

PRESERVATION OF OPHTHALMIC SOLUTIONS A CRITICAL EVALUATION*

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ALTHOUGH the preparation of sterile ophthalmic solutions does not present undue difficulty to trained personnel, the prevention of contamination during use has always been a problem. Denston (1949), in a review of the British National Formulary (1949), stated that:

Serious ill-effects arising from the use of infected eye-drops were reported to the National Formulary Committee.

The inclusion in eye-drops of a suitable preservative would therefore appear necessary. The ideal preservative should be

- (a) bactericidal and fungicidal,
- (b) compatible with most chemicals used in ophthalmic preparations,
- (c) non-irritating and non-sensitizing.

The most commonly accepted preservative is the mixture of methyl and propylhydroxybenzoates used in the "Solution for Eye-Drops" of the British Pharmaceutical Codex. These hydroxybenzoates, being relatively efficient fungicides, will keep the solutions free from mould growth (Hasler, 1939). In the strength used however, they are not bacteriostatic and will allow the growth of pathogenic bacteria. Chlorbutol (0.5 per cent.) is often used (Adelaide Children's Hospital Pharmacopoeia) although it is not very stable to heat (Murphy and Stoklosa, 1952), and may be somewhat irritating to the eye. The use of phenylmercuric nitrate (1 in 100,000) has also been advocated (Hind and others, 1947), but its bactericidal properties have recently been questioned (Engley, 1950), and even at this low concentration it has many incompatibilities. It is therefore difficult to find a chemical which satisfies all requirements, but many of the desired properties are exhibited by certain quaternary ammonium compounds such as cetrimide B.P., and benzalkonium chloride U.S.P.

McPherson and Wood (1949) used 1 in 5,000 benzalkonium chloride as a means of producing "self-sterilizing ophthalmic solutions". As the quaternary ammonium antiseptics inhibit some organisms in concentrations as low as 1 in 500,000 (Quisno, Gibby, and Foter, 1946), and since high concentrations of these substances may be irritating and incompatible with chemicals used in ophthalmology, it appeared to us that a more dilute solution of these compounds might answer most of the requirements.

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Experiments

(1) *Bactericidal Effect.*—Cetrimide 1 in 50,000 was used on *Staphylococcus aureus* and compared with "Solution for Eye-Drops", using distilled water as a control. A pure 18-hr broth culture was diluted 1 in 10 with sterile distilled water, and one drop added aseptically to the test solutions, two bottles of each being used.

The solutions were allowed to stand for 24 hrs before being diluted 1 in 20 with sterile distilled water before plating out. We found that this dilution lowered the strength of the preservative below bacteriostatic strength. The flood-plate method of Anderson and Stuart (1935) was used on nutrient agar, pH 6.8. A small volume (0.7 ml.) of each diluted solution was plated out and incubated at 37°C. for 3 days. The results, shown in Table I, indicate that "Solution for Eye-Drops" supports the growth of *Staphylococcus aureus* to about the same extent as distilled water, whilst cetrimide 1 in 50,000 is lethal to *Staphylococcus aureus* within 24 hrs.

TABLE I
EFFECTS OF CETRIMIDE 1 IN 50,000 AND "SOLUTION FOR EYE-DROPS"
ON *STAPH. AUREUS* AFTER 24 HRS' CONTACT

Plates	Colonies		
	Solution for Eye-Drops	Cetrimide 1 in 50,000	Distilled Water
1	173	None	460
2	230	None	520
3	550	None	390
4	567	None	286
Average No. of Colonies	380	None	414

(2) *Preservative Effect.*—Cetrimide, chlorbutol, and "Solution for Eye-Drops" were studied, using *Staphylococcus aureus*, *Bacterium coli*, *Pseudomonas pyocyanea*, and *Proteus vulgaris* as test organisms.

