ON THE MAINTENANCE OF STERILITY IN EYE-DROPS

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THE use of preservatives in eye drops and ophthalmic solutions is not new, and *p*-hydroxybenzoic esters have been advocated to prevent contamination (for review of earlier references see Klein[1]), replacing chlorocresol (0·03 per cent.), which was included in the earlier editions of the National Formulary. The preservatives were added to eye solutions mainly to prevent the growth of moulds, and laboratory tests were carried out mostly with this object in view.

Recently, however, severe eye infections have been caused with eyedrops contaminated with *Pseudomanas pyocyanea* (*Ps. aeruginosa*). McCullogh[2] reported 18 cases of pyocyanea infection, 5 of which could be traced to contaminated eye drops. On testing the eye drops McCullogh found that almost any commonly used drops could become contaminated with *Ps. pyocyanea*, but fluorescein and eserine bottles collected from the hospital wards were almost always contaminated. In Bignell's[3] series of severe pyocyanea keratitis, most of the cases were due to contaminated penicillin solutions instilled into the eye after superficial injuries. Other authors traced the source of pyocyanea infection to distilled water used in the theatre after an operation, or to the water used to make up solutions, and several cases were reported by different speakers at the meeting of the Ophthalmological Society of the United Kingdom in 1953. Rintelen[4] reported 4 cases of fulminating endophthalmitis due to contaminated tannic silver proteinate solution used after cataract operations. *Escherichia coli* and nonhæmolytic streptococci were isolated from the distilled water used in the preparation of the solution. This was surprising because solutions of silver salts are usually regarded as being bactericidal. Several cases of pyocyanea contamination of cortisone have occurred in this country, and Theodore[5] gave instances of commercial drops having to be withdrawn from sale because of pyocyanea infection.

Soet in 1952 (quoted by King[6]) reported the loss of eyes of several workers in a factory, through pyocyanea infection caused by contaminated eye-drops used in first-aid posts.

Theodore and Feinstein[7] drew attention to the danger of contamination where hospital pharmacists prepare large stocks, and recommend a careful method for the preparation and handling of the solutions.

Other contaminations have also been found, such as *Proteus* in methylcellulose, and Thygesson in 1949 (quoted by Theodore) reported virus infections which were transmitted by eye-drops.

The National Formulary generally follows the line of the British Pharmaceutical Codex regarding the use of bacteriostatic agents, using Liquor pro Guttis B.P.C., as the general solvent for eye-drops, the formula of this being 0·023 per cent. of methyl hydroxybenzoate and 0·011 per cent.

of propyl hydroxybenzoate in freshly boiled and cooled distilled water. It is interesting to observe that the official fluorescein eye-drops are not required to contain any bacteriostatic or germicidal agent whatsoever. The vehicle consists simply of a solution of sodium chloride in sterilised distilled water. The eye lotions in the National Formulary contain no preservative.

The British Pharmacopœia, the British Pharmaceutical Codex and National Formulary require that aseptic precautions shall be observed in the manufacture and dispensing of ophthalmic preparations. Although sterility in any preparation used for treatment is essential, it is difficult to maintain and the dispensing of sterile solutions is not a sufficient safeguard, as the possibility of contamination from aerial sources or from direct contact exists as soon as a bottle is opened. If the eye-drops used in a factory first-aid post or treatment room, or an outpatient department, become contaminated the consequences are especially grave, and several patients may be affected before the trouble is located. There should be adequate safeguards to ensure the continued sterility of all solutions used in the diagnosis and treatment of eye conditions.

