HAEMAGGLUTININS AND FIMBRIAE OF MORGANELLA, PROTEUS AND PROVIDENCIA

D. C. OLD AND R. A. ADEGBOLA

Bacteriology Department, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY

SUMMARY. One hundred and thirteen strains of Morganella, Proteus and Providencia, grown in different cultural conditions, were examined for their ability to produce haemagglutinins (HAs). Three main kinds of HA (MS, MR/K and MR/P) were detected, and 89% of the 112 HA+ strains were capable of producing two or three of the different HAs in the same or different cultures. The properties of the three HAs were partly defined and the difficulties of identifying their separate HA activities when present together are discussed. Electronmicroscopic examination of bacteria from HA+ cultures showed at least six distinct fimbrial types, the properties of which are described. We tried, with limited success, to correlate the presence of the different HAs with that of the different fimbrial types. The significance of our findings is reviewed in the light of recent taxonomic changes for this group of enterobacteria. The distribution of HAs and fimbriae in the species of Morganella, Proteus and Providencia is more complex than that so far described for other genera of Enterobacteriaceae.

INTRODUCTION

Many of the kinds of haemagglutinin (HA) (adhesin) found in Enterobacteriaceae are associated with the presence of fimbriae of different kinds (Duguid and Old, 1980). These adhesins may aid colonisation, and in enterobacteria four main classes have been described (Duguid and Old, 1980): (i) the mannose-sensitive haemagglutinin (MS-HA), produced by many strains of different species and genera, is associated with type-1 fimbriae (Duguid, Anderson and Campbell, 1966); (ii) the mannose-resistant and eluting (MRE) HAs, produced commonly by c. 60% of strains of Escherichia coli, have different erythrocyte specificities (Duguid et al., 1955; Duguid, Clegg and Wilson, 1979) and are generally associated with MRE fimbriae of different kinds though some are non-fimbrial (Duguid et al., 1979; Duguid and Old, 1980; Ip et al., 1981); (iii) the MR/K-HA (Duguid and Old, 1980), first observed in Klebsiella (Duguid, 1959), agglutinates tannic acid-treated, but not fresh erythrocytes and is associated with thin, type-3 fimbriae in MR/K+ strains of Klebsiella and Serratia (Duguid, 1959; Thornley and Horne, 1962); (iv) different MR/P-HAs (Duguid and Old, 1980) which react with different species of untanned erythrocytes are found in strains of Proteus (Duguid and

Received 15 Mar. 1982; accepted 13 Apr. 1982.
Gillies, 1958; Coetzee, Pernet and Theron, 1962; Shedden, 1962). Although different kinds of fimbriae have been described in species of *Proteus* (Duguid and Gillies, 1958; Coetzee et al., 1962; Shedden, 1962; Hashimoto et al., 1963; Hoener, 1965; Silverblatt, 1974; Silverblatt and Ofek, 1978), it is not known which, if any, of them are associated with the MR/P-HAs, the least studied of the enterobacterial HAs.

Preliminary experiments with a few strains of *Proteus* and *Providencia* indicated a complex situation in that some strains simultaneously produced MS-, MR/K- and MR/P-HAs and carried different types of fimbriae (Duguid and Old, 1980; Old and Scott, 1981). Because there is little information about the distribution of haemagglutinins in *Proteus* species, it seemed worthwhile to survey strains of the proteus-providence group for their adhesins and fimbriae. Such an investigation is especially relevant in view of the finding that these organisms are distantly related to other enterobacteria (Brenner et al., 1978). In this paper we follow the recommended nomenclature of Brenner et al. (1978).

