Interactions of 4-chlorophenol and phenol with phosphatidylethanolamine monolayers in relation to antibacterial action

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The introduction of a chlorine atom at the 4-position of the phenol aromatic ring leads to an increase in antibacterial activity. If the antibacterial mode of action of 4-chlorophenol and phenol involves disruption of the bacterial cytoplasmic membrane, 4-chlorophenol itself should be more effective than phenol in disrupting those interactions which contribute to the maintainence of the integrity of the membrane. In order to determine the effect of 4-chlorophenol and phenol on lipid-lipid interactions, which may well contribute to the maintainence of the integrity of a bacterial cytoplasmic membrane, the interactions of 4-chlorophenol and phenol with monolayers of 1,2-dipalmitoyl-L-3-phosphatidylethanolamine at the liquid-air interface has been studies using a film balance technique.

Monolayers of phosphatidylethanolamine were spread and compressed on a sub-phase which was basically a five-fold dilution of McIlvaine buffer, pH 6·0, adjusted to an ionic strength of 0·1 with sodium chloride. The sub-phase temperature was 25 $\pm$ 0·1°. Precautions were taken to ensure that the monolayers attained equilibrium after spreading and during their subsequent compression (Proudfoot and Davdani 1973).

A sub-phase concentration of $0{\cdot}65 \times 10^{-3}$ mol dm^{-3} 4-chloro-phenol was found to expand the phosphatidylethanolamine monolayers. Compression of the expanded monolayers resulted in the 4-chlorophenol molecules being ejected from the monolayers, implying that the interaction of 4-chlorophenol with the phospholipid molecules is of a weak non-specific nature.

The presence of 4-chlorophenol molecules between adjacent phosphatidylethanolamine molecules in a monolayer will interfere with the close packing and hence total lateral cohesion between adjacent phosphatidylethanolamine molecules. In this respect, a sub-phase concentration of $1{\cdot}95 \times 10^{-3}$ mol dm^{-3} 4-chlorophenol reduced the total lateral cohesion between adjacent phosphatidylethanolamine molecules in a monolayer sufficiently that loss of phosphatidylethanolamine molecules occurred from the monolayer. Kaye & Proudfoot (1971) have also shown phenol and a number of alkylated phenols to exert a similar effect on phosphatidyl-ethanolamine monolayers.

4-Chlorophenol was found to be more effective than phenol in expanding monolayers of phosphatidylethanolamine and hence in reducing the total lateral cohesion between adjacent phosphatidylethanolamine molecules. This is probably a consequence of (1) the flat orientation of 4-chlorophenol molecules at the sub-phase-air interface facilitating their ability to interfere with the close packing of adjacent phosphatidylethanolamine molecules and (2) the increased surface activity of 4-chlorophenol compared to phenol.

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Lysozyme—preservative interactions in eye preparations

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The interaction of lysozyme with preservatives used in eye preparations is of importance, mainly because it is undesirable to inactivate the lysozyme present in the tear fluid which plays a major role in the hygiene of the eye. The possibility of lysozyme interactions occurring is greatest with the many contact lens solutions now on the market most of which contain two or three component preservative systems. Some of these solutions are carried into the eye on the contact lens or are instilled separately.

The disruption of Gram-negative cells by lysozyme in the presence of EDTA and tris is well known (Respaske, 1958). We have investigated certain preservatives to ascertain

whether they interact with lysozyme, the body's natural bactericide, either to inhibit or to potentiate the action. The action of such a combination can be expressed as change in the optical density (extinction) of a standardized bacterial suspension. Any decrease in optical density during an arbitrary period of time is referred to as 'lysis'. *Pseudomonas aeruginosa* was selected as the test organism because its presence as a contaminant in ophthalmic solutions can lead to serious eye damage.

Bacterial suspensions were prepared from overnight cultures of *P. aeruginosa* NCTC 6750 which were washed twice in 0·5 M sodium chloride and the pH was brought to 8·0 with tris buffer. Lysozyme and EDTA, with or without a preservative, were added simultaneously and the optical density was measured every minute for 8 min.

It was found that polysorbate 80, sodium lauryl sulphate and phenylethanol did not affect the lysozyme–EDTA system, while benzalkonium and chlorhexidine increased the lytic action. The result with phenylethanol is similar to that of Grote & Woods (1955). This suggests that polysorbate, sodium lauryl sulphate and phenylethanol may not affect the cell envelope external to the peptidoglycan layer, whereas benzalkonium and chlorhexidine do.

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A novel sterility test for chloramphenicol

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The inactivation of penicillin by penicillinase before sterility testing is an elegant solution to the problems which arise during the testing of most other antibiotics by membrane filtration.

Shaw (1967) described the inactivation of chloramphenicol(CM) by a specific enzyme, CM-acetyl-transferase (CAT), and the feasibility of using this enzyme to inactivate CM solutions before they are tested for sterility has been investigated.

There are two sources of CAT, *Staphylococcus aureus* and *Escherichia coli*. Both enzymes perform a similar acetylation of CM in the presence of acetyl Co A. The reaction is followed by recording the increase in extinction at 412 nm caused by the reaction of 5,5-dithiobis-2-nitrobenzoic acid with thiol groups liberated by the breakdown of acetyl Co A (Shaw & Brodsky, 1968).

Crude extracts were prepared of *E. coli* carrying an R factor conferring resistance to CM. After sonication and the removal of cell debris the extracts were dialysed against 0·01 M tris-HCl, pH 7·8 and stored at −20°. 25 μl of this enzyme preparation (containing approx. 10 mg protein ml^{-1}), inactivated 0·5 mmol of CM in 2 min. The inactivation was confirmed biochemically and microbiologically.

For an actual sterility test 5 mmol of CM were inactivated by incubation with 2·5 ml enzyme preparation and 1 μmol acetyl Co A at 37° C for 30 min.

The results indicated that inactivation by this means is a rapid and reproducible method with several advantages over membrane filtration. Other antibiotics which may be amenable to this type of approach are the amino-glycosides, streptomycin, kanamycin.

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