

The Preservation of Ophthalmic Preparations

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Synopsis—The chemical and physical factors involved in ophthalmic formulations have been considered briefly. The problems of sterility for ophthalmic preparations have been discussed and the evidence relating to the activity of several preservatives examined. Chlorocresol, chlorbutol, benzalkonium chloride and chlorhexidine were shown to have scientific support for use as ophthalmic preservatives.

Ophthalmic preparations should be made so as to preserve them against physical, chemical and biological changes which may make them less effective therapeutically. The pharmacist must formulate such preparations with attention to many factors, most important of which are tonicity, pH, stability, viscosity and sterility. It is proposed briefly to consider the chemical and physical factors involved in ophthalmic formulations and to investigate more fully the problems of sterility and preservation against micro-organisms.

PHYSICAL AND CHEMICAL FACTORS

Tonicity

Lachrymal fluid is isotonic with blood and has a tonicity equivalent to that of 0.9% sodium chloride solution. Riegelman and Vaughan (1) have quoted results of their own, and of other workers, showing that an

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ophthalmic solution may safely range from the equivalent of 0.7% to 1.5% sodium chloride. Most ophthalmic drugs have high molecular weights, and may usually be added to an isotonic vehicle without altering the tonicity to painful levels. This applies particularly to eye drops used in small volumes ; large volumes of eye lotions should be approximately isotonic.

Hydrogen ion concentration

Normal tears have a pH of about 7.4. They have the capacity to bring the pH of an unbuffered solution at pH as low as 3.5, or as high as 10.5, to within tolerable limits instantaneously (1). Most ophthalmic drugs, e.g. alkaloidal salts, in distilled water or isotonic saline have a negligible buffer capacity although some drugs, notably salts of pilocarpine and epinephrine, may overtax the buffer capacity. The United States National Formulary XI recommends a 2% boric acid solution (about pH 4.7) as a general ophthalmic vehicle. This solution will tend to neutralize alkali leached from the glass container, it has a satisfactory tonicity, and is at a pH where most ophthalmic drugs are stable to autoclaving as well as being therapeutically active. The United States Pharmacopoeia XVI recommends an isotonic phosphate buffer as an ophthalmic vehicle in addition to boric acid solution.

Stability

The stability of ophthalmic preparations during prolonged storage, and the effects of heat sterilization processes, must be considered during formulation. The effects of temperature and pH are particularly important. Buffering certain drugs in the physiological pH range makes them unstable, particularly at high temperatures. Most of the common ophthalmic drugs, however, in 2% boric acid solution can be autoclaved without seriously affecting their therapeutic activity. The U.S.N.F. XI states that the oxidative discolouration of physostigmine, epinephrine and phenylephrine under these circumstances may be reduced by the addition of 0.2% sodium bisulphite to the vehicle. Alkaline drugs such as the sulphonamides and fluorescein sodium should be prepared in sterile distilled water.

Viscosity

Methylcellulose is sometimes added to ophthalmic solutions to increase contact time with the cornea. Calculated quantities of a concentrated, hydrated stock solution of methylcellulose may be added to normal

ophthalmic vehicles which may then be autoclaved, cooled, and the coagulated methylcellulose re-dispersed by agitation.

PRESERVATION AGAINST MICRO-ORGANISMS

There has recently been much discussion about the difficulties of preserving ophthalmic solutions against micro-organisms. The main problem is the exceptionally high resistance of *Pseudomonas aeruginosa* to chemical antibacterial agents. This pathogen causes serious difficulties in the control of cross-infection in general (2,3) but especially in ophthalmology (4). Some species of *Aerobacter* are eye pathogens (5), and the problem is further complicated by the existence of pathogenic strains of the spore formers *Bacillus subtilis* (6) and *Clostridium welchii* (7).

Pseudomonads have been isolated from most naturally occurring waters (8), and *Ps.aeruginosa* in particular has exceptionally simple requirements and will grow in many ophthalmic solutions. This organism has been shown to use the hydroxybenzoates as a sole source of carbon (9). In addition, the temperature requirements for growth are wide. It is a virulent pathogen causing severe ocular infections (10). Relatively small inocula of about 50–100 cells have been shown to produce infection in rabbits' eyes (11,12). Fisher and Allen (13) have shown that *Ps.aeruginosa* produced an enzyme which destroys the cornea by degradation of corneal collagen.

Ps.aeruginosa has consequently been recommended as the main reference organism when selecting chemical preservatives for ophthalmic solutions. An adequate preservative should also be effective against a wide range of gram-positive and gram-negative organisms.

The case for sterility

An injured eye has less resistance to infection than the bloodstream, so that at least the same precautions should be taken in preparing ophthalmic solutions as in preparing solutions for intravenous use (14). The United States National Formulary XI states that all solutions for surgical use should be sterile, and prepared without a preservative because of danger of chemical damage to the inner structure of the eye. It has frequently been shown that a significant proportion of used, and unused, ophthalmic solutions were contaminated with *Ps.aeruginosa*, and that eye ointments are also liable to contamination (15,16,17). There is now widespread agreement that ideally ophthalmic medicaments should be dispensed in a sterile condition (11,15,18,4).

The United States Pharmacopoeia, the United States National Formulary and the International Pharmacopoeia all specify sterility for ophthalmic solutions. The British Pharmaceutical Codex 1963 stipulates sterility for eye drops only.

Autoclaving in the final container is the method of choice because of the danger from pathogenic spore formers. This is still so with chemically preserved solutions because of the possibility of germination on the cornea, which can apparently eliminate the carry-over activity of a wide range of antibacterial agents (11). It is, however, unlikely that pathogenic spores would germinate in the ophthalmic container in the presence of an antibacterial agent, and at room temperature. Gamma radiation has proved a useful sterilizing agent for some heat labile ophthalmic preparations and may prove to be of wide application (19).

Eye drop containers

Norton (20), and Richards *et al* (21) have shown that eye drop containers available then in Britain for extemporaneous preparations were not satisfactory, and that closure units must be modified to provide satisfactory sealing during autoclaving. British Standards Institution Committee P.188 is now engaged in producing a specification for a suitable multidose eye drop container.

It seems possible, and desirable, that in the field of ophthalmic preparations, as with other classes of medicaments, there will be progressive movement away from extemporaneously prepared products. The time is opportune for bulk-manufactured sterile products of standard formulae, packed in simple plastic single and multidose containers. Several manufacturers do this using methods which would normally be uneconomic for the individual pharmacist in a small business (22). Nevertheless, there is now available relatively inexpensive equipment for small scale extemporaneous sterile filtration employing a modified syringe with membrane filter (15).

Preservation of oils, creams and ointments

There is little published work on the preservation of oils, creams and ointments. Ridley (16) has isolated *Ps.aeruginosa* from eye ointments on several occasions. He proposed to eliminate the use of oils by replacing them with ointments dispensed in sterile tubes. The activity of bactericides in o/w dispersions has been shown to be influenced by many interacting factors and the *ad hoc* addition of preservatives shown to be

unsatisfactory (23). A practical ideal at present would appear to be the aseptic preparation of ophthalmic ointments, using sterile ingredients.