**Materials and methods**

**Bacterial strains.** A collection of 113 strains of different species of *Morganella*, *Proteus* and *Providencia* from various sources and of different biotype and serotype, was examined: (i) 14 strains of *M. morgani* included eight strains from the National Collection of Type Cultures, Colindale Avenue, London NW9 5HT (NCTC nos. 232, 235, 1707, 2815, 2818, 5845, 10041, 10375) and six strains from the collection of Dr B. Senior, Department of Bacteriology, University of Dundee Medical School, Dundee (BS series); (ii) one strain of *P. myxofaciens* ATCC19692 was a gift from Dr D. J. Brenner, Center for Disease Control, Atlanta, GA; (iii) 16 strains of *P. mirabilis* included 10 NCTC strains (nos. 60, 2896, 3177, 5887, 6197, 6369, 7827, 8309, 9559 and 10374) and six strains from the BS series; (iv) 19 strains of *P. vulgaris* included 11 NCTC strains (nos. 401, 4175, 4635, 8311, 8313, 10015, 10020, 10031, 10034, 10376 and 10740) and eight strains from the BS series; (v) 19 strains of *Prov. alcalifaciens* included three NCTC strains (nos. 6933, 6934 and 8113) and 16 strains representative of *Providencia* O serogroups 1, 3, 10, 11, 13, 14, 21, 22, 29, 34, 36, 37, 42, 46, 53 and 62 (Penner et al., 1976) from the collection of Dr J. L. Penner, Department of Medical Microbiology, University of Toronto (JLP series); (vi) 27 strains of *Prov. rettgeri* included nine NCTC strains (nos. 7475, 7476, 7477, 7478, 7479, 7480, 7481, 8893 and 10377), 17 strains from the JLP series, representative of *Prov. rettgeri* O serogroups, 1, 2, 5, 11, 12, 15, 17, 25, 26, 31, 33, 36, 42, 49, 69, 73 and 83 (Penner, Hinton and Hennessey, 1976) and one strain from the BS series; (vii) 17 strains of *Prov. stuarti* included three NCTC strains (nos. 10834, 10836 and 10318) and 14 strains from the JLP series, representative of *Providencia* O serogroups 4, 15, 17, 24, 25, 26, 43, 44, 49, 52, 55, 56, 57 and 63 (Penner et al., 1976; Penner et al., 1979).

Strains of *Morganella*, *Proteus* and *Prov. rettgeri* were subdivided into species by their reactions in tests for urease production, indole production, and fermentation of adonitol, inositol, mannose, mannitol, melezitose or trehalose (McKell and Jones, 1976). Strains of *Prov. alcalifaciens* and *Prov. stuarti* were identified on the basis of their fermentation of adonitol, inositol or trehalose and their gas production from glucose fermentation (Edwards and Ewing, 1972).

**Culture media.** Nutrient Broth No. 2 (CM67) and Nutrient Agar (CM 3) were from Oxoid Ltd. Phosphate-buffered agar (PBA) was nutrient agar buffered at pH 7.0 with 0.36% (w/v) KH$_2$PO$_4$ and 0.64% (w/v) Na$_2$HPO$_4$. Broth was dispensed in 10-ml amounts in cottonwool-stoppered test tubes, and agar media in 20-ml amounts in 90-mm plastic petri dishes.

**Cultural conditions.** Each strain was cultured serially in nutrient broth, in "aerobic" and static conditions, selective for the fimbriate phase of many enterobacteria (Duguid et al., 1966; Old and Duguid, 1970): (i) eight times at 3-day intervals at 37°C; (ii) six times at 4-day intervals at 30°C; (iii) six times at 7-day intervals at ambient temperature (c. 20°C). Broth cultures were
centrifuged at 2000 g for 15 min and the bacteria concentrated (x 100) by resuspension in saline (NaCl 0.85\% w/v).

Each strain was grown also on PBA at 37°C for 24 h and at c. 20°C for 48 h; bacteria from these cultures were suspended in saline to c. 10^{12} colony-forming units (cfu)/ml.

**Erythrocytes.** Bacterial suspensions of all cultures of each strain were tested routinely in haemagglutination tests with erythrocytes of fowl (F), guinea-pig (G), horse (H), man, group O (M), ox (O), and sheep (S). Cultures of a few strains were tested also with erythrocytes from a rhesus monkey, pig, rabbit, rat and toad. Blood samples from laboratory breeds of guinea-pigs, rabbits, rats, toads and domesticated fowls were supplied by the Animal Services Unit, Dundee Medical School; those from oxen and pigs were from unselected breeds after slaughter at the local abattoir; human cells were from the Blood Transfusion Service, Ninewells Hospital, Dundee. Others were from commercial sources: horse (Oxoid); sheep (Gibco Biocult Ltd, Paisley, Strathclyde PA3 4EP); and rhesus monkey (Flow Laboratories Ltd, Irvine, Strathclyde KA12 8NB). Their collection and preparation followed previously described methods (Duguid et al., 1979). The red-cell suspensions in saline were stored at 4°C and used within 7 days.

For tannic acid treatment, equal volumes (5 ml) of washed erythrocytes (usually ox) 3\% (v/v) and tannic acid (BDH Chemicals Ltd, Poole, Dorset BH12 4NN) 0.01\% (w/v) in saline were mixed, incubated at 37°C for 15 min, washed thrice and resuspended in saline to 3\% (v/v).

