LETTERS TO THE EDITOR

Inactivation of *Bacillus subtilis* spores by heating at 100° with phenylmercuric nitrate or acetate

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The British Pharmaceopoeial sterilization process of 'heating with a bactericide' involves heating certain thermo-labile injections containing phenylmercuric nitrate 0·002% at 98 to 100° for 30 min to effect sterility. A similar method involving phenylmercuric nitrate or acetate 0·002% is described in the British Pharmaceutical Codex for the sterilization of eye-drops. This communication describes the assessment of the reliability of this process for the inactivation of bacterial spores. *Bacillus subtilis* spores were chosen as a test organism because of their reported high resistance to heat and bactericides, and because of their frequent occurrence as a contaminant of pharmaceutical products.

The preparation and storage of the stock spore suspension of *B. subtilis* NCTC 8236, the viable count procedure and the utilization of the heat source have been described previously (Deasy, Küster & Timoney, 1968, 1970a, b). Optimum recovery conditions for viable count determinations of spores surviving the various heat-bactericide treatments were found to be incubation at 37° for 48 h on nutrient agar (Oxoid) containing dextrose 1% and L-cysteine 0·025%.

Log survival-time plots constructed to follow the inactivation of the spores by the various treatments were approximately linear. Spores treated with phenylmercuric nitrate 0·002%, with phosphate buffer pH 7 for 30 min at 100° were inactivated through 5·0 log cycles. When the phosphate buffer was omitted the spores were inactivated only through 3·4 log cycles. Reduction of the concentration of phenylmercuric nitrate to 0·001% did not significantly alter the rate of inactivation of the spores. Alteration of the pH of the phosphate buffer in the system from 8 to 6 only altered the inactivation after 30 min to 4·7 and 5·7 log cycles, respectively. Inclusion of sodium chloride 0·4 or 0·8% during the heat treatment with the bactericide did not significantly affect the rate of inactivation. Change of bactericide to phenylmercuric acetate 0·002% did not significantly alter the inactivation rate.

The results qualitatively follow those reported for vegetative organisms, heat treated with similar mercurial bactericides. However, the levels of inactivation achieved with *B. subtilis* spores compare unfavourably with the levels obtainable with other commonly employed sterilization procedures. The results suggest that there is insufficient margin of safety in the official heating with a mercurial bactericide procedures for the sterilization of products heavily contaminated with bacterial spores.

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