Preservation of solutions

The possibility of contamination from pathogenic organisms subsequent to sterilization makes it necessary for multidose containers to be preserved using a suitable antibacterial agent.

The capacity of small inocula of *Ps.aeruginosa* to cause infection, together with the neutralizing properties of the cornea towards antibacterial agents, make it preferable that these agents be capable of maintaining sterility.

Much of the earlier work assessing preservatives is of reduced value because of inadequate experimental procedures. Riegelman *et al* (11) quoted numerous papers which did not state the precautions taken to ensure the absence of bacteriostatic concentrations of preservative in subcultures testing for recovery. They discussed the inadequacy of dilution techniques designed to inactivate preservatives and introduced *in vivo* procedures as well as *in vitro* tests involving chemical antagonists. They consistently found that organisms would produce infections, and could be recovered from the cornea while *in vitro* cultures were apparently negative. Subsequent improvement of the antagonist/recovery broth reduced the discrepancy. Kohn *et al* (24,25) further developed this important principle of comparing *in vivo* with *in vitro* procedures and obtained exact correlation.

Several chemical agents have been proposed as suitable ophthalmic preservatives and it seemed appropriate to consider each separately.

Esters of p-hydroxybenzoic acid

A combination of methyl and propyl *p*-hydroxybenzoates has been used in the form of "Solution for Eye Drops" until this preparation was replaced by chlorocresol in the British Pharmaceutical Codex 1963. Subsequently this Codex was amended (26), and Solution for Eye Drops reintroduced. Brown *et al* (4) have commented on these changes. Klein *et al* (27) tested two combinations of the esters at three concentrations up to 0.16% against 10^8 /ml *Ps.aeruginosa* present in three eye-drop preparations—Sodium fluorescein 2%, atropine sulphate 0.5%, eserine salicylate 0.25%. Contact times were up to 24 hr after which subcultures were made testing for sterility. They found that both combinations 1 (*Nipasept*) and 2 (*Nipa 82121*) were ineffective at all concentrations in fluorescein drops, and growth occurred after subculture at each contact time up to 24 hr. 0.16%

Nipasept was necessary to produce sterility after 1 hr contact in atropine drops, while 0.106% sterilized after 2 hr. In eserine drops 0.16% *Nipasept* sterilized after 3 hr, and 0.106% after 5 hr. Combination 2 was more effective than *Nipasept* in the presence of atropine and eserine, and 0.08% sterilized after 1 hr. The esters were effective in some cases at 0.16%, but nevertheless it was found that this strength caused some burning sensation when introduced on the eye. The value of their results is reduced because they used a broth dilution experimental procedure, and inactivation of the agents was uncertain. Nonionic detergents are generally known to inactivate *p*-hydroxybenzoic acid esters (28) and Kohn *et al* (24) have recommended *Tween 20* as an antagonist of these esters.

Lawrence (29) found that a mixture of 0.16% methylparaben and 0.02% propylparaben sterilized within 1 to 6 hr in every case with 26 strains of *Ps.aeruginosa*, and four species of *Proteus* in simple buffer at pH 6.2. He used undiluted 24 hr cultures as inocula. Activity was also tested against four strains of *Ps.aeruginosa* in the presence of non buffered aqueous solutions of several drugs. Sterility was achieved in less than 30 min in several cases and never after more than 6 hr. He did not use inactivating substances in the recovery broth for the parabens.

Ridley (16) stated that 0.1% methyl *p*-hydroxybenzoate had been used successfully as a preservative in hospital practice for three years. He stated that 0.1% methyl *p*-hydroxybenzoate was "... effective against a wide range of bacteria tested, including *Ps.pyocyanea*, *Streptococcus pyogenes*, and *Staphylococcus aureus* at room temperature, in conjunction with the drugs commonly used and in normal clinical concentrations, in three hours." No experimental evidence was provided to support this statement and the definition of "effective" was not given.

Kohn *et al* (24) found that a mixture of 0.2% methyl paraben and 0.04% propyl paraben sterilized *Ps.aeruginosa* suspensions (2×10^6 /ml) in 3 hr. A mixture of 0.18% propyl paraben sterilized in 6 hr. These workers used 13 strains of *Ps.aeruginosa*, and the effectiveness of the inactivating agents in their recovery broth was established by a correlation between *in vivo* and *in vitro* results.

Anderson *et al* (30) tested 0.1% methyl hydroxybenzoate in six final eye drop preparations using *Staphylococcus pyogenes*, *Proteus vulgaris* and *Ps.aeruginosa* in concentrations of about 5×10^4 /ml. They found that sterility was achieved within one day in every case except two. The viable count increased during 11 days with *Ps.aeruginosa* in gelatin eye drops, and sterility was achieved after two days but not after one day

with *Pr.vulgaris* in glycerine eye lotion. More useful information might have been obtained if tests had been made at intervals less than daily.

Hugo and Foster (9) have shown that the rate of growth of one strain of *Ps.aeruginosa* was little affected by the presence of the concentration of esters used in Solution for Eye Drops B.P.C. (0.034%), and that they could be used as a sole carbon source at the same concentration. These workers, [Foster (31), Hugo and Foster (32)], found that a mixture of two parts methyl and one part propyl *p*hydroxybenzoate sterilized aqueous suspensions containing 10 and 100/ml *Ps.aeruginosa* cells of one strain in 30 minutes at 18° using concentrations of 0.125% and 0.2% respectively. They did not use an inactivator in the recovery medium.

Chlorocresol

*p*Chloro-*m*-cresol was the first official ophthalmic antibacterial preservative used in Britain. Its present replacement by Solution for Eye Drops in the B.P.C. has been discussed (4).

Davis (33) found that 0.05% chlorocresol was as effective against *Staph.aureus* as was 0.5% phenol. In each case, sterility was achieved within one day. Details of reaction menstrum and recovery conditions were not given.

Klein *et al* (27) used a broth dilution procedure and found that 0.1% chlorocresol sterilized broth suspensions (10^8 /ml) of three strains of *Ps.aeruginosa*. They also tested 0.1% and 0.03% chlorocresol in the presence of three eye drop preparations. In each case, sterility was achieved within 1 hr using 0.1%. 0.03% chlorocresol sterilized atropine drops within 4 hr, while fluorescein and eserine were sterilized between 6 and 24 hr. They found that 0.1% chlorocresol caused smarting. 0.05% chlorocresol sterilized inocula of 10/ml *Ps.aeruginosa* cells of one strain in aqueous suspensions at 18° within 30 min, but 0.07% was required to sterilize 100 cells/ml within the same time (32).

Crompton and Anderson (34) have reported that injections of 0.05% chlorocresol in normal saline into the anterior chamber of rabbits' eyes caused the cornea to become opaque. This disadvantage would seem not to apply in ophthalmic solutions preserved and used with intact eyes.

Richards (35) reported that chlorocresol was more active in terms of reduction of viable count in final eye drop preparations at acid pH, than it was in simple solution.

