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# The Effect of Endotoxin on Thin Lipid Bilayer Membranes

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*Summary.* The effect of endotoxin from *Salmonella typhimurium* or *Escherichia coli* was studied on bilayer lipid membranes (1 % lecithin – 1 % cholesterol in n-decane) formed in buffered 0.1 M NaCl solution (pH 6.8). Endotoxin was added to the buffered solution either prior to membrane formation or after stable membranes were formed. In both cases, concentrations of 110 to 720 μg/ml endotoxin initiated a decrease in the electrical resistance of the membranes followed by their rupture. A 50 μg/ml concentration of the agent was unable to elicit any response. Also, the addition of an equal volume of buffer solution, serving as a control, caused no decrease in membrane resistance or survival time. Treatment of the endotoxin with alkaline hydroxylamine to remove ester- and amide-bound fatty acids likewise abolished the membrane effect. This is the first report of an endotoxin effect on lipid bilayer membranes. The potential correlation of this interaction of bilayer and endotoxin with the diverse biologic effects of endotoxin is discussed.

Thin lipid films formed in aqueous solutions have many properties in common with those of cell membranes. Specifically, values for thickness, electrical capacitance, dielectric strength, water permeability, and surface tension are similar [1, 5, 6, 8, 9, 13, 14]. Unlike biological membranes, the resistance of thin lipid films is several orders of magnitude greater than that of cell membranes and reflects ion impermeability. However, high electrical resistance of these membranes can be decreased to physiologic levels by a number of substances [2, 3, 6, 12, 19]. The similarities between lipid bilayers and biological membranes suggest that these membranes may provide an accurate model for studying the interrelationships between certain biologically active substances and membrane structure. The multiple widespread effects of endotoxin on intact organisms may be related to a common denominator, namely, alteration of membrane structure, cellular or subcellular.

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An interaction between endotoxin and membranes has been demonstrated in monolayer experiments [17] and in blood cells [7, 15]. The first demonstration of an endotoxin effect on artificial lipid bilayers is presented in this paper.

## Materials and Methods

With use of the brush technique of Mueller, Rudin, Tien and Wescott [13, 14], membranes were formed across a small (1-mm diameter) aperture in the wall of a polyethylene cup placed within a rectangular, optically clear, plexiglass chamber containing the aqueous solution. The electrical resistance of the membranes was measured by use of a specially designed stimulator/recording electrical instrument (Electronics for Life Sciences, Rockville, Md.) according to the circuitry outlined by Mueller and Rudin [12]. Proper preconditioning of the membrane cup and chamber is essential if membranes are to be stable and long-lasting. The following preconditioning procedure was used: (1) the cup and chamber were soaked several hours in 5 N NaOH, (2) rinsed 10 times in distilled water, (3) soaked in 1 N HCl for 1 to 2 hrs, (4) rinsed 10 times in distilled water, (5) soaked in deionized water several hours, and (6) let dry.

Membranes were formed from 1 % egg lecithin and 1 % cholesterol (both from Nutritional Biochemicals, Cleveland, Ohio) dissolved in n-decane (K and K Laboratories, Plainview, N. Y.). The aqueous phase was 0.1 M NaCl (Fisher Scientific Company, Fair Lawn, N. J.) at pH 6.8. The aqueous phase was degassed by boiling and then cooled prior to use. The NaCl was heated to 550 °C for further purification.

Membranes were formed in buffered 0.1 M NaCl alone or in buffered 0.1 M NaCl to which endotoxin (480 or 720 μg/ml) was added prior to membrane formation. Membranes formed in buffered 0.1 M NaCl alone were allowed to stabilize for 10 to 40 min before endotoxin (50, 110, 220 or 475 μg/ml) was added to both sides of the membrane. In some of these experiments, hydroxylamine-treated endotoxin was substituted in a concentration of 200 μg/ml. All experiments were done at room temperature (23 to 25 °C).

The endotoxin was a phenol-water extract of formalin-fixed whole cells [21] of *Salmonella typhimurium* or *Escherichia coli*. Dr. Herman Beer kindly supplied the endotoxin used in experiments where it was present in the aqueous phase prior to membrane formation. Endotoxin was obtained commercially (Difco Laboratories, Detroit, Mich.) for experiments in which it was added after membrane formation and stabilization. Endotoxin from either source behaved similarly, although potency varied slightly. Difco endotoxin was treated with alkaline hydroxylamine, as described by McIntire, Sievert, Barlow, Finley and Lee [11].

## Results

Table 1 shows the mean survival times of control membranes and of membranes formed in a solution where endotoxin was already present. All control membranes survived longer than 2 hr, whereas experimental membranes lasted approximately 2 min. There was no significant difference between the initial resistance (resistance measured immediately after the membrane lipid was applied) of control and experimental membranes. All control membrane resistances agreed with values previously reported ($\sim 10^7\ \Omega\ cm^2$) [10, 16].

