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THE COMPARATIVE PERFORMANCE OF BETA-LACTAM ANTIBIOTICS AGAINST AMPICILLIN SENSITIVE *ESCHERICHIA COLI* IN CONDITIONS SIMULATING THOSE OF THE INFECTED URINARY BLADDER

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Summary.—The response of an ampicillin sensitive strain of *Escherichia coli* to 6 beta-lactam antibiotics was compared in a mechanical model which simulates the hydrokinetic features of the urinary bladder. The performance of the antibiotics was found to differ in a way that could not be predicted by more conventional *in vitro* techniques. For example, benzylpenicillin was found to be at least as effective as any cephalosporin. Possible reasons for these findings and the relevance of the results to therapeutic practice are discussed.

THE RESPONSE of Gram-negative bacilli to beta-lactam antibiotics depends on a number of factors, the chief of which are the osmolality of the medium, the concentration of the antibiotic and its beta-lactamase stability. These factors have been examined previously in static turbidimetric systems (Greenwood and O'Grady, 1972, 1973) in conditions of high bacterial density such as are found in infected urine. In the hydrokinetic conditions of the urinary bladder, however, the dense culture is exposed to antibiotics in complex, changing conditions because of dilution by ureteric urine and discharge of the bladder contents on micturition. To simulate these complex conditions an *in vitro* model was constructed (Mackintosh, Watson and O'Grady, 1973; O'Grady *et al.*, 1973) and the present work compares the performance of various beta-lactam agents in the bladder model against an ampicillin sensitive strain of *Escherichia coli.*

MATERIALS AND METHODS

An ampicillin sensitive strain of *Esch. coli* (designated ECSA 1) originally isolated from an infected urine in the diagnostic bacteriology laboratory in this hospital was used throughout.

Cephaloridine, cephalothin and cephalexin were provided by Glaxo Laboratories Ltd. ampicillin and benzylpenicillin by Beecham Research Laboratories Ltd; phenoxymethylpenicillin was a standard pharmaceutical preparation. Suitable concentrations of each were freshly prepared as required in sterile distilled water.

Growth medium was the " complete " broth (osmolality *ca* 325 mOsm/kg) previously described (Greenwood and O'Grady, 1973).

Minimum inhibitory concentrations (MIC) of each antibiotic for the strain used were estimated by a conventional serial doubling dilution test in broth using a bacterial inoculum of *ca* 10^4–10^5 organisms per ml.

Model.—In the *in vitro* bladder model (Mackintosh *et al.*, 1973; O'Grady *et al.*, 1973) 20 ml of a fully grown overnight broth culture (representing an arbitrary residual bladder volume) is diluted with fresh broth at 1 ml/min to simulate the ureteric urine flow. At preset intervals a " micturition " episode empties the bladder of all but the residual volume. The culture is gently mixed using a stainless steel paddle and the opacity is continuously monitored photometrically.

For the purposes of the present investigation the machine was set to " micturate " hourly, and sufficient antibiotic to produce a concentration of 500 μg/ml was given as a single pulse after the fourth micturition, *i.e.*, at a point where the culture was growing fairly rapidly, having recovered from the initial lag period when the dilution effect predominates but not having yet entered the " fluctuating equilibrium " state (O'Grady *et al.*, 1973).

RESULTS

The MIC of each of 6 beta-lactam antibiotics for the strain of *Esch. coli* used is shown in the Table.

TABLE.—*Times taken by Cultures in Bladder Model to Re-attain Opacity Level Prevailing at Time of Addition of Single Pulse of 500 μg of Antibiotic per ml*

Antibiotic	MIC for test strain (μg/ml)	Time (h) for opacity to re-attain initial level after antibiotic	
		added	diluted below MIC
Ampicillin	4	10	6·5
Benzylpenicillin	64	8	6·5
Phenoxymethylpenicillin	125	7	6
Cephaloridine	8	8	5
Cephalothin	16	6·5	4
Cephalexin	32	6	4

The general form of the results obtained in the bladder model with antibiotic-free cultures of *Esch. coli* is shown in Fig. 1. A marked fall in opacity occurred during the first hour as the stationary phase culture was diluted out. In subsequent dilution/micturition cycles, growth of the bacteria counteracted the dilution effect until, after about 8 hours, the culture entered a " fluctuating equilibrium " state.

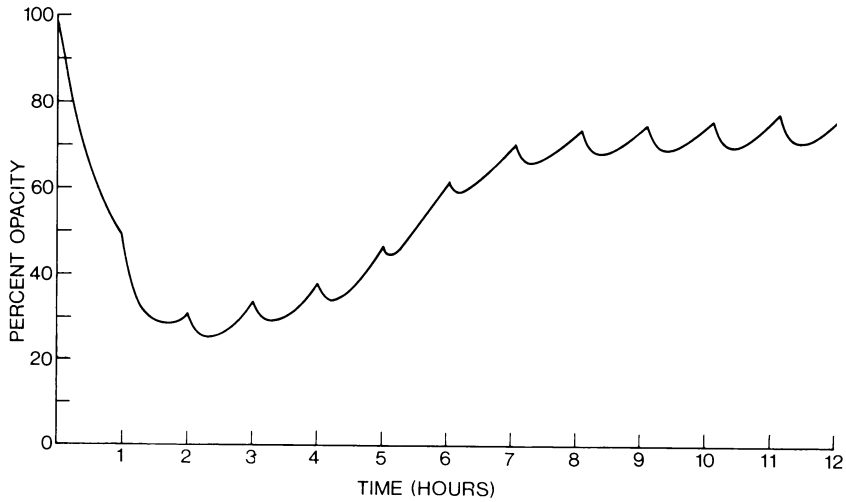

FIG. 1.—General form of results obtained in the bladder model with antibiotic free cultures of *Esch. coli*.

