A NOTE ON THE ACTIVITY OF SIX PENICILLINS AGAINST *ESCHERICHIA COLI*

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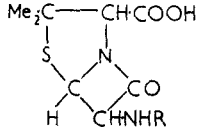
A quantitative comparison of the activity of six penicillins against *Escherichia coli* has been made. The order of their effectiveness in inhibiting growth was benzylpenicillin and phenbenicillin, phenoxymethylpenicillin, phenethicillin, propicillin, methicillin; and in inducing spheroplasts benzylpenicillin, phenoxymethylpenicillin, phenethicillin and phenbenicillin, propicillin, methicillin.

THE isolation of the penicillin "nucleus" (6-aminopenicillanic acid) by Batchelor, Doyle, Naylor and Rolinson (1959) has rightly been acclaimed as a major advance in chemotherapy. Several new penicillins, which differ only in the nature of the side-chain R (I) have been prepared from this and are employed clinically.

One of the main antibacterial effects of benzylpenicillin is the induction of spherical bodies (spheroplasts) in Gram-negative bacteria treated with the antibiotic in a hypertonic medium containing a readily available source of Mg^{++} ions (Lederberg 1956, 1957). Other penicillins induce similar morphological changes in Gram-negative bacteria (Hugo and Russell 1960 a,b) but apart from the results obtained with methicillin (Hugo and Russell, 1960b) no quantitative comparison of this property appears to have been undertaken. The present report compares this function in six penicillins.

MATERIALS AND METHODS

Nutrient broth was prepared from Oxoid No. 2 granules; hypertonic medium for the investigation of spheroplast formation was nutrient broth containing 0·33M sucrose and 0·25 per cent w/v magnesium sulphate, $MgSO_4.7H_2O$; nutrient agar was prepared from Oxoid granules. The pH of all media after autoclaving was 7·4.



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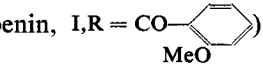
The penicillins used, and their molecular weights, were: sodium benzylpenicillin (I, R=·CO·CH_2·Ph) (356·4), potassium phenoxymethylpenicillin (phenoxymethylpenicillin, Penicillin VK, I, R=·CO·CH_2·O·Ph) (388·5); potassium phenoxybenzylpenicillin (phenbenicillin, Penspek, I, R=·CO·CH(Ph)·O·Ph) (464·8); potassium phenoxypropylpenicillin (propicillin, Ultrapen, I, R=·CO·CH(Et)·O·Ph) (416·5); potassium phenoxyethylpenicillin (phenethicillin, Broxil, I, R=·CO·CH(Me)·O·Ph) (402·5);

and the sodium salt of 6-(2,6-dimethoxybenzamido)penicillinic acid (methicillin, Celbenin, I,R = ) (420·4). The concentrations of antibiotics are expressed as μmoles/ml.

The organism used was a laboratory strain of *Escherichia coli*. It was maintained in nutrient broth and at weekly intervals was plated on to nutrient agar. After overnight incubation of the plate at 37°, a new culture was initiated from a single colony.

When required, 10 ml. nutrient broth tubes were inoculated with one loopful of the stock culture and incubated for 18 hr. at 37°. 0·1 ml. was then added to (*a*) 10 ml. nutrient broth containing the desired concentration of antibiotic, these tubes being incubated at 37° for 24 hr. or (*b*) 10 ml. sucrose-Mg^{++}-broth containing the antibiotic; these tubes were incubated at 37° for 5 hr. when samples were examined by phase-contrast microscopy.

All experiments were repeated on two separate occasions.

TABLE I

MINIMUM CONCENTRATIONS OF PENICILLINS NEEDED TO INHIBIT GROWTH OF, AND INDUCE SPHEROPLASTS IN, *E. COLI*

Antibiotic	Minimum conc. (μmoles antibiotic/ml.) to inhibit growth within 24 hr. at 37°.	Minimum conc. (μmoles antibiotic/ml.) to induce spheroplasts within 5 hr. at 37°.
Benzylpenicillin	Between 0·25 and 0·5	Between 0·1 and 0·25
Phenethicillin	Between 1·0 and 2·0	Between 1·0 and 1·5
Methicillin	>4·0	>4·0
Phenoxymethylpenicillin	Between 0·5 and 0·75	Between 0·5 and 0·75
Phenbenicillin	Between 0·25 and 0·5	Between 1.0 and 1·5
Propicillin	Between 2·0 and 3·0	Between 2·0 and 3·0

RESULTS AND DISCUSSION

The minimum concentration of each penicillin required to inhibit the growth of, and to induce spheroplast formation in, *E. coli* is given in Table I. Benzylpenicillin and phenbenicillin are the most effective in inhibiting growth, although the result with the latter antibiotic must be accepted with some reservation, as the substance was stated by the manufacturers to be 92 per cent purity. Those impurities present could have contributed to the growth-inhibitory effect, while also being of such a nature that they do not affect spheroplast formation. This is supported by the antibiotic concentrations needed to induce spheroplasts (Table I), since phenbenicillin is less effective than either benzylpenicillin or phenoxymethylpenicillin in this respect. Benzylpenicillin is the most effective of the substances tested in inducing speroplasts, and of the phenoxypenicillins, phenoxymethylpenicillin is the most active, followed by phenethicillin, phenbenicillin and propicillin, in that order. Benzylpenicillin is at least 8–16 times as active as methicillin, not only in inhibiting growth but also in inducing spheroplasts. In a study of the antibacterial activity of four penicillins against sensitive and resistant staphylococci, streptococci, *Haemophilus influenzae* and neisseriae,

Williamson, Morrison and Stevens (1961) showed that against the Gram-negative organisms, benzylpenicillin was the most effective, followed by phenoxymethylpenicillin, phenethicillin and propicillin in that order. The present report shows complete agreement with this finding.

A comparison of the concentrations of benzylpenicillin needed to inhibit growth within 24 hr. and to induce spheroplasts within 5 hr. indicates that a lower dose is required in the latter case. This result was confirmed in two replicate experiments, and the apparent anomaly might be explained on the assumption of a penicillinase which would slowly destroy the antibiotic over the longer period of incubation.

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