THE SPECIAL AFFINITY OF PARTICULAR TYPES OF PROTEUS MIRABILIS FOR THE URINARY TRACT

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Infections of the urinary tract with strains of Proteus mirabilis are common and second only to Escherichia coli infections in the frequency with which they occur. However, whereas the majority of E. coli infections are confined to the bladder, Proteus strains have a special predilection for the upper urinary tract where they cause much greater kidney damage than E. coli (Braude, Shapiro and Siemienski, 1959; Braude and Siemienski, 1960; Fairley et al., 1971; Asscher, 1975).

The reservoir of the infecting strain is thought to be the bowel and there has been controversy over many years as to whether the serotypes of the E. coli that are frequently implicated have a special pathogenicity for the urinary tract or simply reflect the predominance of these strains in the gut flora. The recent simple, reliable and highly discriminatory method of typing Proteus by proticine production and sensitivity (P/S typing) (Senior, 1977a) has provided a means of investigating the same problem for the P. mirabilis strains associated with urinary-tract infections. Evidence presented below strongly suggests that particular P/S types of P. mirabilis have a special affinity for the urinary tract.

MATERIALS AND METHODS

Isolation of strains from urine. Urine specimens from local general practices and hospitals were received for routine bacteriological examination. Viable counts were done only on freshly voided or refrigerated specimens by plating a standard loopful (i.e. 4 μl) of a well mixed sample on blood agar (Oxoid CM 55 Blood Agar Base and 5% horse blood) and MacConkey agar (Oxoid CM 7) plates. After overnight incubation at 37°C, six colonies, including representatives of all morphological types of colony, were picked from those plates showing significant, i.e., more than 400 colonies, pure growths of organisms thought to be strains of Proteus species, and inoculated into Tryptone Water (Oxoid CM 87) and incubated at 37°C for 3–6 h. The cultures were subsequently inoculated on to two blood-agar plates as previously described (Senior, 1977b) to test simultaneously the Dienes compatibility of all six isolates from each urine specimen in all combinations. After overnight incubation at 37°C the plates were examined for Dienes lines by reflected light. Tryptone-water cultures of each representative Dienes type found and every non-swarming isolate were inoculated into urea
broth, tryptone water, and glucose, lactose, sucrose and mannitol tryptone water. After overnight incubation at 37°C, cultures giving the biochemical reactions of *Proteus* were inoculated onto nutrient-agar slopes in screw-capped bottles which were incubated overnight at 37°C and stored at 4°C in the dark.

*Isolation of strains from faeces.* Faecal specimens were collected from healthy volunteers 60 years of age and over who lived in local old people's homes or hospitals and were free from urinary-tract infection. The specimens were inoculated at the centre of undried blood-agar plates and incubated overnight at 37°C. From plates showing swarming growth, a loopful of each distinct swarm was plated out on MacConkey agar. After overnight incubation at 37°C a single colony was picked from each plate and inoculated into tryptone water and incubated for 3–6 h at 37°C. The species of each *Proteus* isolated was determined biochemically and the culture was stored as above.

*Proticine production and proticine sensitivity (P/S) typing of strains.* All the strains of *Proteus* species were typed by their ability to produce proticine (P type) and also their sensitivity (S type) to proticines when tested against the standard set of proticine producer and indicator strains described by Senior (1977a). The strains were typed by cross-streaking on 1% (w/v) Proteose Peptone (Difco No. 3) agar plates containing 0.2m~*~ p-nitrophenylglycerol (PNPG), an antiswarming agent which is without effect on P/S typing (Senior, 1977b and 1978) according to the method previously described (Senior, 1977a).

**RESULTS**

During a period of 19 months, 262 specimens of urine, 203 (77%) from hospitals and 59 (23%) from general practices, from 217 individuals were found to be infected solely with significant numbers (i.e., 10^5 bacteria/ml or more) of *Proteus* species. One hundred and and fifty six (60%) specimens were from mid-stream samples of urine, 84 (32%) from catheter specimens, 6 (2%) from bag
urines, 6 (2%) from dip-slides, and 10 (4%) were undefined urine specimens. The sex and age distribution of the patients of whom 72% were female and 28% were male is shown in fig. 1.

Urinary-tract infections with *Proteus* species were commonest in the elderly, 69% of the male and 67% of the female patients being over the age of 60 years. This association was real and not the reflection of the age distribution of patients because when comparisons were made between the age distribution of the patients with *Proteus* infections and that of 200 consecutive urines from patients with significant bacteriuria caused by *E. coli*, the histogram for *Proteus* infections was skewed towards the elderly (fig. 2).

The species of *Proteus* most commonly associated with urinary-tract infections was *P. mirabilis* which was found as the sole infecting species in 253 (96.5%) of the specimens. Three specimens contained a mixture of *P. mirabilis* and *P. morganii* and two specimens a mixture of *P. mirabilis* and *P. vulgaris*. Other species found as the sole infecting organism were *P. vulgaris* in two specimens, *P. morganii* in one specimen and *P. rettgeri* in one specimen.