Addition of ampicillin, benzylpenicillin, phenoxymethylpenicillin, cephaloridine or cephalothin after 4 cycles of dilution and micturition caused rapid lysis of the cultures, similar to that seen in a static system (Greenwood and O'Grady, 1973), but enhanced by the dilution effect. As dilution and micturition continued, growth recommenced and the opacity again began to rise. A typical result is shown in Fig. 2. With cephalexin, lysis was delayed, giving rise to a characteristic

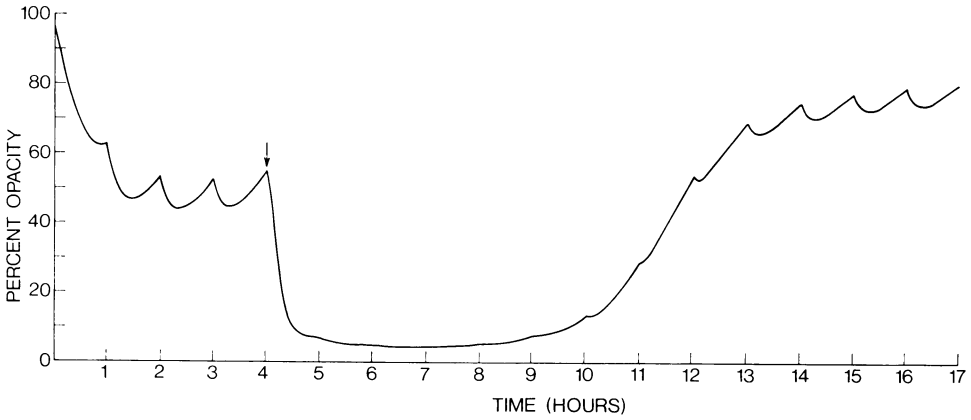

FIG. 2.—A typical result obtained by addition of ampicillin, benzylpenicillin, phenoxymethyl penicillin, cephaloridine or cephalothin after four cycles of dilution and micturition.

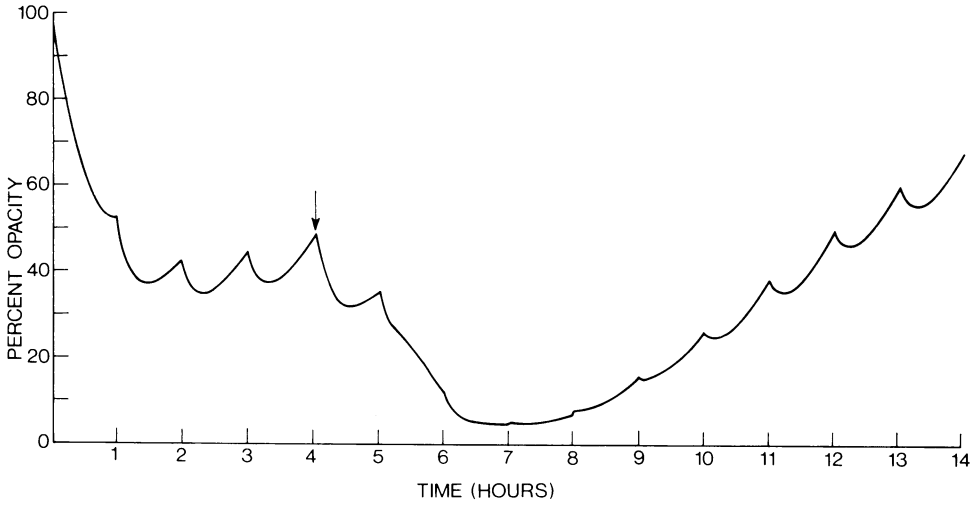

FIG. 3.—Characteristic result obtained after addition of cephalexin following four cycles of dilution and micturition.

trace which is shown in Fig. 3. The time taken in this system for the opacity to re-attain the level at which antibiotic was added is shown for each antibiotic in the Table. This Table also shows the period elapsing between the time when dilution and micturition were calculated to cause the antibiotic to fall below the MIC, and the original opacity level being re-attained.

DISCUSSION

One of the chief questions asked of the bladder model was whether the hydrokinetic features of the urinary bladder would facilitate antibiotic action because of the dilution effect on the organisms or mitigate it because of the concomitant dilution of the antibiotic itself. As might be expected, the answer to this question turned out to be quite complicated.

