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Antibacterial activity of phenylmercuric nitrate in zinc sulphate and adrenaline eye drops B.P.C. 1968

Recent determinations concerning the antibacterial activity of PMN and sodium metabisulphite mixtures in simple solution and in pharmaceutical preparations have been reported (Buckles, Brown & Porter, 1971; Richards & McBride, 1972; Richards & Reary, 1972; Richards, Fell & Butchart, 1972).

The antibacterial activity of PMN contained in zinc sulphate and adrenaline eye drops B.P.C. 1968 (19·8 ml) was examined after sterilization using autoclaving at 115–116° for 30 min; heating at 98–100° for 30 min; filtration using a Millipore membrane (size GSWP 02500, Millipore Limited, London). Killing times for the eye drops against *Pseudomonas aeruginosa* NCTC 7244 were determined at room temperature (20°) using a method similar to that of Richards & McBride (1971). After sterilization, 0·2 ml of an overnight culture containing approximately 5×10^8 bacteria ml^{-1} was added to each bottle and 10, 20, 30, 45, 60, 90, 120, 150 and 180 min after, 1·0 ml samples were removed and added to 10 ml recovery medium consisting of thioglycollate (0·05% w/v) in nutrient broth (Oxoid No. 2). Incubation of the recovery medium was at 37° for 7 days. The concentration of PMN contained in the eye drops was determined before and after sterilization using the polarographic method of Porter (1968).

Killing times and polarographic determinations were also carried out for solutions of PMN (0·002% w/v) + sodium metabisulphite (0·1% w/v) and zinc sulphate and adrenaline eye drops similar to the B.P.C. 1968 formulation but not containing sodium metabisulphite.

Results (Table 1) show that the killing time for zinc sulphate and adrenaline eye drops B.P.C. 1968 varies depending on the sterilization method used. Filtration sterilized drops showed a more rapid killing effect than heat sterilized preparations. Although drops that had been heat sterilized, i.e. either by autoclaving 115–116° for 30 min or heating 98–100° for 30 min, showed a decrease in the concentration of PMN,

Table 1. *Killing times for zinc sulphate and adrenaline eye drops B.P.C. 1968 against* P. aeruginosa *NCTC 7244.*

Sterilization method	Killing time (min)	pH		Concentration PMN (% w/v)	
		before sterilization	after sterilization	before sterilization	after sterilization
Autoclaving 115–116° for 30 min	20–30	3·5	3·3	0·002	0·0002
Heating 98–100° for 30 min	10–20	3·5	3·5	0·002	0·0007
Filtration	<10	3·5	3·5	0·002	0·002

the preparations still maintained antibacterial activity. This may be attributed to the concentration of PMN remaining, the antibacterial complex formed between PMN and sodium metabisulphite on heating as suggested by Richards & Reary (1972) or by a combination of both complex and free PMN. No decrease in concentration of PMN was observed in filtration sterilized drops although Naido, Price & McCarthy (1972) reported a loss of 13% (w/v) PMN from aqueous solution after filtration using a Sartorius membrane filter. Differences in killing times obtained for zinc sulphate and adrenaline eye drops B.P.C. 1968 would appear not to be due to a pH effect since pH showed little or no change after sterilization either by heating or filtration (Table 1).

Zinc sulphate and adrenaline eye drops from which sodium metabisulphite had been omitted showed killing times that were less than 10 min and which were independent of the sterilization method. However, an unexpected decrease in the concentration of PMN was detected in the heat sterilized solutions, the concentration of PMN decreasing from (w/v) 0·002 to 0·0005% (autoclaving) and 0·0011% (heating 98–100°). This may be due to an interaction on heating of PMN with the adrenaline acid tartrate or zinc sulphate in the formulation. No decrease in PMN from filtration sterilized drops was observed.

Heat sterilized solutions of PMN (0·002% w/v) and sodium metabisulphite (0·1% w/v) showed killing times that were slower than those obtained with filtration sterilized solutions. Although no PMN could be detected in autoclaved solutions, the solutions still maintained antibacterial activity. This result is in agreement with that of Richards & Reary (1972) for autoclaved solutions of PMN and sodium metabisulphite.

The results suggest that although the B.P.C. 1968 recommends sterilization of zinc sulphate and adrenaline eye drops by any one of the three methods described, the method of choice is filtration since little or no loss of PMN occurs.

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