**PROTEUS MORGANI IS LESS FREQUENTLY ASSOCIATED
WITH URINARY TRACT INFECTIONS THAN
PROTEUS MIRABILIS—AN EXPLANATION**

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SUMMARY. The metabolic activities of faecal and urinary strains of *Proteus morgani* and *P. mirabilis* were compared. Regardless of origin, the generation time of *P. morgani* strains in urine was approximately twice as long as that of the *P. mirabilis* strains. Urease synthesis was constitutive in *P. morgani* strains but required induction with urea in the *P. mirabilis* strains. In the presence of urea, the *P. mirabilis* strains liberated ammonia more rapidly and produced alkaline conditions more quickly than *P. morgani* strains, although they synthesized much less urease. These characteristics may place *P. morgani* strains at a disadvantage in comparison with *P. mirabilis* strains in their ability to cause urinary tract infections.

INTRODUCTION

Proteus morgani and *P. mirabilis* are commonly found in human faeces. The first isolates of *P. morgani* were cultured from infants suffering from diarrhoea (Morgan, 1906) and the organisms that cause urinary-tract infections originate from the bowel. Urinary-tract infections with *Proteus* spp. are common and some strains have a special predilection for the upper urinary tract (Fairley *et al.*, 1971). Urease is thought to be a major virulence factor because it degrades urea, releasing ammonia, which may cause damage and death to the renal epithelium, inactivation of complement, and conditions favouring the development of renal stones (Braude and Siemienski, 1960; Musher *et al.*, 1975). Urease is produced in large amounts by *P. morgani* and *P. mirabilis* strains (Senior, Bradford and Simpson, 1980).

P. morgani has been isolated from a patient with haematuria (Sevin and Buttiaux, 1939) but nearly all proteus urinary-tract infections are due to *P. mirabilis*, and only rarely to *P. morgani*. In a recent survey (Senior, 1979) of the *Proteus* spp. involved in urinary-tract infections among 217 individuals, 98.5% of infections were due to *P. mirabilis* strains and only 1.5% to *P. morgani* strains.

The aim of this study was to seek an explanation for why the two species differed so markedly in their frequency of association with urinary-tract infection.

MATERIALS AND METHODS

Bacterial strains. Six strains of *P. mirabilis* and four strains of *P. morgani* isolated from samples of urine and faeces sent for routine bacteriological examination were studied (table). They were selected from a larger collection because they were known (Senior, 1979) or believed to be typical of the types found in urine or in faeces. The identity of the strains and their proticene production-proticene sensitivity (P/S) type were determined by biochemical and typing methods described previously (Senior, 1977; Senior *et al.*, 1980).

Determination of the growth rates of strains in urine. Overnight peptone-water (Oxoid L37) cultures of the test strains were diluted tenfold into sterile pooled urine, from which alkali-precipitable material had been removed, and which was prepared and sterilised as described previously (Senior *et al.*, 1980) but the pH indicator was omitted. The cultures were incubated without shaking at 37°C, and at timed intervals samples were removed and their absorbance at 550 µm determined against a blank of sterile pooled urine.

Determination of ammonia formation. The amount of ammonia formed during growth was determined by adding 10 µl of the clear supernate of a centrifuged culture sample to 250 µl of phenolnitroprusside solution on ice. To this mixture were added alkaline hypochlorite 250 µl and water 1.25 ml. After 20 min the E₅₇₀ of the mixture was determined. The ammonia content of the samples was read from a standard curve, prepared from the E₅₇₀ results of known amounts of ammonium chloride in water.

Assay of urease. Samples of culture (5 ml) were centrifuged and the cell pellet washed twice in 2.5 ml of PEM buffer (1mM sodium phosphate, 1mM disodium EDTA and 5mM 2-mercaptoethanol, -pH 7.0). The washed cell pellet was resuspended in 0.5 ml PEM buffer, sonicated for 1 min at iced-water temperature and centrifuged at 12 000 *g* for 1 min in a microcentrifuge (Eppendorf). The clear supernate was removed and its urease activity determined by the modified Berthelot reaction described previously (Senior *et al.*, 1980). One unit of urease was defined as the amount of extract required to liberate 1 µmol of ammonia in 5 min at room temperature.

Protein determinations were made according to the dye-binding method of Bradford (1976) by adding a known volume of extract to 1 ml of a solution of Coomassie Brilliant Blue G 250 0.01% (w/v) in ethanol 5% (v/v) and concentrated phosphoric acid 10% (v/v) in water. The E₅₉₅ of the mixture was measured and the protein content calculated by reference to a standard graph prepared with bovine serum albumin solutions.

