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Application of non-animal models to studies of the chemotherapy of bacterial urinary tract infections

Laboratory experimental models have been used to study both the pathogenesis and treatment of urinary infections. A major advance in the development of in-vitro models and analysis of the dynamics of urinary infection was made by O'Grady and his colleagues. They recognized that the kinetics of the flushing effect of urine were different in the upper and lower parts of the urinary tract. They also considered the implications of bacterial growth rates in urine and of the contamination of urine by organisms originating in or on tissues (O'Grady & Cattell, 1966a, b; MacIntosh et al., 1975; Macintosh, Watson & O'Grady, 1975). Interaction between growth and washout are such, that the upper tract resembles a conventional continuous culture apparatus, whilst in the lower tract the dilution rate of cultures continuously changes due to intermittent emptying of the bladder.

Because of the range of manifestations of natural infections, models are relevant to only limited aspects of infection but have been particularly useful in providing information about the balance between bacterial growth, antibiotic activity, and the tendency of urine to flush organisms out of the tract. Laboratory models developed to date have been especially relevant to uncomplicated cystitis but may be less so to infections involving extensive mucosal or parenchymal involvement. Models of the human lower urinary tract should ideally reproduce the flow kinetics, nutrient supply, antibiotic concentrations, and bacterial population densities found in patients. There should be provision for maintaining appropriate oxygen tensions and for monitoring bacterial populations and antibiotic concentrations. Some of these topics will now be dealt with in detail.

Limited data are available on urinary oxygen tensions in infected patients. Although mean urinary oxygen tensions were relatively high in 18 healthy women (10.9±2.2 kPa) and in 18 infected patients (8.0±4.3 kPa) there was a significant negative correlation between oxygen tensions and bacterial populations in the infected women (Anderson et al., 1979). In 8 out of 11 catheterized hospital patients who had dense urinary bacterial populations the urinary oxygen tension was ≤2.0 kPa (J. D. Anderson, unpubl. data). Reduced oxygen tensions may increase β-lactamase production (Rashtchian et al., 1979), and reduce the activity of aminoglycosides and the growth rates of some organisms. Dense bacterial populations under substrate- or oxygen-limiting conditions may have altered antibiotic susceptibilities (Brown, 1977). Unfortunately control of oxygen tension is technically difficult and there are uncertainties in choosing values that may be representative of natural infections.

Broth or synthetic urine substitutes have been used in lieu of urine in some model systems to avoid technical and aesthetic problems. However, apart from Escherichia coli, most organisms grow at a different rate in urine and laboratory media (Anderson et al., 1979). Limiting substrates, which presumably differ in these media, may affect antibiotic susceptibility (Brown, 1977). Antibiotic activity in broth and urine may differ for other reasons: for example the mean inhibitory and cidal concentrations of gentamicin for Pseudomonas were influenced by pH and osmolality and were up to 40 times higher in urine than in trypticase soy broth (Minuth, Musher & Thorsteinsson, 1976). Urea has been found to enhance the effect of sulphonamides (Neter & Clark, 1944). Urine is a variable product and donors should not take alcoholic beverages, especially beer, or those other drugs which lead to the excretion of antibacterial metabolites. Some foods, such as asparagus or celery, give urine an obnoxious odour. Donors should be healthy to minimize nosocomial infection, especially from viruses which are not removed on filtration. Urine should be cooled to 4°C immediately after voiding and filter sterilized through asbestos-cellulose pads within 2 h (Anderson et al., 1979). Cellulose acetate and
similar materials may be used to sterilize small volumes of urine. Filter-sterilized urine does not form precipitates and retains constant growth supporting properties for at least four days if kept in the dark. There is insufficient evidence at present to indicate when urine must be used in preference to artificial media. However, in any investigation, at least some comparative experiments should be carried out with urine as medium.

Urinary infections differ from those at most other sites in that high urinary concentrations of antimicrobials may diffuse back into infected tissues and may give concentrations higher than those in serum. There is evidence that urine antibiotic activity may be more important than serum activity (McCabe & Jackson, 1965; Klustersky et al., 1974; Stamey et al., 1974). Urine antibiotic concentrations may exceed conventional susceptibility break points. The rate of bacteriolyis of E. coli by some β-lactam antibiotics is determined largely by the antibiotic concentration (Rolinson, McDonald & Wilson, 1977); this could also influence clearance. Realistic urinary antibiotic concentrations should therefore be chosen for bladder models.

Bacterial populations may be monitored in laboratory models by viable count determinations, if necessary, after neutralization of antibiotics. Alternatively, photometric estimations of bacterial populations are much more convenient and permit continuous monitoring. However, photometric observations may not necessarily reflect changes in viable populations, especially in the short term. Photometric methods are unsuitable for suspensions containing less than about $10^3$ organisms per ml, whilst viable counts of $10^3$ to $10^5$ are of particular interest in human infections. An ideal would be to supplement continuous photometric recording with conventional viable count determinations.