Preliminary experiments with *Ps. pyocyanea* indicated that cetrimide 1 in 50,000 was too dilute to produce any rapid lethal effect with this organism, and the concentration of cetrimide was increased to 1 in 20,000. A pure 18-hr broth culture was used in each case, incubation being at 37°C. The test solutions were sterilized by autoclaving at 115°C. for 30 min. before addition of the test organisms. Each culture was diluted 1 in 10 with sterile distilled water before the addition of one drop of diluted culture to each of the following test solutions:

- 10 ml. cetrimide 1 in 20,000;
- 10 ml. chlorbutol 0.5 per cent.;
- 10 ml. "Solution for Eye-Drops";
- 10 ml. distilled water.

Samples (1 ml. each) were taken from the test solutions and subcultured into 9 ml. nutrient broth at intervals of 15, 30, and 60 min. after the addition of the test organism. The nutrient broth used was the neutralizing medium for quaternary ammonium compounds described by Quisno, Gibby, and Foter (1946); it contained lecithin as the chemical inhibitor with Tween 80 as a dispersing agent.

The results of many experiments observed after 5 days' incubation at 37°C. are summarized in Table II.

TABLE II
SURVIVAL OF SOME PATHOGENIC ORGANISMS IN PRESERVATIVE TEST SOLUTIONS AFTER SHORT CONTACT INTERVALS

Organism		<i>Ps. pyocyaneus</i>			<i>Proteus vulgaris</i>			<i>B. coli</i>			<i>Staph. aureus</i>		
		15	30	60	15	30	60	15	30	60	15	30	60
Antiseptic	Distilled Water	+	+	+	+	+	+	+	+	+	+	+	+
	Solution for Eye-Drops	+	+	+	+	+	+	+	+	+	+	+	+
	Chlorbutol 0.5 per cent.	+	+	—	+	+	+	+	+	+	+	+	+
	Cetrimide 1 in 20,000	—	—	—	—	—	—	—	—	—	—	—	—

+ Growth. —No Growth.

Five strains of *Ps. pyocyaneus* were tested: "Sharke", and four other distinct, yet unidentified, strains isolated from tissue. Two strains of *Proteus vulgaris* were tested: OXK and X19. In no case did the organism survive after 15 minutes' contact with the cetrimide solution 1 in 20,000.

(3) *Tests for Irritation.*—Two ophthalmic solutions were prepared, one of sterile distilled water and one of sterile distilled water containing cetrimide 1 in 20,000, and 63 adult persons with normal eyes were tested, one drop of each solution being placed in each eye. The nature of the solutions was not disclosed and the reactions were noted. Of the 63 persons tested, nineteen said they experienced slight irritation with distilled water, and eighteen experienced slight irritation with cetrimide 1 in 20,000. Included in this result are several who apparently experienced irritation with both solutions.

(4) *Tests for Compatibility.*—Cetrimide 1 in 20,000 is compatible with most ophthalmic drugs. No precipitate, turbidity, or opalescence occurred with solutions of boric acid, borax, copper sulphate, pilocarpine nitrate, sodium chloride 0.9 per cent., penicillin, cocaine HCl, zinc sulphate, atropine sulphate, fluorescein sodium 2 per cent., hyoscine hydrobromide, sodium propionate, sodium sulphacetamide, borate, or phosphate buffers. Cetrimide 1 in 20,000 was compatible with physostigmine salicylate 0.5 per cent., although precipitation occurs with higher concentrations of cetrimide (1 per cent.). Precipitation also occurs with solutions of silver nitrate and mercuric chloride.

Discussion

Where ophthalmic solutions are sterilized and used under aseptic conditions it is neither necessary nor desirable to include any preservative. However, where such conditions do not exist, contamination may readily occur and a preservative should be included to prevent the growth of pathogens introduced during use. There appears to be no substance which answers

all the requirements of an ideal preservative. Our studies indicate that cetrimide 1 in 20,000 satisfies most requirements:

- (a) it rapidly destroys common pathogenic bacteria,
- (b) it is compatible with most chemicals used in ophthalmology,
- (c) it is non-irritating.

Furthermore, it is readily soluble in water and is stable to autoclaving. Its value as a fungistatic has not been established, although it is known that solutions of cetrimide will not support the growth of many common moulds.

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