Buffering capacity of cultures. Exponential-phase cultures in nutrient broth (Oxoid CM67) were divided into two 5 ml portions. To one portion was added 0.25 ml of 1M ammonium phosphate. The cultures were incubated at 37°C and at intervals during a 3-h period their pH was measured on a Beckmann Model 3500 Digital pH meter.

Assay of carbonic anhydrase. Carbonic anhydrase was assayed by the method of Wilbur and Anderson (1948) as suggested by Livesey (1977).

Preparation of spheroplasts of *P. morgani*. Spheroplast medium was prepared by mixing equal volumes of autoclaved double strength (5% w/v) nutrient broth and Seitz filtered 1 M sucrose in water. Overnight broth cultures were diluted tenfold into this medium and incubation continued at 37°C until the cultures were growing exponentially. Carbenicillin was then added to the culture to a final concentration of 200 µg/ml and incubation continued until all the cells were seen to have been converted into spheroplasts when samples were examined under the microscope.

RESULTS

The exponential growth rates in urine of all the *P. mirabilis* strains, regardless of their origin, were much faster than those of the *P. morgani* strains, and the strains isolated from urine did not grow significantly faster than the faecal strains. Assuming that the generation time (*g*) is equivalent to the time for a doubling of the optical density, the generation time for the exponentially growing *P. mirabilis* strains was 34–39 min, compared with 70–87 min for the *P. morgani* strains (table). The rate of

TABLE

Growth characteristics of strains of *P. mirabilis* and *P. morgani* in urine and in nutrient broth in the presence and absence of urea 1% (w/v)

Organism	Strain no.	P/S* type	Source	Generation time† (min) at 37°C in pooled urine	Generation time (min) at 37°C in nutrient broth		pH after growth for 2 h in nutrient broth (pH 7-2)	
					With urea	Without urea	With urea	Without urea
<i>P. mirabilis</i>	F40010	P0/S1	Faeces	36	55	55	9.06	7.28
	F51259	P0/S4	Faeces	37	60	55	9.11	7.25
	F35966	P2/S1	Faeces	49	65	60	9.10	7.30
	38116/77	P3/S1,8	Urine	34	60	56	8.90	7.25
	19616	P3/S1,8,13	Urine	45	64	60	8.95	7.35
	50253	P3/S1,13	Urine	37	58	58	8.90	7.34
<i>P. morgani</i>	61900/77	...	Urine	78	62	58	8.11	7.34
	63401/77	...	Urine	87	65	60	7.5	7.30
	63405/77	...	Faeces	84	58	58	7.55	7.32
	63696/82	...	Faeces	70	56	55	7.45	7.30

* Proticene production-proticene sensitivity type;

† Generation time = time for doubling of optical density.

growth of the *P. mirabilis* strains decreased dramatically after incubation for 2 h and ceased well before the growth rate of the *P. morgani* strains started to decline. After 2 h, the pH of the cultures of the *P. mirabilis* strains was *c.* 9 whereas it was between pH 7-8 with the *P. morgani* strains. The highly alkaline conditions, not the lack of nutrients, caused the *P. mirabilis* cells to stop growing.

P. morgani strains were much slower than *P. mirabilis* strains to produce alkaline urine. The reason for this delay was examined by determining the rate of change of pH in conditions in which the strains of both species were growing at approximately the same rate. *P. morgani* strains grew faster in nutrient broth (*g=c.* 60 min) than in urine, and the generation time was similar to that of *P. mirabilis* strains growing in nutrient broth (table). For all strains of both species, the addition of urea 1% (w/v) to nutrient broth did not enhance growth and may have been slightly inhibitory. In the absence of urea there was no significant rise of the pH of the cultures. In the presence of urea there was a substantial and rapid rise of pH in all the *P. mirabilis* cultures whereas in the *P. morgani* cultures growing at the same rate the rise of pH was small and slow (table).

Investigations were made to determine whether the failure of *P. morgani* strains to form very alkaline conditions rapidly in the presence of urea was because of their greater buffering capacity or because the development of alkaline conditions was masked by the formation of carbonic acid by carbonic anhydrase from the CO₂ liberated from urea by urease. The results showed that neither explanation was correct; *P. morgani* strains were no more efficient than *P. mirabilis* strains in lowering the pH of cultures made alkaline by the addition of ammonium ions. Repeated assays of sonicated cells of the *P. morgani* strains failed to detect the presence of any carbonic anhydrase.

