Laboratories of Therapeutical Chemistry,
Istituto Superiore di Sanita,
00161 Rome, Italy.

M. A. IORIO

School of Pharmacy,
Liverpool Polytechnic,
Liverpool L3 3AF, U.K.

A. F. CASY

September 18, 1974

REFERENCES

BOWMAN, R. E., COLLIER, H. O. J., HATTERSLEY, P. J., LOCKHART, I. M., PETERS, D. J., SCHNEIDER, C., WEBB, N. E. & WRIGHT, M. (1973a). *J. mednl Chem.*, **16**, 1177–1180.

BOWMAN, R. E., COLLIER, H. O. J., LOCKHART, I. M., SCHNEIDER, C., WEBB, N. E. & WRIGHT, M. (1973b). *Ibid.*, **16**, 1181–1183.

CASY, A. F. & IORIO, M. A. (1974). *Gazz. Chim. Ital.*, in the press.

CASY, A. F., SIMMONDS, A. B. & STANIFORTH, D. (1968). *J. Pharm. Pharmac.*, **20**, 768–774.

IORIO, M. A. & CASY, A. F. *Gazz. Chim. Ital.*, **104**, 655–664.

KUGITA, H., OINE, T., INOUE, H. & HAYASHI, G. (1965). *J. mednl Chem.*, **8**, 313–316.

NURIMOTO, S. & HAYASHI, H. (1973). *Japan J. Pharmac.*, **23**, 742–745.

OH-ISHI, T. & MAY, E. L. (1973). *J. mednl Chem.*, **16**, 1376–1378.

PERRINE, T. D., ATWELL, L., TICE, I. B., JACOBSON, A. E. & MAY, E. L. (1972). *J. pharm. Sci.*, **61**, 86–88.

The isolation and enumeration of *Pseudomonas aeruginosa* from oily cream

The recovery of micro-organisms from topical non-sterile pharmaceuticals presents many problems for the analyst engaged in the microbiological quality control of such products. Variations of the membrane filtration technique developed by Sokolski & Chidester (1964) are widely used in the pharmaceutical and cosmetic industry to recover micro-organisms from topical preparations. Many solvents have been utilized to dissolve or disperse the ointment before membrane filtration. These include isopropyl myristate (IPM) (Sokolski & Chidester, 1964), n-hexane (White, Bowman & Kirshbaum, 1968), light liquid paraffin (British Pharmacopoeia, 1968) polyethylene glycol ether (Millipore Ltd., 1969), white spirit (Smith, personal communication) and carbon tetrachloride (Tall, personal communication). Recently Hambleton & Allwood (1972 & 1973) reported using a variety of solvents to recover *Bacillus megaterium* spores and *Escherichia coli* from white soft paraffin.

P. aeruginosa has been found as a common contaminant in topical preparations (Savin, 1967) and was isolated from oily cream by Simmons (1969). This report describes the recovery of *P. aeruginosa* from oily cream using a membrane filtration technique and a variety of solvent and dispersion systems.

P. aeruginosa NCTC 7244 was grown for 18h at 35° in nutrient broth (Oxoid Ltd., London) and found to contain approx. 5×10^8 cells ml^{-1}. The culture was diluted in sterile distilled water and 0·1 ml of a suitable dilution was incorporated into 2·0 g of oily cream by adequate mixing using a sterile spatula. The inoculated cream contained approx. 5×10^2 cells g^{-1}.

The following solvents were used in an attempt to recover *P. aeruginosa* cells from the cream; isopropyl myristate (Price's Chemicals Ltd., Bebington, Wirral, Cheshire),

dimethylsulphoxide, n-hexane (both BDH, London), light liquid paraffin B.P., arachis oil B.P., sesame oil (gift from E.R. Squibb Ltd., Moreton, Wirral, Cheshire), peptone water (Oxoid Ltd., London) containing 1 % (w/v) Tween 80 (Koch Light Ltd., London). The peptone water solutions were sterilized by autoclaving at 121° for 15 min and the remaining solvents sterilized by membrane filtration using a 0·22 μm filter.

Each solvent (10ml) was tested for its ability to dissolve or readily disperse a weighed quantity (0·2g) of oily cream at room temperature (25°). The solvent-cream mixture was initially shaken by hand and for the organic solvents, n-hexane and carbon tetrachloride this was sufficient to ensure complete dissolution of the cream. Similarly, IPM readily dissolved the cream. In instances where the cream was not readily soluble one of the following procedures was adopted to aid dispersion: (a) shaking on a Whirlimixer (Fisons Scientific Apparatus Ltd., Loughborough, Leics.), (b) shaking in the presence of a few glass beads (diam. 2mm), (c) homogenization using a MSE homogenizer (MSE Instruments Ltd., Crawley, Sussex). In all instances the cream-solvent solution or dispersion was tested for its ability to pass under vacuum through a membrane filter (0·45 μm) (type HAWPO4700, Millipore Ltd., London) enclosed in a Pyrex 47mm filter holder (type XX1004700, Millipore Ltd., London). Mixtures of n-hexane-cream and carbon tetrachloride-cream filtered rapidly but although adequate dispersions of the cream were obtained after homogenization with sesame oil, arachis oil, light liquid paraffin and the peptone water—Tween 80 or polyethylene glycol soutions, filtration proceeded at a slow rate (>10 min). Dimethylsulphoxide was the only solvent which did not dissolve or disperse the cream even after homogenization.