To keep solutions free from contamination different methods have been suggested, Morris and Truhlsen[8], and others advocated heat sterilisation for drops used in the theatre before and after operations. The potency of the drugs can, however, be affected by heating, and the solution may soon become contaminated once the bottle has been opened. For this reason Haffley and Jensen[9] suggested rubber-capped vaccine vials, the solutions being drawn up by means of a sterilised syringe. This method, however, has limited use. Chemical preservatives such as chlorocresol, chlorbutol and *p*-hydroxybenzoates have been used, but their effectiveness against *Ps. pyocyanea* needs to be verified. Quaternary ammonium compounds were recommended by Hughson and Styron[10], Kedvessy, De Grosz and Szepes,[11] Macpherson and Wood[12] and others. Mostly benzalkonium chloride was used in concentrations varying from 1 in 5000 to 1 in 20,000. Mercurial preparations thiomersalate 1 in 20,000 and metaphen 1 in 7,000 were tried by McCullogh[2].

Comparative Efficiency of Various Antiseptics and Preservatives

In the present work we tested the ability of a number of substances to maintain the sterility of a solution against infection by *Ps. pyocyanea* (*Ps. aeruginosa*). 3 strains were used—N.C.T.C. 7244, N.C.T.C. 5083, and one isolated from a human source that had been used to produce experimental corneal ulcers in rabbits (Klein and Millwood[13]). Testing for bactericidal effect in distilled water was found to be impracticable because no visible difference existed in tubes containing viable organisms compared with tubes in which the organisms had been killed by the test substance. Therefore it was decided to make up the solutions in a simple nutrient medium, and Needham's broth was chosen, the infection being carried out under standardised conditions. A range of dilutions of each substance was made in order to discover the concentration required to prevent infection of the solution when inoculated with 0·02 ml. of a culture of

pyocyanea, maintained as suggested by Needham[14] and containing approximately 14 × 10^9 viable bacteria per ml. After inoculation the solutions were incubated at 37° C. for 48 hours, and then 0·1 ml. was sub-cultured from the tubes showing no obvious growth into 20 ml. of Needham's broth and a similar quantity into a tube of thioglycollate

