Thymine-requiring Mutants of *Proteus mirabilis* Selected by Co-Trimoxazole *in vivo*

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Co-trimoxazole is widely used for the suppression of urinary tract infections (Cattell et al. 1971). The drug contains trimethoprim which can be used *in vitro* to select thymine-requiring mutants of bacteria (Andrew, 1973). This paper reports the isolation of thymine-requiring mutants of *Proteus mirabilis* from the urine of patients who were on long-term co-trimoxazole therapy for urinary tract infections.

METHODS

Routine urine cultures were made by plating a standard loopful on CLED medium (Mackey & Sandys, 1966), and sensitivity tests were performed by the flood-plate method on DST (Oxoid) agar enriched with 5% lysed horse blood. Mastrings (Mast Laboratories Ltd, Liverpool) were used with antimicrobial agents as follows: nitrofurantoin, 200 μg; sulphadimidine, 200 μg; tetracycline, 100 μg; ampicillin, 30 μg; nalidixic acid, 25 μg; and co-trimoxazole, 25 μg. All plates were incubated for 18 to 24 h at 37 °C.

The organisms were identified as *Proteus mirabilis* using standard methods (Cowan & Steel, 1966). The mutants were further tested for growth on routine media such as blood agar, MacConkey agar, nutrient broth, and, in addition, on plates of DST agar enriched with 5% lysed horse blood with discs of p-aminobenzoic acid, sulphonamide, nitrofurantoin and co-trimoxazole. The nutritional requirements of the mutants were determined on minimal salts agar (Clowes & Hayes, 1968). *Escherichia coli* strains W3110 (thymine-requiring, supplied by Dr N. Datta, Hammersmith Hospital, London, W.12) and NCTC10418 (prototroph) were used as controls.

RESULTS

The mutants of *Proteus mirabilis* were isolated from the urine of three patients:

M. P., a woman aged 27, presented in January 1971 with cystinuria, chronic pyelonephritis and renal calculi. A strain of *Proteus mirabilis*, sensitive to co-trimoxazole, was isolated from the urine. She was treated with co-trimoxazole (one tablet of co-trimoxazole contains 80 mg trimethoprim and 400 mg sulphamethoxazole), two tablets twice a day for 7 days: the urine became sterile, and the dose was reduced to one tablet twice a day in February, and to one tablet per night in March. Infection with *P. mirabilis* recurred in May and June 1971; the organisms grew normally and were sensitive to co-trimoxazole, which was then given in therapeutic dose of two tablets twice a day for 21 days, after which it was reduced to one tablet daily. Again the urine became sterile, and the patient was well until September when the infection recurred again. On this occasion the *P. mirabilis* isolated grew normally on the routine culture medium (CLED) but failed to grow on DST (Oxoid) sensitivity agar, and the sensitivity of the organism could not be ascertained normally. The patient continued to take one tablet twice daily until December 1971, and during this time *P. mirabilis* was isolated from the urine. On each occasion the organism grew well on CLED, but not on sensitivity agar. In December the co-trimoxazole was stopped. In February 1972 the patient had a pyelolithotomy under
Table 1. Growth responses of Proteus mirabilis mutants

<table>
<thead>
<tr>
<th>Medium</th>
<th>Proteus mirabilis strains derived from patients</th>
<th>Escherichia coli strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. P.</td>
<td>K. R.</td>
</tr>
<tr>
<td>MA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA + thymine (2.5 µg/ml)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA + thymine (25 µg/ml)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MA + thymine (250 µg/ml)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MA + PABA* (20 µg/ml)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA + PABA* (1000 µg/ml)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA + sulphonamide (200 µg/ml)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA + trimethoprim (200 µg/ml)</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

* p-Aminobenzoic acid,
−, No growth; +, growth, and single colonies after 48 h incubation.

Discussion

Thymine-requiring mutants of various organisms, such as Escherichia coli (Okada, Yagishita & Ryan, 1960), Salmonella typhimurium (Okada, Homma & Sonohara, 1962), Aerobacter aerogenes (Harrison, 1965) and Streptococcus faecalis (Andrew, 1979), have been selectively isolated using trimethoprim in vitro. Our findings suggest that in vivo selection of thymine-requiring mutants of Proteus mirabilis may occur in the presence of co-trimoxazole, probably due to its trimethoprim content. It is likely that such mutants occur only rarely, because further similar isolates have not been obtained from more than 100 other patients known to have had co-trimoxazole for periods of a year or longer.

Enhancement of growth with p-aminobenzoic acid, nitrofurantoin and sulphanomides...
Short communication

occurred only in the presence of blood, and the explanation for this is not clear to us. A similar observation has also been made by McGhie, Hutchison and Finch (1972).

There are two practical implications of these findings. First, in routine bacteriological work, the failure of an organism such as a Proteus species to grow on sensitivity agar (in marked contrast to its normal behaviour) might lead to its being reported as sensitive to all the agents tested on the sensitivity plate. Secondly, the observation that the mutants grew in the vicinity of sulphonamide and co-trimoxazole discs suggests the possibility that in these patients, co-trimoxazole may be helping to maintain infection rather than suppressing it. This suggestion may be of clinical importance, and can be supported theoretically since it is known that thymine-requiring mutants of Escherichia coli are able to metabolize exogenous thymine (Crawford, 1958), and gain advantage over wild-type bacteria when thymine is present with trimethoprim (Bertino & Stacey, 1966). Andrew (1973) also showed that wild-type Streptococcus faecalis was able to utilize exogenous thymine to reverse trimethoprim inhibition.

It is interesting that 1 to 3 months after the co-trimoxazole was stopped in two of the patients (M.P. and K.R.), Proteus mirabilis isolated from the urine grew normally on sensitivity agar, suggesting that wild-type bacteria again predominated over thymine-requiring mutants.

We are grateful to Professor A. Polak and Dr H. A. Lee for permission to publish details of these patients and to Dr N. Datta for advice and supply of Escherichia coli strain W3110.

REFERENCES