0·2 g of the inoculated cream was added to each solvent (10 ml) and dispersed by the most suitable means as described above. After filtration the membrane was rinsed with sterile peptone water (100 ml) and then transferred aseptically onto a sterile absorbent pad (47 mm) moistened with nutrient broth. Both membrane and pad were incubated at 35° for 48 h. The resultant colonies were counted after incubation.

The toxicity of each solvent system against *P. aeruginosa* was determined by a viable counting method. Only n-hexane showed some toxicity towards the organism, since the viable count was reduced over one hundred fold during the 60 min incubation period (Table 1). Since only a small number of cells for isolation (i.e. approximately 500 g^{-1}) were contained in the experimentally contaminated oily cream, a similar

Table 1. *Effect of solvent system on the isolation, enumeration and viability of* P. aeruginosa.

Solvent system	% cells isolated	Number of viable organisms ml^{-1}[a] Initially	After 30 min	After 60 min
Isopropyl myristate	56	$4·0 \times 10^7$	$3·6 \times 10^6$	$2·8 \times 10^7$
Peptone water + 1% (w/v) Tween 80	52	$3·5 \times 10^7$	$2·5 \times 10^7$	$3·0 \times 10^7$
Peptone water + 1% (w/v) polyethylene glycol	4	$1·3 \times 10^7$	$8·0 \times 10^7$	$7·0 \times 10^7$
n-Hexane	2	$1·3 \times 10^8$	$2·0 \times 10^6$	$1·0 \times 10^6$
Light liquid paraffin	2	$9·6 \times 10^7$	$9·0 \times 10^7$	$3·5 \times 10^7$
Arachis oil	0	$1·3 \times 10^7$	$1·1 \times 10^7$	$9·0 \times 10^6$
Sesame Oil	0	$6·0 \times 10^6$	$5·0 \times 10^6$	$2·0 \times 10^6$

a Mean of three determinations

reduction of viable count at this inoculum level would have a marked effect on the results. Therefore the results (Table 1) using n-hexane as the recovering solvent system indicate the unsuitability of this particular solvent for the isolation of *P. aeruginosa* from oily cream and possible similar preparations.

Viable counts for other solvents used (Table 1) indicate no similar toxicity since the count remained relatively constant during the 60 min incubation period. Results obtained (Table 1) for the isolation and enumeration of *P. aeruginosa* from oily cream indicate that for some solvents (e.g. light liquid paraffin, arachis oil, sesame oil) other factors and not toxicity of the solvent may affect the result.

Results (Table 1) show that the greatest recovery of *P. aeruginosa* from oily cream was obtained using IPM and a solution of peptone water containing 1% (w/v) Tween 80 although these recoveries were only approximately 50% of the original inoculum level. This is in contrast to the results of Hambleton & Allwood (1972) who obtained complete recovery of *B. megaterium* spores from white soft paraffin using IPM, but obtained only poor recoveries of *E. coli* from the paraffin using the same solvent (Hambleton & Allwood, 1973). Similarly, Hambleton & Allwood (1972, 1973) obtained varying recoveries of *B. megaterium* spores and *E. coli* from white soft paraffin using a peptone water–Tween 80 system. Although Tsuji, Stapert & others (1970) showed that IPM was toxic to a varying degree towards several different strains of *P. aeruginosa*, no toxicity was demonstrated for the sample of IPM used in the above experiments and therefore the recovery level of *P. aeruginosa* from oily cream was not due to toxicity of the IPM.

Recoveries of *P. aeruginosa* from the cream with other solvent systems were poor by comparison with IPM and the peptone water–Tween 80 system. n-Hexane was shown to be toxic towards the organism and therefore only low recoveries were obtained with this solvent. Since no recovery of *P. aeruginosa* was obtained with either arachis oil or sesame oil this might be due to the encasement of the bacterial cells in a film of oil as suggested by Tsuji & others (1970) for IPM.

The results obtained indicate that *P. aeruginosa* may be difficult to recover from preparations similar to oily cream, using the conventional membrane filtration technique and the commonly used dispersion solvents and systems.

School of Pharmacy,
Liverpool Polytechnic,
Byrom Street,
Liverpool L3 3AF, U.K.

A. HART
M. B. RATANSI

October 2, 1974

REFERENCES

British Pharmacopoeia 1968, The Pharmaceutical Press, London.

HAMBLETON, R. & ALLWOOD M. C. (1972) *J. Pharm. Pharmac.*, **24**, 671–672.

HAMBLETON, R. & ALLWOOD M. C. (1973). *Ibid.*, **25**, 559–562.

Millipore (U.K.) Limited (1969). Microbiological analysis of toiletries and cosmetic products. Application Report AR-16. p. 4 and p. 6.

SAVIN, J. A. (1967). *Pharm. J.*, **199**, 285–288.

SIMMONS, N. A. (1969) *Br. med. J.*, **1**, 842.

SOKOLSKI, W. T. & CHIDESTER, C. G. (1964). *J. pharm. Sci.*, **53**, 103–107.

TSUJI, K., STAPERT, E. M., ROBERTSON, J. H. & WAIYAKI, P. M. (1970). *Appl. Microbiol.*, **20**, 798–801.

WHITE, M., BOWMAN, F. W. & KIRSHBAUM, A. (1968). *J. pharm. Sci.*, **57**, 1061–1063.