![Fig. 2.—The age distribution of patients with urinary-tract infections due to *Proteus* species (---) compared with that due to *Escherichia coli* (-----).](image-url)
In 240 (95%) of the 253 specimens infected solely with *P. mirabilis*, a single strain was judged to be involved because the six colonies examined from each specimen gave Dienes reactions of compatibility with each other when tested in all combinations. The remaining 13 (5%) specimens were each infected with two strains of distinct Dienes-compatibility types which were subsequently found to be of different P/S types.

The results of P/S typing of the strains isolated from urine are presented in the table. Only one representative of every different P/S type isolated from each individual has been recorded here to avoid errors arising from repeated isolations of strains of the same P/S type in patients from whom more than one specimen was obtained. From the 241 urinary strains so recorded, 97 different P/S types were found and 75% of the strains were proticinogenic. The most commonly occurring proticinogenic strains in urine (21% of the total), were those producing proticine type 3. Proticine type 1-producing strains were isolated somewhat less frequently (13% of total) followed by producers of proticine type 5 (10%) and proticine type 2 (8%). There was no correlation between the P/S type of a strain and the type of urine specimen or the sex or the age of the patient. Proticine type 3-producing strains were isolated from every ten-year age group ranging from 20 months to 96 years.

Although 97 distinct P/S types were found among the 241 urinary strains it was observed that a significant proportion of the strains (14%) belonged to only three of these P/S types—namely, P3/S1,8, P3/S1,13, and P3/S1,8,13. Other P/S types frequently found were PO/S4 and P1/S0 (fig. 3).

To investigate whether or not the predominant *Proteus* strains in the gut were of the same P/S types as these frequently associated with urinary-tract infections, analyses were made of the P/S types of the *Proteus* strains found in faeces. Because urinary-tract infections with *Proteus* were frequently found only in patients over 60 years of age it was thought more appropriate to restrict isolation attempts to stool specimens from individuals within this age range who were without urinary-tract infection.

During a period of nine months 118 specimens of faeces, 95 (80.5%) from patients in hospital and 23 (19.5%) from those at home, from 100 individuals (30% male and 70% female), were found to be infected with swarming strains of *Proteus* species; 98 (83%) of the specimens yielded *P. mirabilis*, 18 (15%) specimens yielded *P. vulgaris*, and 2 (2%) contained a mixture of *P. mirabilis* and *P. vulgaris*. In only 3 (2.5%) specimens were strains found belonging to different Dienes types and P/S types.

The results of the P/S typing of the strains from faeces are presented in the table alongside the results of the urine strains for ease of comparison. As before, only one representative of each P/S type from each individual has been recorded. Among the 106 strains so recorded were 52 different P/S types, and 65% of the strains were proticinogenic. The most commonly occurring proticinogenic strains in faeces, unlike the urinary strains, were those producing proticine type 2 (12.5%), whereas proticine type 3-producing strains, comprising 21% of the urinary strains, were much less frequently isolated from faeces (8.5%). Proticine type 1-producing strains were similarly less frequent in
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**TABLE**

The proticine production/proticine sensitivity (P/S) typing results for Proteus strains isolated from urine and faeces

<table>
<thead>
<tr>
<th>Proticine type (P type) of producer strain</th>
<th>Strains from urine</th>
<th>Strains from faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of strains in P type (%) out of 241 isolated</td>
<td>Number of different P/S types</td>
</tr>
<tr>
<td>P0</td>
<td>60 (25)</td>
<td>17</td>
</tr>
<tr>
<td>P1</td>
<td>32 (13)</td>
<td>13</td>
</tr>
<tr>
<td>P2</td>
<td>20 (8)</td>
<td>8</td>
</tr>
<tr>
<td>P3</td>
<td>51 (21)</td>
<td>13</td>
</tr>
<tr>
<td>P4</td>
<td>8 (3)</td>
<td>5</td>
</tr>
<tr>
<td>P5</td>
<td>24 (10)</td>
<td>10</td>
</tr>
<tr>
<td>P6</td>
<td>9 (4)</td>
<td>5</td>
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<tr>
<td>P7</td>
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<td>P8</td>
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<td>4 (2)</td>
<td>3</td>
</tr>
<tr>
<td>P11</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>P12</td>
<td>5 (2)</td>
<td>4</td>
</tr>
<tr>
<td>P13</td>
<td>2 (1)</td>
<td>2</td>
</tr>
<tr>
<td>Atypical</td>
<td>14 (6)</td>
<td>8</td>
</tr>
<tr>
<td>All P types</td>
<td>241 (100)</td>
<td>97</td>
</tr>
</tbody>
</table>

faeces than in urine. The remaining proticine types were found with similar frequency in urine and in faeces. A comparison between the frequency of occurrence of the different common P/S types of strains, i.e. those found in 1% of patients or more, isolated from urine and faeces is given in fig. 3.