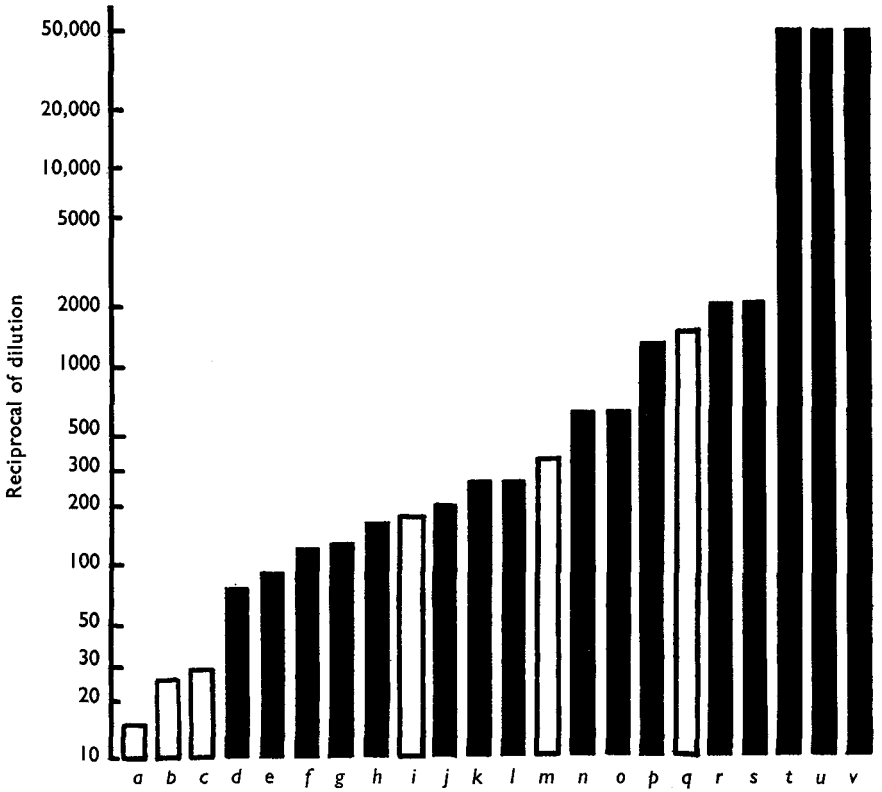

FIG. 1. Relative efficiency of antiseptics in Needham's broth, incubated at 37°C. for 48 hours.
(*a*) methanol; (*b*) ethanol; (*c*) urethane; (*d*) β-phenoxyethylalcohol; (*e*) β-phenoxypropyl-alcohol; (*f*) *iso*octylhydrocupreinotoxin; (*g*) benzyl alcohol; (*h*) chlorobutol (dissolved without heat); (*i*) lysol B.P.; (*j*) *p*-chlorophenyl-α-glycerol ether; (*k*) β-phenylethyl alcohol; (*l*) methylphenyl carbinol; (*m*) phenol; (*n*) hydroxybenzoic acid ester; (*o*) hydroxybenzoic acid ester (Nipa 82121); (*p*) chlorocresol; (*q*) sodium azide; (*r*) benzalkonium chloride; (*s*) cetrimide; (*t*) phenylmercuric acetate; (*u*) phenylmercuric nitrate; (*v*) thiomersalate.

medium, these then being incubated for a further 48 hours. The results obtained from the 3 strains of pyocyanea did not vary greatly and the dilution of the substance which maintained sterility against the least sensitive strain was recorded.

In Figure 1, which shows the relative efficiency of substances, the white columns represent compounds used for comparison only. They are methanol, ethanol, urethane, lysol B.P., phenol, and sodium azide. It may be seen that this test is a strict one, since the organism was placed in a

medium favourable to its growth, and if the same substances were used for the preservation of eye-drops a lower concentration should suffice. Phenoxyethyl alcohol, and phenoxypropyl alcohol were effective in 1 in 80 dilution. *iso*Octylhydrocupreinotoxin which has been recommended as a preservative for injection solutions, and which kills staphylococci and streptococci in over 1 : 100,000 dilution, was only moderately effective, and in the effective concentration the solution was cloudy and its use is not recommended. Benzyl alcohol 0·9 per cent. is used as a preservative in the original stock solution of cortisone acetate. In that concentration it proved effective against pyocyanea. When, however, cortisone eye-drops are made up, the cortisone is diluted in the proportion of 1 : 4, and the concentration of benzyl alcohol falls below the effective level. This would explain the pyocyanea contamination of cortisone eye-drops. It seems rational therefore to use 0·9 per cent. of benzyl alcohol in distilled water or saline solution in making up cortisone eye-drops.

Chlorbutol enjoyed widespread use as a preservative for some time. In many tests our results were inconsistent and on looking up previous publications we found that the recommended concentration varied from saturated solutions (approximately 0·8 per cent.) to 0·3 per cent., and even lower, and by some it was regarded as unreliable. This erratic behaviour of chlorbutol may be explained by the fact that it is a volatile substance and if a solution is made up by heating, or the water preserved with chlorbutol is boiled before making the eye-drops, the chlorbutol may escape or be decomposed by heating. The practical effect of this is shown in Table I.

p-Chlorphenyl-α-glyceryl ether (Gecophen) was effective in 0·5 per cent. solution. Phenylethyl alcohol and its isomer methylphenyl carbinol were both effective in 0·4 per cent. solution. Our findings agree with those of Brewer, Goldstein and McLaughlin[15]. The *p*-hydroxybenzoates are recommended in the National Formulary as inhibiting and not as bactericidal agents. A combination of these esters (Nipasept) was used, and against pyocyanea it was effective at 0·16 per cent., which is the limit of its solubility and is several times the strength recommended by the N.F. A new combination of *p*-hydroxybenzoate (Nipa 82121) was supplied by the makers, and in Needham's broth its effectivity was the same, but when used in eye-drops it proved more potent against pyocyanea than the commercially available "Nipasept." Chlorocresol is perhaps the most time-honoured preservative for eye-drops. It was found that in Needham's medium it was efficient at less than 0·1 per cent. In this concentration it killed a heavy contamination of pyocyanea in a very short time.