Chlorbutol

Klein *et al* (27) used a broth dilution test, and found that three strains of *Ps.aeruginosa* (10^8 /ml) were sterilized by 0.2% chlorbutol. They found that three eye drop preparations inoculated with 10^8 /ml *Ps.aeruginosa* were sterilized after 1 hour's contact with 0.5% chlorbutol. Recovery media did not contain inactivators. These workers found that heat significantly reduced the antibacterial activity of this compound. Chlorbutol is slowly soluble in water, and heat is often used. This phenomenon may account for the contamination of 0.25% chlorbutol preserved eye drops reported by Crompton (6).

All of 35 strains of *Ps.aeruginosa* failed to grow on subculture after 24 hours' contact with 0.5% chlorbutol (36). These workers measured the percentage hydrolysis of buffered 0.5% chlorbutanol solutions after autoclaving at 121°. There was about 5% hydrolysis after 15 min at 121° at pH less than 5. At about pH 6, and higher, the percentage rose considerably. They found that autoclaving at acid pH (up to pH 6) did not inactivate the antibacterial effect of chlorbutanol. Murphy *et al* (36) reported an absence of endothelial damage or other ill effect upon the tissues within the eye, despite repeated use of 0.5% chlorbutol lotions during anterior chamber washes. This preservative had been in continuous hospital use for twenty-nine years.

Lawrence (29) found that 0.5% chlorbutol sterilized heavy inocula of 26 strains of *Ps.aeruginosa* and four species of *Proteus* in simple buffer after contact times of up to two days. The bactericidal action was more rapid in the presence of non-buffered solutions of several drugs, and sterility of four strains of *Ps.aeruginosa* took up to 6 hr. Recovery media did not contain inactivators.

Riegelman *et al* (11) found that 0.5% chlorbutol apparently sterilized broth suspensions of *Ps.aeruginosa* in times which varied with the inactivating recovery broth. Negative *in vitro* tests were obtained between 8 and 24 hr using optimum recovery broth, and these were correlated with *in vivo* tests. Apparent sterilization occurred after 45 min when recovery was made in nutrient broth alone. These workers found that concentrations in excess of 0.5% were irritating to the eye.

Anderson and Stock (37) found that chlorbutol 0.5% in water sterilized three strains of *Ps.aeruginosa* (about 10^5 /ml) within 15 min, but was ineffective against one strain of *Staph.aureus* even after 1 hr. These workers recovered in nutrient broth without any inactivator.

Kohn *et al* (24) used 13 strains of *Ps.aeruginosa* and found that 0.5%

chlorbutol sterilized suspensions of about 2×10^6 cells/ml in 12 hr. These workers used inactivators in their recovery media and obtained good agreement between *in vivo* and *in vitro* experiments.

Organic mercurials

Phenylmercuric nitrate, phenylmercuric acetate and thiomersalate all sterilized three strains of *Ps.aeruginosa* (10^8 /ml) at a dilution of 1 in 50,000 in broth (27). It was found that 0.005% thiomersalate sterilized this organism in 2 hr in fluorescein drops but a contact of 6 hr was necessary to sterilize atropine and eserine drops. These sterilizing times must be viewed critically because the efficiency of the recovery media was apparently not demonstrated.

Lawrence (29) found that organic mercurials were less active in the presence of common ophthalmic drugs than in their absence. 0.01% P.M.N. and 0.01% thimerosal sterilized four strains of *Ps.aeruginosa* in several ophthalmic solutions in contact times varying up to 48 hr. In the absence of any drug, 0.01% P.M.N. sterilized in times varying up to 3 hr. Recovery was in Brewer's fluid thioglycolate medium.

Riegelman *et al* (11) showed that 0.01% P.M.N. produced apparent sterility after 1 hour's contact with *Ps.aeruginosa* (10^8 /ml) when sub-cultured into thioglycolate broth. However, corneal ulcers were produced by these apparently sterile suspensions. The use of lecithin-polysorbate 80-thioglycolate medium eliminated the discrepancy between *in vivo* and *in vitro* results, and the sterilizing time was in excess of a week.

0.01% P.M.N. failed to sterilize one strain of *Staph.aureus* and three strains of *Ps.aeruginosa* in contact times up to 1 hr using about 10^5 /ml cells (37).

Ridley (16) stated that 0.004% P.M.N. had been used successfully in hospital practice and had been shown to be effective against several pathogenic species but omitted to give experimental procedures.

In vitro tests using inactivating recovery media together with *in vivo* tests showed that P.M.N. 0.01% and thimerosal 0.02% sterilized *Ps.aeruginosa* (2×10^6 /ml) in aqueous suspensions in 6 hr. A two-fold dilution of these mercurials had little effect upon the sterilizing time (24).

Anderson *et al* (30) tested the activity of P.M.N. (0.001-0.004%) against *Staph.pyogenes*, *Proteus vulgaris* and *Ps.pyocyanea* (10^3 - 10^4 /ml) using 18 eye drop formulations involving 12 drugs. Sterility was achieved in less than one day in all cases excluding fluorescein. P.M.N. 0.002% did not sterilize fluorescein drops after several days contact. In the one formu-

lation using 0.004% P.M.N. in fluorescein drops, sterility was achieved within a day. These workers did not use contact times of less than a day and the recovery media did not contain inactivators.

Phenylethyl alcohol

Lilley and Brewer (38) showed that 2-phenylethyl alcohol was particularly active against gram-negative bacteria including *Pseudomonads*.

Klein *et al* (27) tested phenylethyl alcohol against *Ps.aeruginosa* in the presence of atropine, eserine and fluorescein. In each case 0.6% sterilized within 1 hr while 0.5% required contact of up to 3 hr. Inactivators were not used during recovery.

This preservative was rejected by Murphy *et al* (36) on the grounds that two strains of *Ps.aeruginosa* could be serially transferred in the presence of 0.5%, and four strains of *Staph.aureus* grew in the presence of 0.6% in broth.

Lawrence (29) tested the activity of 0.5% phenylethyl alcohol against 26 strains of *Ps.aeruginosa* and four species of *Proteus* using inocula of undiluted broth culture. Sterilization occurred after contact times of up to 6 days in the presence of a series of ophthalmic drugs. In the absence of drugs, sterilization occurred after longer contact periods and in some instances had not occurred by the end of the experimental period (6 days).

Corneal ulcers were produced from intracorneal injections of a *Ps.aeruginosa* contaminated solution of 2% phenylethyl alcohol after 8 hours' exposure (longest experimental period) (11). These workers found that 0.75% solutions were irritating to the eye.

Phenylethyl alcohol 0.6% failed to sterilize three strains of *Ps.aeruginosa* in aqueous suspension during exposures up to 1 hr, but sterilized one strain of *Staph.aureus* within 1 hr using cell concentrations of about 10^5 /ml (37).

Kohn *et al* (24) tested 0.5% phenylethyl alcohol against 13 strains of *Ps.aeruginosa* (2×10^6 /ml) using *Tweens* as inactivating agents in the recovery media. Sterilization was not effected after 24 hours' contact, and *in vivo* experiments confirmed these *in vitro* results.

