and erythrocyte lipid) in the presence of alkali
lipopolysaccharide underwent sudden decreases in $\Delta V$ as compression proceeded.
A likely explanation was that two phenomena were occurring simultaneously; (a)
penetration of the film by alkali lipopolysaccharide (lipid up, polysaccharide down)
causing a positive contribution to $\Delta V$ and (b) adsorption of alkali lipopolysaccharide
to the undersurface, causing a negative contribution to $\Delta V$. As compression of the
film proceeded, some of the penetrating molecules were squeezed out of the mono-
layer. This can most easily by inferred from the graphs in Fig. 4. The decrease in
these molecules making a positive contribution would allow the negative contribution
of adsorbed molecules to manifest itself. Of interest, the net negatively charged film
phosphatidylserine did not show these inflections in the presence of alkali lipopoly-
saccharide, suggesting that a net negative charge repelled adsorbing lipopoly-
saccharide.

The experiments summarized by Table I were undertaken to test the latter
hypothesis. Films at a high state of compression would allow little penetration and
only adsorption of lipopolysaccharide would contribute to the $\Delta V$. The decrease in
$\Delta V$ which occurred for all films except phosphatidylserine, which showed no change,
are consistent with the previously suggested hypothesis. The degree of penetration
was small in all cases a as determined by changes in $\pi$, except in the case of cholesterol.
This can be explained by the fact that cholesterol has a very steep $\pi$–area curve.
Therefore, very few molecules of lipopolysaccharide would have to penetrate this
film to cause a large increase in surface pressure. The fact that the decreases in $\Delta V$
for the ionic phospholipids phosphatidylcholine and phosphatidylethanolamine were
greater than that for cholesterol films, which are nonionic, suggests that adsorption
to the undersurface may be accompanied by ionic interaction.

Adsorption to the undersurface might also play a part in the interaction of
native lipopolysaccharide with monolayers since its interaction with cholesterol
caused no change in surface pressure (Fig. 4) but caused a significant decrease in
surface potential (Fig. 7).

The results of this study suggest, as have the results of similar studies, that
the affinity of lipopolysaccharide for cell surfaces may be explained by the penetration
of amphipathic lipopolysaccharide particles into the cell membrane. This interaction
appears to be characterized by a lipid–lipid attraction between lipopolysaccharide
lipid A and membrane lipids[9]. In addition, this study suggests a surface adsorptive
type of interaction which may be dependent upon a charge–charge attraction which
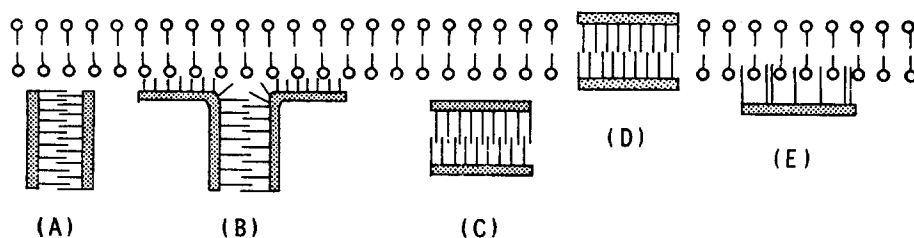


Fig. 8. Possible models of the interaction of lipopolysaccharide (A, B, C, D) and alkali lipopoly-
saccharide (E) with a phospholipid membrane.

can be inhibited by a net negative charge of the membrane. However, such a hypothesis can only be conclusively proven by pH or ionic strength variation studies.

Fig. 8 is a schematic representation of these interactions where A and C depict the adsorption of native lipopolysaccharide to the membrane surface, B and D represent penetration of the membrane by native lipopolysaccharide and E penetration by alkali lipopolysaccharide. A model for the adsorption of alkali lipopolysaccharide is not depicted.

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