Among the quaternary ammonium compounds benzalkonium chloride was effective in 1 in 2000 and several commercial preparations gave comparable readings. Cetrimide was effective against pyocyanea in 1 : 2000, but one batch had a very moderate bactericidal effect even at a concentration of less than 1 in 100. Quaternary ammonium compounds as a preservative for eye-drops should be used only in exceptional cases. Ginsburg and Robson[16] found that detergents could prove harmful by causing "solubilisation" of the intercellular cement of the corneal

epithelium. For the sterilisation of surgical instruments or acrylic implants its use is permissible if followed by a thorough rinse in distilled water or saline solution.

Of the mercurial group phenylmercuric nitrate, phenylmercuric acetate and thiomersalate were tested, and all of them found to be effective in high dilutions. Their use has been recommended for eye-drops and also for solutions for injections. They are not only potent bactericides, but are also effective fungicides.

Tests on Eye-drops

The tests previously described were made in Needham's medium, but from a practical point of view it seemed desirable to test some of these substances on eye-drops as used in everyday practice. For the tests we selected 0·5 per cent. atropine sulphate, 0·25 per cent. eserine salicylate and 2 per cent. sodium fluorescein dissolved in distilled water. The last two are readily contaminated with pyocyanea. From the many substances in the comparative efficiency diagram we selected arbitrarily a few only and serial tests were made with them. The eye-drops were prepared with preservative and then infected with 0·02 ml. of an 18-hour culture of *Ps. pyocyanea*. Subcultures were then taken at intervals up to 24 hours. The results are recorded in Table I.

Chlorocresol (Table I) was tested in 0·1 per cent. and 0·03 per cent. concentration. The 0·1 per cent. kills *Ps. pyocyanea* almost instantaneously and in spite of the massive infection it remains sterile. With the 0·03 per cent. solution atropine became sterile in between 2 and 4 hours, while fluorescein and eserine needed more than 6 hours.

Chlorbutol (Table I) 0·5 per cent. was used and the difference between the heated and unheated solutions is significant. It seems that chlorbutol if used as a preservative for eye-drops must be dissolved without heat. Theodore's suggestion that undissolved chlorbutol crystals should be present in the dropper bottle, and when the crystals have dissolved, that new ones should be placed in the bottle with sterilised forceps, is a safeguard, otherwise, since chlorbutol is so volatile its concentration in solution may be dropping below the effective level.

Phenylethyl alcohol was recommended in 0·5 per cent. solution, but the tests (Table I) show that 0·6 per cent. is safer, killing pyocyanea within 1 hour. With the 0·5 per cent. phenylethyl alcohol, pyocyanea was viable for more than 3 hours. When the drops are used in an outpatient department this difference may be of importance.

Thiomersalate (Table I) 0·005 per cent. solution worked best with fluorescein, where it killed pyocyanea in less than 2 hours, while in atropine it needed over 5 hours.

The *p*-hydroxybenzoates—Combination 1 ("Nipasept") and Combination 2 (Nipa 82121) were tested in 3 different strengths. The tabulated results (Table I) show that in fluorescein their antipyocyanea effect is not satisfactory. In atropine and eserine, the new combination is better than the old one.