**Haemagglutination tests.** Suspensions of bacteria grown in broth or on PBA were tested for:
(i) agglutination in the presence and absence of D-mannose, on rocked tiles at 4°C and at ambient temperature (c. 20°C) (Duguid et al., 1979); (ii) settling tests with the two strains for which agglutination tests failed with all erythrocytes; thus, equal volumes (0.5 ml) of a series of doubling dilutions of bacterial suspensions and 1\% (v/v) erythrocyte suspension in saline, or in 2\% (w/v) D-mannose in saline, were mixed in 7.5 x 1-cm tubes and allowed to settle at 4°C for 18–24 h. Haemagglutination was indicated by diffuse settling of the erythrocytes.

**Haemagglutinating power (HP) of cultures was calculated by the method of Duguid (1959).**

**Electronmicroscopy.** Two methods were used for the preparation of bacteria before staining. (a) Bacteria were washed once in saline and then twice in deionised water by centrifugation. Samples of resuspended bacteria (c. 4 x 10^8 cfu/ml) and bacitracin solution (100 \mu g/ml) were mixed and applied to copper grids covered with carbon-coated Formvar. (b) In most experiments, however, bacteria from cultures were centrifuged, resuspended in deionised water (c. 4 x 10^8 cfu/ml), mixed with a portion of bacitracin solution (100 \mu g/ml), and the bacteria washed once or twice (depending on the amount of extraneous material in the background of control preparations) in situ on the grid. Bacteria were stained negatively for 1 min with either 0.3\% (w/v) uranyl acetate (Agar Aids, Bishop's Stortford, Herts) in deionised water (pH 4.6) or 1\% (w/v) methylamine tungstate, pH 6.5 (EMscope Laboratories, Ashford, Kent). Grids were examined with a Jeol 100CX microscope and micrographs taken of the different kinds of fimbriae observed on bacteria from different preparations.

**RESULTS**

**Haemagglutinin production**

Each of the 113 strains was assessed for its ability to produce HAs in tests with bacterial cells grown in various conditions. In the agglutination tests, 111 strains (98\%) were haemagglutinating and the only two that were not were strains of *Prov. stuartii* (table I). The types of haemagglutinin detected were MS, MR/K and MR/P; 99 (89\%) of the 111 HA+ strains were capable of producing more than one kind of haemagglutinin.

**MS-HA.** The presence of MS-HA, detected usually by the MS agglutination of fowl or guinea-pig erythrocytes, was demonstrated in bacterial suspensions of cultures of c. 23\% of the 113 strains (table I). The production of MS-HA occurred usually in aerobic, static broth cultures of MS-HA+ strains independently of temperature, but,
unlike other MS-HA+ enterobacteria, serial broth culture did not significantly enrich for the MS-HA+ phase of MS+ strains in this series. Accordingly, MS-HA was produced and detected irregularly and, at best, in moderate amounts in broth cultures that had HP values of from 1 to 300 only. The ability to produce MS-HA, however, was different for the different species examined. None of the 14 strains of *M. morgani* or the single isolate of *P. myxofaciens* was MS-HA+ in any broth culture of the several series; and, whereas only four of the 35 strains of *P. mirabilis* and *P. vulgaris* were MS-HA+, 22 of the 63 strains of *Providencia* produced MS-HA. Strains that were MS-HA+ invariably produced other HA's (table I).

**MR/K-HA.** The MR/K-HA, active against tanned erythrocytes, was the HA most widely distributed in this series of strains, being produced by all but two of them. When grown serially in broth, cultures of MR/K-HA+ strains were usually strongly haemagglutinating with HP values of 400–1600, for tanned erythrocytes, in the first or second of the broth cultures in a series. In addition, the MR/K-HA was produced by agar-grown cultures of MR/K-HA+ strains. MR/K-HA+ strains generally produced MR/K-HA more regularly in broths cultured at low (20° or 30°C) rather than high (37°C) temperature. Indeed, c. 21% of MR/K-HA+ strains were MR/K-HA− when bacteria from 37°C-grown cultures were tested.

