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.

REFERENCES

GROTE, I. W. & WOODS, M. (1955). *J. Am. Pharm. Ass.* (*Sci. Edn*), **44**, 9–16.
RESPASKE, R. (1958). *Biochem. biophys. Acta*, **30**, 225–234.

A novel sterility test for chloramphenicol

ANTHONY S. BREEZE AND G. ALSTON MACADAM
Department of Pharmacy, Heriot-Watt University, 79 Grassmarket, Edinburgh EH1 2HJ, U.K.

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.

REFERENCES

SHAW, W. V. (1967). *J. biol. Chem.*, **242**, 687–693.
SHAW, W. V. & BRODSKY, R. F. (1968). *J. Bact.*, **95**, 28–36.