Phenylethanol 0.9% sterilized inocula of 100 cells/ml of one strain of *Ps.aeruginosa* in aqueous suspension in 30 min at 18°. Inactivators were not used in the recovery medium (31, 32).

Quaternary ammonium compounds

There is much published evidence about the use of these compounds as ophthalmic preservatives. Klein *et al* (27) found that a concentration

of 0.05% benzalkonium was required to sterilize broth suspensions (10^8 /ml) of three strains of *Ps.aeruginosa*. Subcultures were taken after two days. These workers found that although 0.5% cetrimide sterilized, there was one instance when cetrimide was shown to have comparatively little effect against *Ps.aeruginosa*.

Benzalkonium chloride was found not to be a uniformly effective bactericide against *Ps.aeruginosa*. Five out of 30 strains were not sterilized by contact for 24 hours with 0.01% of the agent, at pH 7.4 (36).

Lawrence (39) in his review concluded that benzalkonium chloride, chlorbutanol and P.M.N. were the most suitable agents on the basis of published evidence. The same worker (29) tested benzalkonium chloride 0.02% and 0.01% against 26 strains of *Ps.aeruginosa*, and four species of *Proteus* using inocula of undiluted 24 hr broth cultures. Tests were made in (1) simple buffer, (2) in the presence of several ophthalmic drugs in buffer, and (3) in the presence of several drugs in aqueous solution. In every instance sterility was achieved in less than 30 min with 0.02% benzalkonium chloride. The majority of strains were sterilized by 0.01% in less than 30 min in all three systems but a few strains needed up to 6 hr. Chemical inactivators were used in the recovery broth.

These results conflicted with those of Riegelman *et al* (11) who found that benzalkonium chloride was only slowly bactericidal. They suggested that the discrepancy between their results, and those of earlier workers, was because of inadequate inactivation of benzalkonium chloride in the recovery broth used by other workers. They found that 0.01% benzalkonium chloride failed to sterilize 10^8 /ml *Ps.aeruginosa* cells in saline in one week when tested by *in vivo* methods whereas the use of nutrient broth subculture indicated sterility after five minutes contact. The use of a lecithin-*Tween* 80-thioglycolate medium resulted in concordant *in vivo* and *in vitro* tests. Riegelman *et al* (11) reported incidents when resistance to benzalkonium chloride by *Ps.aeruginosa* was demonstrated. In one case, active growth occurred and pigment was produced in the presence of un-neutralized 0.02% benzalkonium chloride from an inoculum of 1 ml of a 24 hr culture.

Kohn *et al* (24) found that benzalkonium chloride 0.02% sterilized 13 strains of *Ps.aeruginosa* (2×10^8 /ml) in aqueous suspension within 45 min, and that 0.01% required up to 9 hr. The effectiveness of the inactivators used in the *in vitro* recovery media was demonstrated by correlation with *in vivo* results. These workers found that of seven commonly used preservatives, only 0.02% benzalkonium chloride sterilized in less than 1 hr.

Inocula of 10 and 100/ml *Ps.aeruginosa* cells were sterilized in aqueous suspension at 18° in 30 min by benzalkonium chloride 0.05% and 0.08% respectively (32). Recovery was on media containing an inactivator.

Benzalkonium chloride has been accepted officially in the U.S.A. for many years but has not been widely used in Britain despite the large amount of favourable published work. There have been criticism against benzalkonium chloride on grounds other than bacteriological. Klein *et al* (27) stated "Quaternary ammonium compounds as a preservative for eye-drops should be used only in exceptional cases. Ginsburg and Robson found that detergents could prove harmful by causing 'solubilization' of the intercellular cement of the corneal epithelium." This reference to the work of Ginsburg and Robson (40) has been quoted by others (41). Recently, Anderson *et al* (30) have also quoted this work as evidence that benzalkonium chloride dissolves the intercellular cement substance of the cornea. Ginsburg and Robson (40) did not use a quaternary ammonium compound but worked with anionic and nonionic wetting agents. These workers found that the anionic detergent, dodecyl sodium sulphate (1%) damaged the cornea. They also found that although 1% nonionic *Lissapol N* caused slight temporary oedema, 0.5% caused no observable effect. These results are in accord with those of Buschke (42) who made direct measurements on intercellular cohesion in corneal epithelium. He found that six out of seven anionic detergents " . . . produced marked effects in concentrations of 1% or sometimes even in lower concentrations." Not one of a wide selection of nonionic or cationic detergents caused loss of intercellular cohesion. Benzalkonium chloride was used in concentrations up to 10%.

Recently, Anderson *et al* (43) have stated " . . . Swan has shown that wetting agents such as benzalkonium chloride are injurious to the corneal epithelium and especially to the endothelium. . . ." Swan (44) found that intraocular concentrations of benzalkonium chloride in excess of 0.01% caused serious damage. He also found that instillation of 0.1% benzalkonium chloride produced a severe reaction, and that 0.03% to 0.04% used over periods of up to 8 weeks produced a less severe reaction. In most cases the conjunctiva and cornea were normal within 12 hr. Nevertheless, Swan concluded, "The minimal concentrations of wetting agents producing irritation in the conjunctival sac are greater than those that are effective."

Hughson and Styron (45) replaced the aqueous humour of rabbits' eyes with benzalkonium chloride in saline, and found that concentrations of

1 in 3000 and 1 in 6000 produced endothelial oedema which disappeared in 6 to 8 weeks. The use of a concentration of 1 in 7500 gave no visible evidence of damage. Bell (46) used a 1 in 10,000 concentration of a quaternary ammonium agent as a routine irrigant in eye surgery for three years, and observed no hypersensitivity or allergic reaction, nor was there any evidence of irritation. Lawrence (39) has quoted numerous other workers with similar experiences using quaternary ammonium compounds.

Polymyxin

Wiggins (47) found that 85 strains of *Ps.aeruginosa* were sensitive to polymyxin B sulphate. He found that the minimum inhibitory concentration at 37° in broth lay between 0.08 to 2.5 µg/ml.

Antibiograms for 152 strains of *Ps.aeruginosa* showed that while there was marked resistance to several antibiotics, all strains were sensitive to polymyxin B (3).

Riegelman *et al* (11) found that 1000 units/ml polymyxin B sulphate sterilized *Ps.aeruginosa* (10^8 /ml) in aqueous suspension within 30 min. Lecithin was found to be an effective inactivator for polymyxin. *In vivo* tests confirmed the *in vitro* results.

Polymyxin B sulphate was found to be only slowly bactericidal in aqueous solution against 13 strains of *Ps.aeruginosa* (2×10^6 /ml). Sterility was achieved in 12 hr using 2000 units/ml, and in 18 hr using 1000 units/ml. The results of *in vivo* experiments were in agreement with results of recovery using lecithin broth (25).

