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The recovery of *Bacillus megaterium* spores from white soft paraffin

The isolation and enumeration of viable contaminant organisms from topical non-sterile preparations is particularly difficult. The efficiency of recovery of micro-organisms will depend upon the solvent or dispersal system used to liberate the cells from the fatty base. The following systems have been reported: isopropyl myristate (Sokolski & Chidester, 1964), n-hexane (White, Bowman & Kirshbaum, 1968), Tween 80 (Buhlmann, 1968), polyethylene glycol-ether (Millipore Corporation, 1969) and a liquid growth medium (Woodward & McNamara, 1971). A polyethylene glycol, aqueous buffer 2 phase system has also been used to purify bacterial spore suspensions (Sacks & Alderton, 1961). This report describes the relative merits of five solvent or dispersal systems in the recovery of spores from white soft paraffin experimentally contaminated with *Bacillus megaterium*.

Spores of *B. megaterium* ATCC 8245 were heat activated at 80° for 10 min and 2 ml of a diluted aqueous suspension was added to 18 g of molten white soft paraffin held at 50° in a glass screw-capped jar. The mixture was shaken mechanically until cold to disperse the spores in the ointment base. The concentration of spores in the ointment base was about 2000/g. A comparison between the total count of the spores by the haemocytometer counting chamber method (Marshall & Rigby, 1970) and colony counts on membrane filters indicated that at least 90% of the heat activated-spores could produce colonies.

The following solvent or dispersal systems were tested: isopropyl myristate, n-hexane and light petroleum (b.p. 60–80°), which were filter sterilized before use; a 1·0% v/v Tween 80 solution in peptone water and a 50% v/v mixture of polyethylene glycol 200 in peptone water, which were sterilized by autoclaving. Each solvent and dispersal system was tested for efficiency by placing about 0·5 g of white soft paraffin in 10 ml of the system at 37° and shaking mechanically for up to 10 min. Where solution or dispersal took place, the resultant mixtures were tested for their ability to pass through a 0·45 μm pore size membrane filter.

Neither the Tween 80 nor polyethylene glycol systems dissolved the base. Isopropyl myristate, n-hexane and light petroleum rapidly dissolved up to 5·0% w/v of base but only the isopropyl myristate and n-hexane solutions filtered sufficiently rapidly to be of practical use.

Recovery of spores from the base was achieved by the following method. Approximately 0·5 g of contaminated ointment, accurately weighed, was added to 20 ml of solvent or dispersal system at 37°. This was then either homogenized for 1 min in a blender or shaken mechanically,with the addition of 1 g of 0·5 mm sterile glass beads, for 5 min. Samples of the resulting mixture were added to 100 ml of a sterile filtration vehicle consisting of 0·1% v/v Tween 80 in peptone water, and the whole filtered through a 0·45 μm pore membrane filter followed by 100 ml of peptone water, then the membrane was transferred to a Petri dish containing an absorbent pad moistened with nutrient broth enriched with 1·0% w/v Difco yeast extract and incubated for

Table 1. *Effect of solvent/dispersal system on the recovery of* B. megaterium *spores from white soft paraffin.*

Solvent/dispersal system	% recovery of spores[a] after Mechanical shaking	Homogenization	Standard deviation
Isopropyl myristate	100	86	6·8
n-Hexane	29	56	8·2
1·0% v/v Tween 80	9	44	5·4
50% v/v Polyethylene glycol	12	13	5·6

[a] Mean of four separate determinations.

24 h at 32°. Control counts were obtained from the same spore suspension as that used to inoculate the base.

Table 1 shows the relative efficiencies of the solvent-dispersal systems examined in recovering the spores. In systems shaken mechanically, complete recovery was achieved when isopropyl myristate was the solvent. With the other systems the proportion of spores recovered was greatly reduced. Homogenization increased the recovery of spores from Tween 80 and n-hexane significantly ($P = 0{\cdot}05$). Homogenization of the isopropyl myristate-ointment mixture slightly though significantly reduced the recovery. This reduction might be due to the more efficient removal of water from the spores with a consequent lowering of its protective effect (Tsuji, Stapert & others, 1970). The rate of solution of up to 2·5% w/v of white soft paraffin when shaken in isopropyl myristate is, however, sufficiently rapid to make homogenization unnecessary as a means of dispersion.

The low recovery from Tween 80 and polyethylene glycol systems is attributable to the poor dispersion of the base in these systems.

The technique described provides a rapid means of recovering spores from white soft paraffin and should be sufficiently sensitive to allow the detection of 100 spores per gram in ointments of which it forms the base.

Department of Pharmacy,
The University,
Manchester M13 9PL.

ROGER HAMBLETON
M. C. ALLWOOD

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