In further tests we used thiomersalate 0·002 per cent. and chlorocresol

0·1 per cent. with 0·5 per cent. atropine sulphate. 2 bottles of each solution were prepared, one being kept at room temperature and the other at 37° C. to see if temperature affected the efficiency of the preservative. For a period of 31 days the bottles were opened at intervals of 2 to 3 days and the contents tested for sterility, and one drop of an 18-hour culture of *Ps. pyocyanea* containing approximately 14×10^9 viable bacteria then added. By the end of the test period the bottles had been opened and

TABLE I

Eyedrops	Preservative, per cent.	Hours after infection with 0·02 ml. of 18-hour culture of *Ps pyocyanea*. + indicates growth when subcultured. 0	1	2	3	4	5	6	21 to 24
Sodium Fluorescein 2 per cent.	Chlorocresol 0·1	−	−	−	−	−	−	−	−
	Chlorocresol 0·03	+	+	+	+	+	+	+	−
	Chlorbutol 0·5 (dissolved without heat)	+	+	−	−	−	−	−	−
	Chlorbutol 0·5 (dissolved with heat)	+	+	+	+	+	+	+	−
	Thiomersalate 0·005	+	+	−	−	−	−	−	−
	β-Phenylethyl alcohol 0·5	+	+	−	−	−	−	−	−
	β-Phenylethyl alcohol 0·6	+	−	−	−	−	−	−	−
	Hydroxybenzoates 0·16	+	+	+	+	+	+	+	+
	Hydroxybenzoates 0·106	+	+	+	+	+	+	+	+
	Hydroxybenzoates 0·053	+	+	+	+	+	+	+	+
	Hydroxybenzoates (Nipa 82121) 0·12	+	+	+	+	+	+	+	+
	Hydrozybenzoates (Nipa 82121) 0·08	+	+	+	+	+	+	+	+
	Hydroxybenzoates (Nipa 82121) 0·04	+	+	+	+	+	+	+	+
	No preservative	+	+	+	+	+	+	+	+
Atropine Sulphate, 0·5 per cent.	Chlorocresol 0·1	+	−	−	−	−	−	−	−
	Chlorocresol 0·03	+	+	+	+	−	−	−	−
	Chlorbutol 0·5 (dissolved without heat)	+	+	−	−	−	−	−	−
	Chlorbutol 0·5 (dissolved with heat)	+	+	+	+	+	+	+	+
	Thiomersalate 0·005	+	+	+	+	+	+	−	−
	β-Phenylethyl alcohol 0·5	+	+	−	−	−	−	−	−
	β-Phenylethyl alcohol 0·6	+	−	−	−	−	−	−	−
	Hydroxybenzoates 0·16	+	−	−	−	−	−	−	−
	Hydroxybenzoates 0·106	+	+	−	−	−	−	−	−
	Hydroxybenzoates 0·053	+	+	+	+	+	+	+	+
	Hydroxybenzoates (Nipa 82121) 0·12	−	−	−	−	−	−	−	−
	Hydroxybenzoates (Nipa 82121) 0·08	+	−	−	−	−	−	−	−
	Hydroxybenzoates (Nipa 82121) 0·04	+	+	+	−	−	−	−	−
	No preservative	+	+	+	+	+	+	+	+
Eserine Salicylate, 0·25 per cent.	Chlorocresol 0·1	−	−	−	−	−	−	−	−
	Chlorocresol 0·03	+	+	+	+	+	+	+	−
	Chlorbutol 0·5 (dissolved without heat)	+	+	−	−	−	−	−	−
	Chlorbutol 0·5 (dissolved with heat)	+	+	+	+	+	+	+	−
	Thiomersalate 0·005	+	+	+	+	+	+	−	−
	βPhenylethyl alcohol 0·5	+	+	+	+	−	−	−	−
	β-Phenylethyl alcohol 0·6	+	−	−	−	−	−	−	−
	Hydroxybenzoates 0·16	+	+	+	−	−	−	−	−
	Hydroxybenzoates 0·106	+	+	+	+	+	−	−	−
	Hydroxybenzoates 0·053	+	+	+	+	+	+	+	+
	Hydroxybenzoates (Nipa 82121) 0·12	−	−	−	−	−	−	−	−
	Hydroxybenzoates (Nipa 82121) 0·08	+	−	−	−	−	−	−	−
	Hydroxybenzoates (Nipa 82121) 0·04	+	+	+	−	−	−	−	−
	No preservative	+	+	+	+	+	+	+	+

infected 12 times. On one occasion the bottle containing atropine and thiomersalate kept at room temperature was found to be infected, but subsequent tests of the bottle showed it to be sterile again. The other solutions remained sterile throughout and when tried at the end of the experiment the atropine of all bottles gave good mydriasis in rabbits. Thus while maintaining sterility, the pharmacological effect of atropine was also retained in the presence of chlorocresol and thiomersalate.

