Methicillin-induced lysis of some methicillin-resistant strains of *Staphylococcus aureus*

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The effects of inhibitory and sub-inhibitory doses of methicillin on five strains of *Staphylococcus aureus* have been studied at different temperatures. Cells grown overnight at 37° were treated with methicillin at 25, 30 or 37°, and cells grown overnight at 25 or 30° were treated at 37°. The strains of *S. aureus* consisted of two methicillin resistant (MR) β-lactamase producers, 9254 + and 7270 +; a MR non β-lactamase producer, 7270–; and the penicillin-sensitive, methicillin-sensitive Oxford strain, N.C.T.C. 6571. Inhibitory and sub-inhibitory doses of methicillin used were calculated as fractions (8/5, 3/5 and 1/5) of the minimum inhibitory concentration (MIC) of each strain at 37°. Methicillin was added to cultures at zero time, or to shaken logarithmic phase cultures.

The initial inoculum in each experiment was standardized to give $\sim 5 \times 10^8$ viable cells ml^{-1}. Growth and lysis were followed spectrophotometrically, and viable counts were made by the pour-plate technique. From the results obtained, the temperature of treatment with methicillin, rather than the pretreatment temperature at which the cells were grown, was the important factor. Annear (1968) has previously shown that the resistance of MR strains to methicillin increases with a lowering of the incubation temperature. Inhibitory doses of methicillin caused some lysis of shaken MR cultures, followed by regrowth; preliminary findings have indicated that a higher MIC value of methicillin is obtained against shaken than against non-shaken cultures. With the shaken MR cultures in the present experiments there was an increase in viable numbers after the addition of methicillin, followed by a decrease in viability; with the Oxford strain, in contrast, an initial rapid decrease in viability with concurrent lysis occurred following treatment with methicillin.

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REFERENCE

ANNEAR, D. I. (1968). *Med. J. Aust.* **1**, 444–446.

Inactivation of β-lactam antibiotics by *Pseudomonas aeruginosa*

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Eight strains of *Pseudomonas aeruginosa* have been examined for their ability to destroy ampicillin, carbenicillin, cephaloridine and cephacetrile. Antibiotics were added to suspensions of whole cells, broken cells and to cell filtrates from overnight cultures grown in a synthetic medium; drug concentrations used were approximately half the minimum inhibitory concentration (MIC) for each particular strain. Cephaloridine and cephacetrile were assayed spectrophotometrically at 255 nm and 263 nm, respectively (Russell & Furr, 1973) whilst the penicillins were heated in an acid buffer containing a trace of copper salt and assayed at 320 nm (Smith, de Grey & Patel, 1967).

Antibiotic concentrations did not decrease in any of the cell-free filtrates, thus ruling out the presence of a constitutive extracellular β-lactamase. Four strains (NCTC 8203 and 6750 and the two highly carbenicillin-sensitive strains NCTC 10701 and 10490), whether as whole or disrupted cell preparations, caused no antibiotic destruction, despite the fact that cells of these strains are highly resistant to ampicillin, cephaloridine and cephacetrile (Russell & Mills, 1974). Thus, no constitutive β-lactamase is present, and resistance in these strains appears to be due to the inability of the drugs to enter the cells.

Two R-factor bearing strains, 1822 R^+ and 3425 R^+, and two substrains (designated 1822 R^- and 3425 R^-) which have been derived from these strains and which have either lost the R-factor or the ability to transfer it, were able, when used as whole or disrupted cell preparations, to reduce markedly the antibiotic concentrations. These results might suggest that the so-called R-strains still possessed the R-factor but were unable to transfer it to our

test recipient (*Escherichia coli* strain W 3110). However, it is possible that a permeability barrier also exists in these four strains which may be a major factor contributing to their resistance to β-lactam antibiotics. A comparative study of cell envelope constituents is in progress which should help to clarify this.

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REFERENCES

RUSSELL, A. D. & FURR, J. R. (1973). *J. clin. Path.*, **26**, 599–603.
RUSSELL, A. D. & MILLS, A. P. (1974). *Ibid.*, in the press.
SMITH, J. W. G., DE GREY, G. E. & PATEL, V. J. (1967). *Analyst*, **92**, 247–252.

A comparative study of the gamma-radiation responses of *Bacillus megaterium* spores suspended in aqueous solutions of ethanol and ethylene glycol

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Standard techniques that maintain equilibrium between cells *in water suspensions* and the gases above them during continuous ^{60}Co gamma-irradiation (Tallentire & Jones, 1973) have been used for measurement of the radiation responses of *Bacillus megaterium* spores while suspended in aqueous solutions of alcohols.

Ethanol was chosen as typical of a mobile, monohydric alcohol. The observed responses (slopes of dose-ln survival curves) of spores suspended in concentrations of ethanol in the range 0–95% under air or nitrogen were similar to those seen with spores in water, but at higher concentrations, the oxic response exceeds that seen with water and the anoxic response is less. These changes in response at high ethanol concentrations have been attributed to dehydration of the spores (Stratford & Tallentire, 1973). The similarities in responses at low concentrations clearly show that ethanol in oxic or anoxic aqueous solution is not playing a role in the radiation chemical events that determine spore response.

Ethylene glycol was chosen as an example of a dihydric alcohol of simple chemical composition. It is a viscous liquid, miscible with water in all proportions, resembling more closely those non-aqueous liquids used as vehicles in topical preparations. Changes in ethylene glycol concentration within the range 0–95% have no effect upon the anoxic response, which lies very close to that seen for water alone; 100% ethylene glycol gives a small but significant reduction in response. Thus *in anoxia*, the effects of ethylene glycol resemble closely those of ethanol, leading us to suppose that dehydration is responsible for the reduction in response and that ethylene glycol has no chemical involvement in effective processes during anoxic irradiation. This contrasts with events occurring when irradiation is in air. Under these conditions over the concentration range 0–95% the responses progressively decrease to a minimum level that is almost characteristic of anoxia, while between 95 and 100% the responses increase steadily to a level appreciably greater than that of oxic water. The increase in response observed at high ethylene glycol concentrations is probably due to spore dehydration, but the fall in response below that of water seen at low concentrations indicates functional chemical involvement of this alcohol. Tests carried out with 50% ethylene glycol under air at four different dose rates (1·8, 3·6, 5·4 and 7·2 krads min^{-1}) gave responses that were inversely related to rate, a clear indication that this involvement results in oxygen depletion from the system. The absence of depletion in water and ethanol suggests strongly that the mobility of the suspending liquid is one determinant of radiation response when the irradiation system is in contact with air.

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REFERENCES

TALLENTIRE, A. & JONES, A. B. (1973). *Int. J. Radiat. Biol.*, **24**, 345–354.
STRATFORD, I. J. & TALLENTIRE, A. (1973). *J. Pharm. Pharmac.*, **25**, 130P.