The occurrence of variants of *Pseudomonas aeruginosa* NCTS 6750

MICHAEL W. BROWN, *School of Pharmacy, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.*

The widespread use of *Ps. aeruginosa* NCTC 6750 as a test organism for antimicrobial compounds, together with the inconsistency of reported results, has led to the proposal that standard conditions for growth and preparation of test inocula should be employed. Davies & Norton (1975) commenting on the variations of sterilization times reported for contact lens solutions, using this organism, suggested such a set of parameters. However they did not take into account the well-documented evidence that many pseudomonads have several variant forms (Zierdt & Schmidt, 1964; Shionoya & Homma, 1968; Phillips, 1969), and that these variants could have different sensitivities to antimicrobial compounds.

When *Ps. aeruginosa* NCTC 6750 was grown on nutrient agar (Oxoid C.M.3) at 35° for 48 h, two colony forms were observed, i.e. (i) a smooth, grey-green, glistening colony with entire margins, producing large amounts of green pigment, and (ii), a small khaki-green, rough-surfaced colony with indented margins giving a purple-green pigment. These differences were only discernible after 48 h incubation, when the phenomenon of iridescence also occurred, on all occasions with variant (ii) but only occasionally with variant (i). Separate subcultures of these colonial variants into nutrient broth (Oxoid No. 1) incubated at 35° for 24 h also produced cultures of a dissimilar nature. Thus variant (i) gave a uniformly-turbid suspension with little sediment and a thin pellicle which was easily dispersed by shaking; whereas variant (ii) yielded a very heavy sediment and a thick viscid pellicle which was not easily dispersed by shaking, giving the culture a lumpy granular appearance. Phase-contrast microscopy of the two live cultures showed that in (i) the cells were present as individuals, pairs and very small clumps, while the (ii) culture exhibited only a few individual cells and many large clumps of organisms. When single colony isolates of both variants were made into nutrient broth and incubated at 35°, then daily surface colony counts of diluted aliquots of the cultures on nutrient agar gave results as follows. The variant (i) culture initially gave rise to only variant (i) colonies on the agar, but there was a gradual replacement of the (i) colonies by (ii) colonies until after 6 days when the (i) culture yielded only (ii) colonies on the agar. The variant (ii) culture produced only (ii) colonies on agar suggesting that under these conditions the (ii) form was the most stable. As stated this is not a new observation as these two colonial variants described were also observed by Shionoya & Homma (1968) who designated the variants sm (small) and la (large), equivalent to the (ii) and (i) variants respectively. Examination of their cultures using electron-microscopy by Shionoya & Homma (1968) demonstrated that the sm (ii) cells were linked to one another by a string-like material while the la (i) cells were not. This would account for the difficulty encountered when mixing the (ii) broth culture and its clumped microscopical appearance.

Since the difference in the (i) and (ii) broth cultures was probably due to the presence of some extracapsular material or slime (Brown & Richards, 1964) the presence of which could affect the sensitivity of the organisms to bactericidal action, the sensitivity of the two cultures to benzalkonium chloride was examined. Separate 24 h cultures of both variants were prepared in nutrient broth, thoroughly mixed and the concentrations of cells in both cultures adjusted to 5×10^6 ml^{-1}. The sterilization times for inocula of these cultures in solutions of benzalkonium chloride (0·01%, w/v) at pH 7, 25° were measured, using Letheen Broth (10 ml, B.B.L. Ltd), incubated at 35° for 7 days to recover any surviving bacteria. The sterilization times obtained were 0–5 min and 10–15 min for the (i) and (ii) cultures respectively. The greater resistance of the (ii) culture was probably due to the protection of cells in the large clumps observed microscopically. Thus it appears that of the two colonial variants of *Ps. aeruginosa* NCTC 6750 described here, the (ii) variant is the most stable form in normal culture media and also the most resistant to benzalkonium chloride.

If cultures are prepared by single colony isolates from nutrient agar plates incubated at 35° for only 24 h it is not possible to select the (i) or (ii) colonial variant since at this stage both forms have similar colonies. These observations should be considered when establishing standard conditions for the production of test inocula as suggested by Davies & Norton (1975).

The problem of pellicle formation has been circumvented in the Kelsey & Sykes Test (1969) which requires the removal of the pellicle by filtration before preparing the inoculum. Another approach is that reported by Tyrrell, Moss & Davies (1972) who filtered 1 ml of a well mixed broth culture through a 0·45 μm membrane filter and resuspended the retained organisms in diluent. It may be argued however that neither of these techniques give a truly representative culture since, in the first case the apparently more resistant cells in the pellicle are removed while in the second case the more resistant cells are selected. Hence results obtained using either of these two methods should not be compared with those obtained using unfiltered, unwashed broth cultures.