Chemical Compatibility

Benzalkonium chloride was found to be incompatible with argyrol, boric acid, silver nitrate, sodium fluorescein, and with the radicals nitrate and salicylate, e.g., pilocarpine nitrate and eserine salicylate (Macpherson and Wood[12]). McEwan and McMorran[17] tested the compatibility of chlorocresol, chlorbutol, and phenylmercuric nitrate with several substances: adrenaline, cocaine hydrochloride, ephedrine hydrochloride, homatropine hydrobromide, hyoscine hydrobromide, penicillin, pilocarpine and physostigmine. All these gave clear solutions with all the above preservatives, excepting homatropine and hyoscine, which were faintly opalescent, but which cleared on heating. These authors used very weak solutions of the drugs mentioned and it is possible that the precipitation with haloids did not become manifest. The B.P.C. and B.P. mention that these mercurial preparations cause precipitation with alkaloids. It seems therefore that the mercuric preparations are suitable mainly with eserine salicylate, pilocarpine nitrate and sodium fluorescein. Phenylethyl alcohol was tested by Brewer, Goldstein and McLaughlin[15] and was compatible with most of the ophthalmic solutions, and this is true of the *p*-hydroxybenzoates.

Tolerance of the Eye

Chlorocresol in 0·1 per cent. solution and the *p*-hydroxybenzoates esters in 0·16 per cent. solution cause some burning sensation. Chlorbutol and phenylethyl alcohol have a slight anæsthetic effect which is of some advantage. The mercurial preparations are used in such dilution that no stinging or burning is caused by them. Sensitisation to mercury is extremely rare in that dilution.

Conclusion and Summary

A number of substances were tested against *Ps. pyocyanea* (*Ps. æruginosa*) and their bactericidal concentration determined.

The quaternary ammonium compounds, although bactericidal, are not recommended because of their effect on the cornea.

The mercurial compounds:—thiomersalate 0·005 per cent. and phenylmercuric acetate and nitrate 0·005 per cent., are safe, and are recommended for eserine and sodium fluorescein, which are the most liable to pyocyanea contamination, also for methylcellulose eye-drops, which are liable to contamination by moulds. These compounds are not only powerful bactericides but are also fungicides. Chlorbutol in saturated solution (about 0·8 per cent.) is safe and recommended, but owing to the fact that the solution cannot be heated without detriment to the preservative its use needs great care. Chlorcresol 0·1 per cent. is safe, but in that concentration it causes smarting, and 0·03 per cent. kills pyocyanea within 24 hours. The *p*-hydroxybenzoates in 0·1 per cent. solution are reliable for most of the eye-drops used except fluorescein. Phenylethyl alcohol is safe in 0·5 per cent. concentration but much quicker in its action at 0·6 per cent., and further clinical trials are needed with this promising preservative.

For cortisone eye-drops 0·9 per cent. of benzyl alcohol, the one used in the original solution, is recommended.

Eye-drops used in hospital wards, outpatient departments and factory medical rooms, should be prepared with bactericidal preservatives, but for eye-drops used by individual patients, a bacteriostatic agent may be permissible. However, for reasons of safety and to make it as foolproof as possible a uniform procedure using a bactericidal preservative is recommended.

Up to now the National Formulary has recommended the use of bacteriostatic agents only. In view of the increasing incidence of eye infections reported by different authorities from many parts of the world, more stringent standards seem desirable.

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