All P/S types commonly found in urine were found with a similar or greater frequency in faeces with the exception of P/S types P1/S0, P3/S1,8, P3/S1,13 and P3/S1,8,13. Strains of P/S type P1/S0 were common in faeces and the slightly greater frequency of isolation from urine may not be significant (see below). However, strains of P/S types P3/S1,8, P3/S1,13 and P3/S1,8,13, which are related but distinct were seldom found in faeces (3%) whereas they were by far the commonest (14%) P/S types in urine. These differences were significant and suggested that strains possessing these P/S types had a special affinity for the urinary tract.

This possibility was investigated further by subdividing the 217 patients with infected urine into three groups on the basis of their clinical symptoms. One group of 86 patients (55% in hospital) consisted of those with clinically manifest symptoms of urinary-tract infection including frequency, loin pain, dysuria, pyrexia and haematuria. A second group of 106 patients (94% in hospital) consisted of those who had infected urine as a result of surgery and were symptomless. No clinical information was provided about the remaining group of 22 patients. A comparison between the common P/S types of strains isolated from patients with symptoms of urinary-tract infection with those from
post-operative infections is presented in fig. 4. Strains of P/S type P0/S4 which were common in urine and faeces (see fig. 3) were almost exclusively found in patients with post-operative urinary-tract infections, whereas strains of P/S type P1/S0 were found in similar frequency in both groups of patient. However, 90% of the 30 patients infected with strains of P/S types P3/S1,8, P3/S1,8,13 and P3/S1,13 had symptoms of urinary-tract infection. Clinical details given for the remaining 10% (3 patients) in whose infected urine these P/S types were found as a result of surgery, included "bowel malignancy", "bile in urine" and "pelvic floor repair". These findings supported the evidence that strains with P/S types P3/S1,8, P3/S1,8,13 and P3/S1,13 had a special affinity for the urinary tract.

**DISCUSSION**

When *Proteus* strains isolated from the urine and faeces of groups of individuals having similar distributions of age and sex from within and outside hospitals were P/S typed, it was found that three particular P/S types of *P. mirabilis* were associated with urinary-tract infections at a frequency higher than could be explained by the frequency of their occurrence in the gut. It
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Fig. 4.—The frequency of occurrence of the 23 commonest P/S types of Proteus found in patients with symptoms of urinary-tract infection compared with those found in infections of urine arising post-operatively.

would appear therefore that these particular P/S types have some special predilection for the urinary tract. The unusually infrequent occurrence of proticine type-3-producing strains in faeces was also observed by Senior (1977a) in a previous survey in which among 58 strains of P. mirabilis from faeces, all proticine producer types except type 3 were found. Nevertheless, it is presumed that the source of infection for these patients was faecal although, because stool specimens were not available, this was not confirmed.

The mechanisms whereby the strains with these three P/S types are pathogenic are to be investigated. Perusal of the medical records of patients infected with them seems to indicate that when the urinary tract is infected, these strains are particularly difficult to eradicate; many of them were repeatedly isolated from individuals over a period of 1–6 months. Furthermore when the antibiotic sensitivities of all the urinary strains were tested, only 14% of the 217 individuals had strains resistant to more than one antibiotic, whereas the figure for individuals infected with proticine type-3 producer strains was 40%, and for those individuals infected with strains of P/S type P3/S1, 13, 56% of the strains were multiply resistant to antibiotics. There was no association between R factors and the genetic determinants for proticine type-3 production or for proticine sensitivity
and it seems that in attempts to eliminate these strains from the urinary tract, many different antibiotics had been tried and as a result many strains had become resistant to them.

The role of the P/S type, if any, in the infective process is uncertain. The S-type may characterise a particular cell envelope with increased resistance to immunological processes. Proticine type 3 may itself act as a toxin as do some other bacteriocines (Brock and Davie, 1963; Jacob, Douglas and Hobbs, 1975) or, alternatively, there may be closely associated virulence determinants as with the virulent bacteriocinogenic strains of Yersinia pestis and colicine V-producing E. coli strains. These possibilities are to be investigated.

**Summary**

The strains of Proteus species found in significant numbers and as pure cultures in urine from 217 individuals were isolated, identified to species level and typed for proticine production (P type) and proticine sensitivity (S type) to give their P/S type. Urinary-tract infections with Proteus, principally P. mirabilis, were associated with the elderly. Ninety seven distinct P/S types were found but three P/S types P3/S1,8, P3/S1,8,13 and P3/S1,13 were isolated at a much higher frequency (14%) then could be explained from their faecal carriage rate. These types were almost without exception restricted to patients with clinical symptoms of urinary-tract infection and it is suggested therefore that they have some special affinity for the urinary tract.

**References**