Agents not widely used as preservatives

Anderson and Stock (37) showed that chlorhexidine acetate 0.01% in aqueous solution sterilized three strains of *Ps.aeruginosa* within 15 min, and one strain of *Staph.aureus* within 30 min using cell concentrations of about 10^5 /ml. Egg yolk medium was used to inactivate the chlorhexidine on recovery. Much useful work on formulation of ophthalmic solutions with chlorhexidine has been done in Australia (48,49), and it has been included in the Australian Pharmaceutical Formulary. No instance of sensitivity had been detected after several years' hospital use of chlorhexidine preserved drops (6). Crompton (6) has stated that "... Chlorhexidine at present is the bacteriostatic of choice." He quoted evidence of the personal communications of two workers to support this belief but omitted to give sufficient detail to allow evaluation of experimental methods. Anderson *et al* (30) tested chlorhexidine against single strains of *Staph.*

pyogenes, *Proteus vulgaris* and *Ps.aeruginosa* using inocula of about 10^3 – 10^4 cells/ml. Tests were made at 28° in the presence of numerous ophthalmic drugs in 75 formulations. Counts were made of recovered cells, and the preservative was regarded as satisfactory if the count remained static or was reduced. In most instances sterility was achieved within the shortest test period of one day. Increased counts occurred with both serum and methylcellulose formulations in the presence of 0.005% chlorhexidine. Growth occurred in the presence of lignocaine in one out of two formulations. It was suggested that growth in the presence of pilocarpine was due to incompatibility with the 0.005% chlorhexidine. Chlorhexidine is not in fact used in the Australian Pharmaceutical Formulary for eye drops containing pilocarpine (50). The experimental procedures of these workers are open to question. They did not use conventional inactivators in their recovery medium but attempted to inactivate the agents tested, including chlorhexidine, using a washing procedure, the efficiency of which apparently was not demonstrated. They recovered chemically treated *Pr.vulgaris* cells in medium containing 0.1% phenol which might well reduce recovery as well as swarming. One other unusual aspect of their experimental procedure was an 18 hr recovery incubation period. Without evidence to the contrary, this would seem to be a short time for the recovery of chemically treated organisms. Hugo and Foster (32) found that inocula of *Ps.aeruginosa* (100/ml) were sterilized in aqueous suspension at 18° by 0.005% chlorhexidine, and at 30° by 0.002%.

Kohn *et al* (25) tested 51 chemicals not previously employed widely in ophthalmic solutions as preservatives. Thirteen strains of *Ps.aeruginosa* (2×10^6 /ml) were used, and *in vitro* and *in vivo* methods employed. Six out of 37 quaternary ammonium compounds were found to possess equal or superior activity to benzalkonium chloride which they used as a standard. All of eight amphoteric surfactants were found to be inadequate while three iodophors were found to possess sterilizing times of less than 1 hr. Chlorhexidine and Colistin were each found to sterilize in less than 1 hr.

Richards (35) found that chlorhexidine was less active in final eye drop preparations at acid pH than it was in simple solution.

Wiseman (51) attempted to increase the resistance of several gram-positive and gram-negative species to chlorhexidine by training in its presence. He failed to increase the resistance of gram-positive organisms but found that gram-negative species showed a substantial increase in resistance. In particular the minimum inhibitory concentration against

Ps.aeruginosa increased progressively from an initial 4 $\mu\text{g/ml}$ to 64 $\mu\text{g/ml}$ after 8 subcultures in the presence of chlorhexidine diacetate.

Bronopol (2-bromo-2-nitropropane-1, 3-diol) is a new antimicrobial agent shown to be active against numerous strains of several gram-positive and gram-negative species including 22 strains of *Ps.aeruginosa* (52). Preliminary experiments showed that bactericidal concentrations are not irritant to the skin or mucous membranes. The authors failed to increase the resistance of *Ps.aeruginosa* and *Staph.aureus* to Bronopol by repeated subculture in its presence.

SUMMARY AND CONCLUSIONS

Consideration of the available published evidence reveals that at present there is no single ideal preservative for ophthalmic preparations. The capacity of strains of *Ps.aeruginosa* or closely related species to become resistant to quaternary ammonium compounds (11), chlorbutol (39), phenylethyl alcohol (36) chlorhexidine (51) and even to decompose phenol (53) and *p*hydroxy-benzoates (9) would suggest caution in accepting any new agent supposed not to have this defect against this enigmatic organism.

Many of the results in the literature are conflicting. Some of the discrepancies may be accounted for by reason of variation in such important factors as reaction temperature, composition of reaction mixture, and recovery medium. Importance must be attached to the results of those workers who have clearly defined their experimental conditions, and who have related the efficiency of the inactivators used in their recovery media to the results of *in vivo* tests. The capacity or not to cause disease would appear to be the ultimate, objective assessment, particularly when activity is measured in a final ophthalmic formulation.

An appraisal of the scientifically acceptable data on the *p*hydroxy-benzoates would suggest that nearly saturated solutions are capable of sterilizing heavy inocula (10^8) of *Ps.aeruginosa* in several hours. Such concentrations are too irritant for normal ophthalmic use. A concentration of about 0.1% mixture may be useful in some eye preparations excluding, in particular, fluorescein. The capacity of *Ps.aeruginosa* to utilise (9) these compounds as a source of carbon would seem to preclude their recommendation for widespread use.

The available evidence suggests that the concentration used in Solution for Eye Drops B.P.C. (0.0229% methyl, 0.0114% propyl) is unlikely to sterilize, and may possibly allow growth of *Ps.aeruginosa*.

The small amount of available evidence supports the use of chlorocresol 0.05% for use with intact, but not damaged eyes.

The considerable volume of work on chlorbutol has shown that almost all workers have found it to be consistently an effective, but relatively slow, sterilizing agent. It would seem that formulation aspects of this agent have been adequately investigated (36,54).

The mercurials have been shown to be effective sterilizing agents by nearly all workers. Riegelman *et al* (11) found 0.01% P.M.N. required longer than a week to sterilize a heavy inoculum of *Ps.aeruginosa*, but Kohn *et al* (24), using several strains and similar procedures to those of Riegelman and his co-workers, found that sterilization was effected in 6 hr.

It has been asserted that these mercurials may give rise to mercurial sensitization ("mercurialentis"), and that mercury may deposit on the lens capsule (6,26). There is little published evidence to support these assertions. Ridley (16) found very few cases of dermatitis medicamentosa with P.M.N. preservation. These few cases were with drops used for a long period of time, and in every case the reaction was slight. Work is in progress in our laboratory to elucidate this situation. Rabbits' eyes have been irrigated with mercurial solution, subsequently sectioned, and examined with an electron probe microanalyser. It is hoped that these procedures will provide useful information about local concentrations of mercury.

The available evidence offers little support for the use of phenylethyl alcohol as an ophthalmic preservative. The existence of resistant *Ps.aeruginosa* strains would not appear to be the result of inadvertent training due to faulty procedures.

The evidence about quaternary ammonium compounds is clouded by numerous quotations of opinions, assertions unsupported by data, and even errors in quoting the literature. A literature search has revealed very few instances where resistance to benzalkonium chloride has been demonstrated. Murphy *et al* (36) and Riegelman *et al* (11) have shown the existence of highly resistant strains of *Ps.aeruginosa*. On the other hand, it has been shown that faulty procedures may enable contaminants to acquire resistance, particularly to quaternary ammonium compounds (55).