Table 1. *Survival times of thin lipid membranes in the presence and absence of endotoxin in solution where membranes are formed*

| Solution | Mean survival time ±S.E.M. | No. of experiments |
|---|---|---|
| Control | 130.0±9.0 min | 10 |
| Endotoxin (480 or 720 μg/ml) | 2.1±0.3 min | 9 |

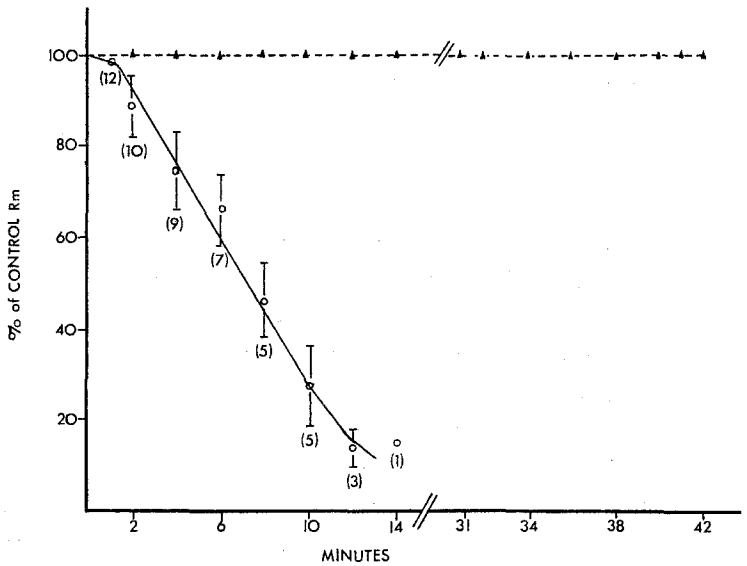


Fig. 1. *Solid line*: Time (min) after addition of endotoxin (110, 220 or 475 μg/ml) plotted against % of pre-addition value of the membrane resistance (*Rm*). Since all three concentrations had the same effect, they are plotted together. S.E.M. is calculated for the number of membranes surviving at the designated times (given in parentheses). *Dotted line*: Time (min) after addition of hydroxylamine-treated endotoxin (200 μg/ml) or of a volume of buffer equal to the volume of endotoxin added in the other experiments plotted against % of pre-addition value of *Rm*. The line represents six experiments, three utilizing buffer and three utilizing hydroxylamine-treated endotoxin; all behaved identically

Fig. 1 shows the effect of endotoxin added to the aqueous phase after membranes had been formed, turned black, and resistances were constant for 10 to 40 min. After addition of endotoxin, membrane resistances fell to a fraction of their pre-addition stabilized values. The effect was apparent within 2 min after addition of endotoxin. The addition of buffer only or of 200 μg/ml of the hydroxylamine-treated endotoxin was without effect (Fig. 1).

Table 2. *Survival times of thin lipid membranes after addition of endotoxin to stable formed membranes in buffer solution*

| Dose of endotoxin (μg/ml) | Mean survival time ±S.E.M. after addition | No. of experiments |
|---|---|---|
| Control[a] | 38.3±3.2 | 3 |
| 50 | 41.0±2.2 | 4 |
| 110 | 6.0±4.0 min | 4 |
| 220 | 5.0±2.0 min | 4 |
| 475 | 9.0±2.6 min | 4 |

[a] An equal volume of buffer was added as a control.

### *Dose-Response*

Table 2 presents the effect of different concentrations of endotoxin on membrane survival time. A concentration of 50 μg/ml behaved in a manner identical to addition of buffer alone. Concentrations of 110, 220 and 475 μg/ml had indistinguishable effects on membrane survival time; all of these concentrations caused a marked decrease in survival time, as compared to the control. This data supports a steep dose-response curve.

It was impossible to achieve a stable low resistance when endotoxin was added. When a decrease in resistance occurred, it progressed to membrane disruption at any effective concentration of endotoxin. In other words, any concentration of endotoxin that produced a significant resistance change caused the membrane to break. Low concentrations (50 μg/ml) had no effect on resistance or survival time.

## Discussion

Endotoxin produces a fall in electrical resistance and a decrease in survival time of bilayer membranes. The difference in survival times observed when endotoxin was present prior to membrane formation and when it was added to stable formed membranes might be due to variations in mixing or to some direct effect of endotoxin on the phospholipid of the membrane during its formation. The latter possibility seems unlikely because the initial resistances of membranes formed in the presence of endotoxin did not differ from those formed in buffer alone.

The results of the dose-response data suggest that a critical concentration of endotoxin (between 50 and 110 μg/ml) initiates a process which results first in a resistance drop and inevitably in disruption of the entire membrane.

The mechanisms by which endotoxin causes a fall in resistance and a decrease in survival time cannot be deduced from these experiments. However, evidence for an interaction between endotoxin and phospholipid monolayers has been demonstrated, and the agent has been shown to completely disrupt organized phospholipid leaflets by electron microscopy [17]. Endotoxin may interact with the surface phospholipids of the bilayer, either chemically or electrically, altering their structural array. The lipids of the membrane would then most probably seek a more stable micellar arrangement. Small areas of such micellar formation would cause an increase in permeability and a decrease in electrical resistance of the membrane. Further micellar formation could take place spontaneously in the now weakened membrane structure, finally resulting in complete membrane disruption. Specificity was exhibited by the endotoxin-bilayer interaction as indicated by the hydroxylamine-treated endotoxin experiments. If, as suggested by Shands, Graham and Nath [18], a major determinant of the morphology of the lipopolysaccharides is the lipid moiety, then hydroxylamine treatment would be expected to produce morphological changes in the endotoxin through its removal of ester- and amide-linked fatty acids. The treated endotoxin had no effect on membrane electrical resistance or survival time.

Endotoxin produces diverse biological effects in experimental animals [4]. Some of these effects could be interpreted as the result of disruption of cellular or subcellular integrity at the membrane level. For example, endotoxin releases such enzymes as cathepsin and $\beta$-glucuronidase from lysosomes [20].

Although an interaction of endotoxin with phospholipid monolayers has been demonstrated, the effect of this agent on lipid bilayers may be more relevant to its activity *in vivo*. The similarities between lipid bilayers and cell membranes suggests that the former may serve as a more accurate model of cell membranes than the monolayer. Experiments are now in progress to establish the relationship between the effect of endotoxin *in vitro* and its activity *in vivo*.

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