An analysis of the urease levels of strains of both species growing in nutrient broth showed that in *P. mirabilis* strains only trace amounts of enzyme were synthesised in the absence of urea, but that urease synthesis was induced by the addition of urea which brought about the immediate liberation of large quantities of ammonia (figure). However, the *P. morgani* strains synthesised urease in the absence of urea in amounts

FIG.—Ammonia formation (●) by and urease specific activity (○) of strains grown in nutrient broth in the presence (—) and absence (---) of urea; (a) *P. mirabilis* F51259, (b) *P. mirabilis* 61900/77, (c) *P. mirabilis* 38116/77 and (d) *P. mirabilis* 63405/77. The results obtained with the other strains were similar to those illustrated for organisms of the same species.

and at a rate far in excess of that of the *P. mirabilis* strains and the addition of urea caused only a small increase in the amount and rate of urease synthesis. Nevertheless, the amount and rate of ammonia formation by the *P. morgani* strains were much smaller and slower than by the *P. mirabilis* strains (figure).

These observations would be explained if urea did not pass easily into the cells of *P. morgani*. However, spheroplasts of *P. morgani* were stable in a solution of 0.5 M sucrose in water but lysed within seconds when suspended in an equiosmolar solution of urea in water. Thus, although urea rapidly passed into *P. morgani* cells that had high internal concentrations of urease, only small amounts of ammonia were formed. The reason for this is still being investigated.

DISCUSSION

The ability of a strain of *Proteus* to initiate and establish infection in the urinary tract is the outcome of the interplay of several factors that include the dynamics of urine flow, bacterial adhesion to and survival upon the epithelium of the urinary tract, the formation of ammonia and the development of alkaline conditions by urea degradation and the growth rate and survival of the infecting organism. The adhesins and fimbriae of *P. mirabilis* and *P. morgani* are very similar (Old and Adegbola, 1982) and it is unlikely that *P. morgani* strains are less likely than *P. mirabilis* strains to establish urinary-tract infections because of inability to adhere to epithelial cells. However, it is clear from the results of the present study that *P. morgani* is at a major disadvantage in establishing infection for two reasons. Firstly, unlike *P. mirabilis*, *P. morgani* cells cannot make urine alkaline rapidly by liberating ammonia from urea and thereby promote conditions favourable for the development of renal stones and tissue damage. This finding was surprising; although the urease of *P. morgani* is different from that of *P. mirabilis* (Senior *et al.*, 1980) it is formed constitutively and is more active than *P. mirabilis* urease. Others have reported similar findings (Rosenstein, Hamilton-Miller and Brumfitt, 1981). It is possible that *P. morgani* cells produce an inhibitor that prevents urease degrading urea and as cells grow in the presence of urea the synthesis of the inhibitor is repressed and the restraint upon urea degradation slowly overcome. This hypothesis has not been investigated experimentally.

Secondly, the mean generation time for the *P. morgani* strains growing in urine was about twice as long as that for the *P. mirabilis* strains. Because the growth of bacteria in the urine of the bladder *in vivo* takes place under "semi-continuous" cultural conditions, the only parameters of bacterial growth important for infection are the mean generation time during logarithmic growth and the maximum population reached in the stationary growth phase. Clearly, the longer generation time puts *P. morgani* at a disadvantage as a potential pathogen in urinary-tract infection.

Studies of the relatedness of the DNA from *P. mirabilis* and *P. morgani* strains suggest that these organisms are quite distinct (Brenner *et al.*, 1978); this may explain their different growth rates in urine. Moreover, *P. morgani* has an absolute requirement for pantothenic acid and L-cystine (Porter and Meyers, 1945) and the difference in growth rates will be more pronounced if urine is deficient in either metabolite. The shorter generation time for *P. morgani* strains grown in broth may indicate that the amounts of pantothenic acid and L-cystine in urine are growth-limiting factors for *P. morgani*, but this was not investigated further.

Unfortunately it was not possible to determine the maximum stationary population reached by the *P. mirabilis* and *P. morgani* strains grown in urine; in the absence of the physiological buffering system that exists *in vivo*, *P. mirabilis* cells grown in urine in batch culture reached a stationary population within a few hours because the pH of the culture had reached an unphysiological value (>9). Such pH values would not be reached *in vivo* and the stationary population might be higher than that permitted by in-vitro conditions.

This in-vitro study has shown that *P. morgani* strains have two main disadvantages that may affect their ability to cause urinary-tract infections. The slower growth rate in urine and poor ability to form alkaline conditions, compared with *P. mirabilis* strains, have been considered as separate entities, but it is possible that *in vivo*, they may interact to produce an even greater disadvantage for *P. morgani* than the mere sum of their effects.

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