The available evidence supports the conclusion of Riegelman and Vaughan (1) "with all its limitations, benzalkonium chloride is among the most effective and rapidly acting preservatives when the conditions of its use are properly controlled."

There is no evidence of resistance by *Ps.aeruginosa* to polymyxin B sulphate. This antibiotic is limited by its relatively low activity against gram-positive organisms, and some species of *Proteus* (47). The U.S.P. XI suggests the use of a combination of 1000 units/ml polymyxin B sulphate and 0.01% benzalkonium chloride.

The newer recommended ophthalmic preservatives include several promising compounds, nearly all of which require more work before their acceptance. Chlorhexidine is an exception in that it has been introduced into the Australian Pharmaceutical Formulary, and experience in practice has been gained. Nevertheless there are problems of compatibility with this compound, and it is not uniformly satisfactory with all ophthalmic solutions (50).

When suitably formulated, the following compounds have reasonable scientific support for their use as ophthalmic preservatives in some instances: 0.05% Chlorocresol, 0.5% chlorbutol, 0.01% benzalkonium chloride, 0.005% chlorhexidine. Experimental evidence is required about mercury deposition on the lens before the mercurials may be used with confidence.

Little attention has been paid to the use of combinations of agents. The recommendation of a combination of polymyxin B sulphate and benzalkonium chloride by U.S.N.F. XI would seem to be based on attaining broad spectrum activity rather than an expectation of synergism against any particular organism. Attention has been drawn to the dangers of indiscriminately combining antimicrobial agents (56,57). Preliminary work has shown synergism between phenylethyl alcohol and the organic mercurials (58).

It would appear that the future lies more in the understanding of the nature of the resistance of *Ps.aeruginosa* to chemical inactivation than in the discovering of other new agents. The literature possesses several monuments to the capacity of this beast, literally to eat the agents used against it. The acquired resistance to a quaternary ammonium compound has been eliminated in the presence of E.D.T.A. (59), and the U.S.N.F. XI has suggested its use for that purpose. It has been shown that cells grown in the presence of *Tween 80* are much less resistant to the action of several agents than are cells grown in plain broth (60,61). It has been suggested that the resistance of *Ps.aeruginosa* is connected with its slime production and its membrane permeability, both of which may be affected by *Tween 80*.

The problems of sterilization and preservation have for too long been

overshadowed in pharmacy curricula by consideration of tonicity, and the attendant calculations now shown to be largely unnecessary. The time would seem appropriate, and even overdue, to adjust long held ideas relating to ophthalmic formulations.

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Introduction by Dr. M. R. W. Brown

I would like to draw attention very briefly to some of the more important elements. The ideal preservative should be effective against a wide range of organisms, must not be irritant, or in any way harmful to eye tissues, must be compatible with other medicaments and should withstand sterilization, preferably by a heat method, without production of harmful degradation products. Furthermore, careful distinction must be made between materials used for continuous application to intact eyes, and the solutions used on damaged eyes or during surgery. The latter solution should be sterile and should not contain preservatives.

Since this paper was submitted, we have been made aware of the recent work of Abrams (62) related to mercurialentis. He found that mercurialentis was slow to develop and no instance was found in glaucoma patients using myotics containing P.M.N. for less than three years. He furthermore stated, "as far as is known the appearance (mercurialentis) is not associated with any visual impairment and seems to be quite innocuous." There is scientific evidence to support the use of the following preservatives in appropriate instances: Benzalkonium chloride, phenylmercuric nitrate, thiomersal, chlorbutol, chlorocresol. At present there appears to be no scientifically based evidence to completely preclude their use under the controlled conditions of an ophthalmic formulation. Reports from Australian workers indicate decomposition of chlorhexidine on autoclaving, and the production of degradation products the freedom of which from toxicity has not been fully established and this would suggest caution regarding heat sterilization of preparations containing this compound.

The employment of these substances must be subject to normal considerations of formulation and compatibility and, of course, to correct clinical usage. There

(62) Abrams, J. D. *Trans. Ophthalm. Soc.* **83** 263 (1963).

is little evidence so far to confirm effectiveness of combinations of preservatives, and there are dangers in indiscriminately combining antimicrobial agents. There is no simple answer to the problem of preservation and each eye drop formulation must be considered individually.

In conclusion I would like to draw your attention to a reference which may possibly be misleading. In p. 003 reference (6) is mentioned and refers to the work of Roemer, cited by Crompton, regarding pathogenicity of *Bacillus subtilis*. It is in fact stated correctly as a citation in the reference. However, in p. 016, reference (6) was used in connection with mercurialentis and it was intended to refer directly to Crompton's paper. (Our attention to this was kindly drawn by Professor Neuwald.)

DISCUSSION

PROF. DR. F. NEUWALD: We have heard from Mrs. Wedderburn that every drug and every formulation needs special preservation. This applies especially to eye drops, and this is illustrated by *Table I* (63).

Table I

Eye-drop medicament	Buffer solution	Preservative
Ethylmorphine hydrochloride	P 5.3	Bz 0.02%
Silver diacetyltannin protein	B 6.8	Ph 0.002%
Silver protein	B 6.8	Ph 0.002%
Atropine sulphate	P 6.45	Bz 0.02%
2-Benzylimidazoline hydrochloride	P 6.85	HB 0.1%
Calcium chloride	B 6.8	Ph 0.002%
Carbamide	B 6.8	Ph 0.002%
Cinchocaine hydrochloride	P 6.05	Bz 0.02%
Cocaine hydrochloride	P 6.05	Bz 0.02%
Ephedrine hydrochloride	P 6.05	Bz 0.02%
Homatropine hydrobromide	P 6.45	Bz 0.02%
Mercuric oxycyanide	P 6.85	—
Potassium iodide	P 6.85	HB 0.1%
2-(Naphthyl-1-methyl)imidazoline nitrate	P 6.85	HB 0.1%
Sodium iodide	P 6.85	HB 0.1%
Sodium salicylate	B 6.8	Ph 0.002%
Physostigmine salicylate	P 6.05	HB 0.1%
Pilocarpine hydrochloride	P 6.85	Bz 0.02%
Procaine hydrochloride	P 6.05	Bz 0.02%
Resorcinol	P 6.05	Bz 0.02%
Scopolamine hydrobromide	P 6.45	Bz 0.02%
Adrenaline acid tartrate solution	B 5	Ph 0.002%
Tetracaine hydrochloride	P 5.3	Bz 0.02%
Zinc sulphate	B 6.3	Ph 0.002%

Abbreviations

B 5	Boric acid solution pH 5	P 6.85	Phosphate buffer solution pH 6.85
B 6.3	Borate buffer solution pH 6.3	Bz	Benzalkonium chloride
B 6.8	Borate buffer solution pH 6.8	Ph	Phenylmercuric acetate
P 5.3	Phosphate buffer solution pH 5.3	HB	Mixture of two parts of methyl
P 6.05	Phosphate buffer solution pH 6.05		hydroxybenzoate and one part
P 6.45	Phosphate buffer solution pH 6.45		of propyl hydroxybenzoate

DR. BROWN: Thank you. It is interesting to see that they are regarding each formulation as a separate entity rather than hoping that one preservative can be used for everything.