O'Grady, Greenwood, and their colleagues have developed models of the lower urinary tract in which artificial media are pumped into a culture chamber maintained at 37°C with facilities for continuously monitoring bacterial numbers photometrically. These models were initially used to study the balance between growth rate and washout and were extended to investigations with antibiotics (e.g. O'Grady & Pennington, 1966, 1967; O'Grady et al., 1973; Maclntosh et al., 1973). Titles of a selection of papers by the group indicate problems amenable to study with such apparatus (see References under Greenwood, Kawada, Macintosh, and O'Grady).

The author's group constructed a similar apparatus, which reproduces the flow characteristics of the human bladder, but employed urine as medium and determined viable counts directly (Anderson et al., 1979; Anderson, Johnson & Aird, 1980). There was a good correlation between the response of 16 infected patients to amoxicillin in a clinical trial with the behaviour of organisms in this model, and also in conventional laboratory tests (Anderson et al., 1980). This model was also used to study synergy between mecillinam and ampicillin against ten urinary isolates of Enterobacteriaceae (Anderson et al., 1981). There was a good correlation between response in the model and that in a mouse infection protection test even though both models showed a very poor correlation with conventional in-vitro tests. This finding indicates that the model may be reliably used to assess patient response to new antibiotics.

O'Grady and his colleagues, and others, have demonstrated the importance of residual urinary volume in the hydrodynamic clearance of infections from the urinary tract. An increased residual volume decreases the efficacy of clearance and leads to denser bacterial populations. An unexpected finding with the author's bladder model system was that some amoxycillin-resistant Enterobacteriaceae were capable of destroying high urinary antibiotic concentrations and that this effect was enhanced when the residual volume was increased from 4 ml to 50 ml (Anderson, Banerjee & Eftekhar, 1982). β-Lactamase destruction by Gram-negative bacilli may thus contribute to the poor response to therapy which is frequently seen in patients with a high residual volume. Although β-lactam destruction did not occur in these experiments in the presence of clavulanic acid, this agent had intrinsic activity at urinary concentrations and the antibacterial and anti-enzymic activities could not be clearly distinguished.

Simple models may suffice for some purposes: for example, shake culture in urine has been used to show that sulphamethoxazole and trimethoprim which may be synergistic in some media (Bushby, 1973), are not so in urine (Anderson et al., 1974), or that mecillinam-resistant mutants which arise at high frequency in vitro, grow relatively slowly in urine and are more likely to be flushed
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Partial treatment and partial diagnosis in pyogenic meningitis

In patients with pyogenic meningitis, antibiotic therapy given before admission to hospital can reduce the chances of detecting organisms by culture or by Gram's stain of the CSF smear. The ability of oral penicillins to interfere with the results of these investigations is well known, and contrasts rather strangely with their poor CSF penetration and with the large parenteral doses that are used to ensure adequate CSF levels during definitive therapy. It may be that the blood/CSF barrier operates particularly inefficiently during the early stages of pyogenic meningitis.

The most widely quoted clinical study of this problem is that of Dalton & Allison (1968) who investigated 310 patients with proven or probable pyogenic meningitis, 152 of whom had received partial treatment mostly with an oral penicillin either alone or in combination. These workers found that the numbers of expected positive CSF smears and cultures in the partially treated group were both reduced by one third; positive blood cultures were reduced by 64%. Of the three major pathogens (Haemophilus influenzae, Streptococcus pneumoniae and Neisseria meningitidis) the greatest deficit in results among the partially-treated patients was in the culture of N. meningitidis. In retrospect, it is not clear to what extent this gap was due to suppression of diagnosis by treatment, or to a genuine deficiency of meningococcal cases in the partially-treated group, since this infection often presents very acutely and leads to early or immediate hospital admission. There was nevertheless a noticeable reduction in positive CSF smears amongst partially-treated patients with culture-proven meningococcal infection.

Other investigators have documented a similar, though variable, suppressant effect of partial treatment. In the case of CSF smears, for example, the reduction in the total number of expected positive results has been reported as 28% in the U.S.A. (Jarvis & Saxena, 1972) and in Manchester as 18% (Mandal, 1976). It is probably unwise to define the size of the problem too precisely; when we reviewed our patients in Liverpool we found that partial treatment had no significant effect on the likelihood of either the CSF culture or smear proving positive. Epidemiological, clinical and laboratory variables differ considerably between series, as does the extent to which patients with probable but unproven pyogenic meningitis are included in the analyses.

A CSF neutrophilia is the most reliable laboratory marker of pyogenic infection in patients with meningitis, even when they have been partially treated. Occasionally, however, partial therapy induces a CSF lymphocytosis (Converse et al., 1973; Mandal, 1976) which can be genuinely misleading, particularly in parts of the world where both pyogenic and tuberculous meningitis are common. The CSF protein and glucose levels are less affected by partial treatment but are poorer guides to diagnosis. The CSF glucose concentration is of limited value in early management since low absolute levels are more often encountered when the CSF smear reveals bacteria than when it fails to do so (Nye, F. J., in press): pyogenic meningitis is in any case more reliably diagnosed by using the criterion of a low CSF/blood glucose ratio (≤40%).

The role of antigen detection in the management of partially treated meningitis is a