It is suggested that in establishing criteria for the preparation of standard inocula of *Ps. aeruginosa* NCTC 6750 for assessing antimicrobial activity, care

should be exercised to ensure that the (i) and (ii) variant is used and that the methods used to prepare such inocula do not prejudice the results by removing either the more or less sensitive fraction of the bacterial culture.

July 9, 1977

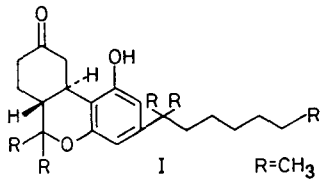
REFERENCES

BROWN, M. R. W. & RICHARDS, R. M. E. (1964). *Pharm. J.*, **16**, 517–519.
DAVIES, D. J. G. & NORTON, D. A. (1975). *J. Pharm. Pharmac.*, **27**, 383–384.
KELSEY, J. C. & SYKES, G. (1969). *Pharm. J.*, **202**, 607–609.
PHILLIPS, I. (1969). *J. med. Microbiol.*, **2**, 9–16.
SHIONOYA, H. & HOMMA, J. Y. (1968). *Japan J. exp. Med.*, **38**, 81–94.
TYRRELL, R. M., MOSS, S. H. & DAVIES, D. J. G. (1972). *Mutation Res.*, **16**, 1–12.
ZIERDT, C. H. & SCHMIDT, P. J. (1964). *J. Bact.*, **87**, 1003–1010.

Solid dispersion approach for overcoming bioavailability problems due to polymorphism of nabilone, a cannabinoid derivative

ARVIND L. THAKKAR*, CLARENCE A. HIRSCH, JOHN G. PAGE, *The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, U.S.A.*

Nabilone, (±)-3-(1,1-dimethylheptyl)-6,6aβ,7,8,10aα-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo[b,d]-pyran-9-one (I) is a potential anti-anxiety agent (Lemberger & Rowe, 1975a & b; Stark & Archer, 1975). This synthetic (Archer, Blanchard & others, 1977) cannabinoid is crystalline, unlike naturally occurring cannabinoids which tend to be resinous.



The aqueous solubility of nabilone is extremely low, less than 0·5 μg ml^{-1} at 25°.

Initial pharmacological testing of nabilone was done using an aqueous colloidal suspension prepared by mixing an acetone solution of the compound with 1% aqueous polysorbate 80 followed by removal of the acetone by evaporation. This preparation, given orally or parenterally, elicited pronounced cns activity in dogs at very low doses. A characteristically high-gaited ataxia was observed approximately 2·5 h after oral dosing. Other effects noted soon thereafter included body sway, head nod, hypothermia and sedation. The maximum intensity of these effects occurred within 4 h of dosing.

*Correspondence.

Since a dry dosage form of nabilone was desired we prepared a formulation as follows: 1 g of nabilone was dissolved in ~50 ml of acetone, 9 g of starch U.S.P. was mixed in and the acetone removed by evaporation under vacuum. The residue was ground and filled into gelatin capsules. This dry formulation, when freshly prepared, elicited the same intensity of pharmacological response in dogs as did the aqueous colloidal preparation. The onset and duration of the cns effects were similar to those after an oral dose of the aqueous suspension.

After storage at room temperature (~25°) for 2 or 3 days, the dry formulation appeared much less active, and after 5 days became inactive. We extracted nabilone from the starch formulation and found, by t.l.c. and g.c., that no chemical degradation of nabilone had occurred. Evidently one bioavailable form of nabilone had converted to a non-bioavailable form. We, therefore, decided to investigate in detail the polymorphism of nabilone and to determine its impact on bioavailability.

Nabilone can occur in at least four distinct polymorphic forms depending upon the crystallization conditions and solvent. All the forms appeared to be equally hydrophobic and insoluble. Table 1 summarizes the bioavailability characteristics of the various forms. The polymorphs were characterized by DTA and X-ray diffraction powder patterns.

All the bioavailable forms tend to convert upon heating, grinding or prolonged storage to the non-bioavailable Form A, which is evidently the thermodynamically-stable form. An effective way to prevent this conversion is to keep nabilone dispersed in the water-soluble matrix of polyvinylpyrrolidone (PVP).