(63) *Österreichisches Arzneibuch* 9 (1960).

DR. E. E. BOEHM : It has recently been pointed out by several authors (64-65) that a *phydroxybenzoate* concentration of 0.0343%, as recommended in the B.P.C. for eye drops preservation is insufficient. It has already been pointed out by Williams *et al* (65), that the addition of at least 0.08% of *phydroxybenzoates* is necessary for the successful inhibition of microbial growth in eye drops. They further pointed out that *phydroxybenzoate* concentrations of the order as recommended by the B.P.C. were ineffective against less resistant micro-organisms and therefore it could hardly be expected that at these low concentrations of the *phydroxybenzoates* they would be effective against a much more resistant organism such as *Pseudomonas aeruginosa*, which is also resistant against benzethonium chloride (67).

We have also shown that the addition of 0.06% *Nipasteril*, another *phydroxybenzoate* combination, does not only inhibit the growth of an especially resistant strain of *Pseudomonas aeruginosa* in a buffered aqueous solution, but also killed the organism between 5 and 24 hr.

Recently Müller (68), on the basis of his own experience, recommended the addition of 0.07% methyl and 0.03% propyl *phydroxybenzoates* to eye drops, and Montgomery *et al* (64) the addition of 0.063% methyl and 0.023% propyl *phydroxybenzoates*.

DR. BROWN : I think you will find in the paper that we agree that the concentration recommended in the B.P.C. is not likely to produce sterility with such organisms as *Pseudomonas aeruginosa*. However, I personally think that this field has been cluttered up with all sorts of opinions and assertions. One can pick almost any chemical and it is possible to find three or four references to workers recommending it. If, however, one critically evaluates the experimental procedures the recommendation often lacks weight. We have to give particular emphasis to those workers who have precisely stated their experimental conditions, have stated how many cells they used, what the medium was, recovery conditions and have correlated the efficiency of their inactivating agents with *in vivo* tests. Few people have done this. Riegelman *et al* (1) in the U.S.A. have done this. Their work was followed by Kohn *et al* with two excellent papers (24,25). I am unable to comment on your reference to the experience of Müller because I have not had an opportunity to see this reference. I have seen the reference by Montgomery and Halsall (64), and although they recommend the hydroxybenzoates they give no experimental details about their findings. I am not criticising them for omitting details because their views were merely expressed in a short letter.

MR. J. E. JEFFRIES : In your summary you state that at present there is no single ideal preservative for ophthalmic preparations and in particular that strains of *Pseudomonas aeruginosa* or closely related types become resistant to all known preservatives. Referring to the *Achromabacter* species, there would again appear to be no effective preservative and in any case differentiation between the two types is very difficult. Would you please indicate if, in fact, these organisms are very thermolabile, since I believe their optimum growth temperature is of the order of

(64) Montgomery, M. F. and K. G. Halsall, *Pharmaceut. J.* **192** 407 (1964).

(65) Sabalitschka, Th., *Pharmaz. Ztg.* **108** 1723 (1963).

(66) Williams, R. and Boehm, E. E. *Lancet* **2** 790 (1963).

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(68) Müller, F. *Pharmaz. Praxis* **71** (1964).

25°C whilst at 37° they can be killed off, certainly in the presence of preservatives such as the esters of *ph*hydroxybenzoic acid at normal concentration after not more than two days' incubation. This might possibly be a method of getting out of difficulty if one had a batch of material that was subsequently shown to be contaminated heavily with *Achromobacter*, although it would of course suffer from the serious drawback that the products of metabolism would still be present in the finished product.

DR. BROWN: I would just like to make one slight correction. In our paper we did not state that strains of *Pseudomonas aeruginosa* become resistant to all known preservatives. People from several quarters have been saying that compound X is the thing. Exaggerated claims have been made for chlorhexidine. What we state above (p. 015) is rather different from claiming that *Pseudomonas* can become resistant to all known preservatives. The point we are making here is that we should be cautious in accepting any new chemical that is heralded as a new wonder preservative. I do not believe there are any. I am puzzled about your second point. In page 003 of our paper we mention *Pseudomonas*, we mention *Aerobacter subtilis* and *Clostridium*. We do not, in fact, mention *Achromobacter*. Do you in fact mean *Achromobacter*?

MR. J. E. JEFFRIES: Yes, I do mean *Achromobacter*. Surely this is a fairly common contaminant of water used in the production area and it is in fact extremely difficult to get rid of once you have it there. It appears particularly on columns from demineralized water.

DR. BROWN: I must confess I am not an expert in this field and as far as I know *Achromobacter* is not pathogenic, and is not particularly thermolabile. I looked it up in Bergley and the original type species is reported at 20° to 25° optimum growth. Unfortunately this was a very early work and certain important tests that now seem to be necessary were not carried out. There are many other species listed where the optimum growth temperature is more normal. As far as the heat resistance is concerned, I do not think it is particularly heat labile. You may have come across some odd strains that are, but I certainly have not.

DR. O. D. PRIDDLE: What, in your opinion, is a good preservative for fluorescein sodium solutions? Fluorescein, as a high molecular weight anion, should react with a cation such as benzalkonium and the phenylmercurate ion.

DR. BROWN: Mr. Norton and I agree that the ideal would be a sterile single dose unit for fluorescein sodium, which is mainly used as a diagnostic agent. I would also like to make the point that although I have in fact worked at one eye hospital I am informed that because fluorescein is used frequently in eye hospitals, it is quite common to have a bottle of fluorescein used for months and I suspect that because of this there have been so many reports of fluorescein contaminated by *Pseudomonas*. I suspect that if it were customary for cups of tea to be left around for months there would be numerous reports of tea being a marvellous medium for *Pseudomonas*. I think that the problem could be eliminated in this particular case where it has a specific use as a diagnostic agent by using sterile, single unit drops.

The Australian formulary which has just been published, uses 0.004% P.M.N. with fluorescein, and the B.P.C. of course used 0.002% P.M.N. It would appear

that they should react. I am not sure that anybody has shown whether in fact it does happen at this concentration.

DR. O. D. PRIDDLE : Can you make any comments on the physical stability of comparatively low molecular weight preservatives such as chlorobutanol or phenyl-ethanol in solutions contained in polyethylene or other plastic bottles ?

DR. BROWN : It seems very likely that there is absorption of some sort between plastics and phenols and as far as we know, not that we know very much, very little is published on this. I think it is very important and we, at Bristol, are certainly coming to the conclusion that we should be progressing towards mass produced, small volume eye drop preparations, rather than extemporaneous preparations as the norm. Information about the effect of these low molecular weight preservatives in the presence of plastics would be useful.