The response to beta-lactam agents depends on the concentration of the antibiotic. At low concentrations division of Gram-negative bacilli is inhibited, giving rise to filamentous organisms which slowly lyse after several hours. At higher concentrations the bacterial population responds in a trimodal manner: part rapidly lyses, part transforms to the spheroplast forms over a 1–2 hour period and a third part (the so-called " persister " fraction) is affected in a purely bacteristatic manner (Greenwood and O'Grady, 1970; Greenwood, 1972). The antibiotic concentration ranges over which filamentation on the one hand and the trimodal response on the other occur, varies with different beta-lactam antibiotics and may or may not be related to the conventionally determined MIC (Greenwood and O'Grady, 1973). In the trimodal response the numbers of bacteria rapidly lysing or transforming to the spheroplast form can be varied by altering the osmolality of the growth medium, more spheroplasts being formed as the osmolality is raised (Greenwood and O'Grady, 1972), while the persister fraction remains constant at about 1 in 10,000 cells (Greenwood and O'Grady, 1970).

At the initial concentration of antibiotic used in the present experiments (500 μg/ml) all the agents tested here (with the exception of cephalexin see below) give rise to the trimodal response, and at the osmolality used (*ca* 325 mOsm/kg) the predominant response of *Esch. coli* was, as previously described (Greenwood and O'Grady, 1972), rapid lysis, very few bacteria transforming to the spheroplast form.

The performance of antibiotics in the bladder model may be expressed in two ways: (A) comparative " therapeutic efficacy "—the time to recovery from the same antibiotic dose or (B) the MIC-related effect—the time taken for recovery to occur after dilution and micturition alone were calculated to have caused the antibiotic concentration to fall below the MIC. Expressing the results as the MIC-related effect indicates whether factors other than hydrokinetic washout are operating. If washout is the only factor involved, the MIC-related time to regrowth should remain virtually constant, provided that a comparable lytic effect is initially caused. As the Table shows (final column), the MIC-related recovery time is less for cephalosporins than penicillins. Even in terms of " therapeutic efficacy " benzylpenicillin was as effective as cephaloridine and more effective than cephalothin or cephalexin in the dynamic conditions of the urinary bladder, despite its considerable lower activity, as judged by experiments in a static turbidimetric system (Greenwood and O'Grady, 1973) or conventional MIC titration. Phenoxymethylpenicillin, the least active in MIC titrations of the test agents, was also more effective than cephalothin or cephalexin. This unexpected difference is probably due chiefly to the fact that ampicillin-sensitive *Esch. coli* elaborate a beta-lactamase which slowly hydrolyses a number of other beta-lactam agents. Cephalosporins appear more sensitive to this enzyme than penicillins and ampicillin appears completely resistant (Greenwood and O'Grady, 1973). A similar pattern of susceptibility (ampicillin less than benzylpenicillin less than cephalosporins) has been described on several occasions using enzymes prepared

from *Esch. coli* strains (Hamilton-Miller, Smith and Knox, 1965; Jack and Richmond, 1970; Sawai, Mitsuhashi and Yamagishi, 1970; Dale and Smith, 1971). This result would seem to indicate that, at the very high beta-lactam antibiotic concentrations attainable in the urine, stability to this " slow " beta-lactamase is as important as intrinsic activity in obtaining an optimal antibacterial effect against ostensibly " sensitive " organisms which possess the enzyme. At a concentration of 500 μg/ml (which is readily achieved) benzylpenicillin was as effective as cephaloridine or cephalothin in causing rapid lysis of the bacteria and leaving a mere persister fraction, while its superior resistance to slow beta-lactamase enables it to act bacteristatically on the " persister " fraction for longer than cephalothin and as long as the highly active cephaloridine.

In the case of cephalexin, an additional important factor influences the result. This antibiotic is anomalous among beta-lactam agents in that the chief effect on *Esch. coli* over a very wide concentration range is to inhibit cell division but not cell growth, giving rise to long filamentous forms which are only slowly killed (Muggleton *et al.*, 1969; Russell and Fountain, 1970; Greenwood and O'Grady, 1973). The washout effect in the bladder model thus reduced the efficacy of cephalexin considerably, because the antibiotic level fell below the inhibiting concentration when relatively few organisms had succumbed and the persister fraction was consequently large.

Overall, the therapeutic performance in urinary tract infection of the agents tested here does not appear to be graded in the way their behaviour in the model indicates. The reasons for this are probably two-fold: (1) Therapeutic trials in urinary tract infection tend (quite properly) to be conducted among domiciliary patients, many of whom would probably respond to minimum treatment; (2) ampicillin-sensitive strains of *Esch. coli* are probably sufficiently sensitive to all broad spectrum beta-lactam agents to respond adequately even to the less active representatives of the group. It remains to be seen from a study of patients with either impaired hydrokinetic clearance or infections with more resistant organisms, whether these *in vitro* models provide a valuable guide to likely therapeutic performance in more demanding circumstances *in vivo*. The response of more resistant *Esch. coli* strains in the bladder model will form the subject of a separate paper.

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