MR. M. J. GROVES : We were interested to note that you refer to *Bronopol* under the heading of agents not widely used as preservatives, the inference being that it could possibly be considered as a preservative for eye drops. We are also interested in this but we would like to sound a note of warning since *Bronopol* is not stable at an alkaline pH. We have examined the eye drops officially formulated in the British Pharmaceutical Codex. A number of these are alkaloidal solutions and hence acidic in reaction. But of the 20 eye drops in the Codex, 10 would appear to be either directly incompatible with *Bronopol* or contraindicated because of the pH. The use of *Bronopol* as a preservative is therefore somewhat limited and in addition it would be necessary to establish beyond reasonable doubt, that this new antibacterial, and I emphasize the word new or untried, is completely safe to use in the eye. Some work in our own laboratories which has been undertaken on the effect of *Bronopol* in rabbit eyes had given encouraging results so far, but there is limited information at present on the effects on human eye. We can only reiterate Dr. Harold Davis' comment at the recent British Pharmaceutical Conference, to the effect that reliable information about safety must be obtained under the conditions in which the materials can be used.

DR. BROWN : I agree and I would like to point out that *Bronopol* is mentioned under the heading of agents not widely used as preservatives. In the summary and conclusions we have not made any reference to it at all. We have ignored it, because more work will clearly have to be done on it, as you have suggested.

MISS B. CROSHAW : There is a further word of caution I would like to add regarding *Bronopol*. Although we have shown that it is bacteriostatic against both gram-negative and gram-positive bacteria at similar concentrations, the bactericidal activity is much greater against gram-negative organisms than against gram-positive. For this reason we would not recommend the use of *Bronopol* alone as a satisfactory preservative for ophthalmic solutions. Although you do not like combinations, we would only consider it in combination with benzalkonium chloride or something similar. I do not think we should be arguing whether *Achromobacter* is or is not pathogenic to eyes. I think any organism in an eye drop must be regarded as a potential pathogen, and a preservative that does not deal with it can not really be considered as satisfactory.

Concerning the use of chlorhexidine and its breakdown on heating : Are the breakdown products bacteriologically active?

DR. BROWN : I agree completely with the first part of your remarks. I would just like to comment on your remark that we do not like combinations, as I do not think this is correct. We have stated that they should not be used indiscriminately. A great deal of work has been done on synergism, antagonism, and additive action. It was originally thought that if you had one antibiotic that killed cells in the body and another one that did the same, then obviously the two together would be twice as good. This, of course, was not true and we were merely referring to the fact that they should not be combined indiscriminately.

I agree that any organism which is happily surviving in the presence of the preservative, should be regarded as potentially dangerous. I only made the remark because I thought that Mr. Jeffries was perhaps referring to *Aerobacta*, and not *Achromobacter*.

We were rather timid about our reference to breakdown of chlorhexidine because the literature contains too many personal communications and statements of opinion. Nevertheless we have it on good authority that heating chlorhexidine in solution and autoclaving it, did in fact produce compounds which could well be toxic and that this would have to be examined before using this method. This would not preclude the use of chlorhexidine in a filtered solution, or sterilizing in some other way. I do not know whether these degradation products are harmful to the bacteria.

MR. A. G. HOPKINS : At the present time quaternary ammonium compounds are becoming suspect as causing indurated ulcers, especially in damaged tissue. Does this not rule out substances like benzalkonium chloride, cetrimide, etc.?

DR. BROWN : We have heard about many defects of the quaternary ammonium compounds, particularly benzalkonium chloride. It has been stated, for example, that it dissolves the intercellular cement of the cornea. I have never heard of this particular problem but I think perhaps the problem is eliminated if, when using the damaged eye, no preservative at all is used unless it is absolutely necessary. Certainly during an operation when you are irrigating, say, the anterior chamber, you should use sterile saline or sterile water, or whatever it is that is required, without any preservative. What real evidence there is about this type of ulcer I do not know. Is it more than a report?

MR. A. G. HOPKINS : It is quite factual and there have been some reports in the last two or three weeks on this. In fact the use of Dequadin, Dequalinium chloride and even cetrimide is being curtailed because of these reports. I do not know whether anyone else has heard anything about this.

DR. BROWN : In America benzalkonium chloride has been used for a good number of years as a preservative of choice, and has been the official preservative. There are a good number of hospitals in America and I should have thought that benzalkonium chloride had been exposed to sufficient possibilities causing problems, to have really stood the test. We have only been able to find about two cases where there was proof that a resistant strain to benzalkonium chloride had arisen. I think it is easy, if you abuse the techniques, to produce resistant strains to benzalkonium chloride.

MR. J. A. MYERS : Are you now in a position to suggest a suitable preservative for any of the eye drops in the British Pharmaceutical Codex?

DR. BROWN : We would like to suggest, and I am not sure, that the B.P.C.

Committee agrees, that each preparation be regarded in its own right and to eliminate the attitude of "which is the best preservative?" Not to say, "we will use chlorhexidine, or we will use *p*hydroxybenzoates," but to look at each particular compound, and at each formulation, because each formulation could be different and could involve the necessity of using a different kind of preservative. It is therefore advisable to have in one's armoury as many preservatives as possible and not to eliminate any unless one has to. I think that this is the approach that will be taken in England from now on, i.e. that we will look at each preparation and consider it on its own merits. This, as Professor Neuwald has mentioned, occurs in Austria.

MR. W. TRILLWOOD : It seems to me that we are very little further forward than we were 20 years ago. We do not have a suitable container, we have one or two eye drops that are available as single dose containers, if we can call it that. I think we have surely reached the stage when, for surgical theatre work on damaged eyes, it must be a single dose container and yet we are limited to perhaps five drugs if we do this. We then turn to the things which are available commercially; none of these, as far as I know, are available in a single drop container. The point has been made about the improper use in hospital of injections by nurses. I wonder if it is generally realized what kind of a moving population we have to deal with in hospitals. The nurses are moving all the time they are in training. We have moving populations of medical staff, too. And whatever drill one lays down today is obsolete tomorrow unless people are continuously watched. With eye drops it is even worse. They are left in the hands of patients. They are liable to touch the margin which may be contaminated, and after the first drop is used, the dropper is put back again and the contents are contaminated. These are the practical problems we are up against in hospitals. For damaged eyes one must suggest single drop containers as far as possible, and the smallest possible container.

DR. BROWN : I believe there are single dose units on the market, and I believe that a B.S.S. for multidose containers is on its way. Mr. Norton has details of it.

MR. F. BROPHY : I confirm that there are already a small number of 2 ml single dose phials available on the British market, which are completely sterile. A balanced salt solution, which is completely sterile, is also shortly to be available here.

MR. D. A. NORTON : A committee of the British Standards Institution has been investigating pharmaceutical containers for the last two years or so, and one of the subcommittees has been dealing with the multidose eye drop container. We recognize, I think, that the need for extemporaneously prepared preparations will go on for some time and this standard recognizes it. The main aim of the specification is to produce a bottle which will withstand autoclaving when properly sealed and with the medicaments contained therein. This was the requirement for autoclaving laid down some time ago in earlier papers. I hope that this specification will be published quite shortly, and I understand that almost final agreement has been reached on this standard.

DR. HARRIS : The question concerning corneal ulceration following quaternary ammonium compounds is, I think, one of concentration. Some work has been published on this. Some work was done in Australia where I believe it was shown fairly clearly that the concentration normally used to preserve eye drops does not really give any trouble, not even over long periods.