**MR/P-HA.** The MR/P-HAs—detected by their mannose-resistant haemagglutination of some or all of the following species of untanned erythrocytes: fowl, guinea-pig, horse, pig and sheep—were widely distributed, being produced by 85 (75%) of the 113 strains. The distribution, however, was not uniform; thus, they were present in all but one of the 50 strains of *Morganella* and *Proteus* but in only 36 of 63 strains of *Providencia* (table II). The MR/P-HAs were formed in serial broth cultures and were more regularly produced at low (20° or 30°C) than at high (37°C) temperature. Indeed, some 26% of MR/P+ strains did not form MR/P-HAs in cultures grown at 37°C. Although a few strains formed their MR/P-HAs in large amounts, i.e., HP values of MR/P-HA+ cultures of from 350 to 1400 in tests with the

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of strains tested</th>
<th>Number (and percentage) of strains forming haemagglutinins in the following combinations*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MR/K only</td>
</tr>
<tr>
<td><em>M. morgani</em></td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><em>P. myxofaciens</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td><em>Prov. alcalifaciens</em></td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td><em>Prov. rettgeri</em></td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td><em>Prov. stuarti</em></td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>All</td>
<td>113</td>
<td>12 (10-6)</td>
</tr>
</tbody>
</table>

* None of the strains produced haemagglutinins in the following combinations: MS only, MR/P only or MS with MR/P.
† Of two *Prov. stuarti* strains that were HA− in agglutination tests, one gave weak MR haemagglutination of fowl and guinea-pig cells in a settling test.
HAEMAGGLUTININS AND FIMBRIAE OF PROTEI

**TABLE I**

Patterns of haemagglutination given by MR/P+ strains of Morganella, Proteus and Providencia

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of strains with MR/P-HA pattern* that was</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FGHMS</td>
</tr>
<tr>
<td>M. morgani</td>
<td>14</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>15</td>
</tr>
<tr>
<td>P. myxofaciens</td>
<td>0</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>5</td>
</tr>
<tr>
<td>Prov. alcalifaciens</td>
<td>0</td>
</tr>
<tr>
<td>Prov. rettgeri</td>
<td>2</td>
</tr>
<tr>
<td>Prov. stuarti</td>
<td>0</td>
</tr>
</tbody>
</table>

* Erythrocytes agglutinated were species of: F = fowl; G = guinea-pig; H = horse; M = man; S = sheep.

most sensitive erythrocyte species, most of them produced MR/P-HA only in moderate amounts—HP values in positive cultures of 60–350.

The pattern of haemagglutination given by MR/P-HA+ cultures, whether broad or narrow, was characteristic for any MR/P+ strain. For example, a strain that gave MR/P-HA for erythrocytes of fowl, guinea-pig, horse, man and sheep (type FGHMS) consistently gave that MR/P-HA pattern, although quantitative differences observed with different cultures sometimes made it difficult to recognise weak reactions with some erythrocytes (table I).

The bacteria of different species showed recognisable trends in the different MR/P-HA patterns demonstrable. Thus, the MR/P-HAs of Morganella and P. mirabilis (98% of which were MR/P-HA+) were, with one exception, of the broad-spectrum type (FGHMS, table I). The exceptional isolate of P. mirabilis was unusual not only in its MR/P-HA (type FGS) but also in its production of MS-HA. The one isolate of P. myxofaciens produced an MR/P-HA of narrow spectrum (table I). The diversity of MR/P-HA types in the other species was notable: P. vulgaris exhibited seven patterns, Prov. alcalifaciens six, Prov. rettgeri ten and Prov. stuarti two. The MR/P-HAs of Providencia tended to be narrower in spectrum of activity than those of P. vulgaris (table I).

Other HAs. Dense bacterial suspensions of the two Prov. stuarti strains, which were HA– in agglutination tests, were examined with 11 erythrocyte species by the settling-test method. One strain gave weak MR agglutination of fowl and guinea-pig erythrocytes; the other did not agglutinate any of the 11 erythrocyte species. The other 111 strains, HA+ by the agglutination test, were not routinely screened by the settling method.

**Fimbriae**

Various types of fimbria were observed on bacteria from haemagglutinating cultures of different species of Morganella, Proteus and Providencia.

(a) The predominant type of HA produced was the MR/K type (table I).
Electronmicroscopic examination of bacteria from MR/K-HA\textsuperscript{+} cultures of strains of \textit{Providencia} that produced the MR/K-HA only, showed peritricously arranged non-channelled fimbriae of external diameter of 4–5 nm (fig. 1). ‘Thin’ fimbriae, indistinguishable from those shown in fig. 1, were present on at least 60\% of the bacterial cells from MR/K-HA\textsuperscript{+} cultures (HP values of 350–1600) of other strains of \textit{Providencia} and strains of \textit{Morganella} and \textit{Proteus} that formed other HAs along with MR/K-HA (table I). On those bacteria, however, other types of fimbria were often

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures1-4.png}
\caption{Figs 1–4.—1. Thin (4–5 nm) fimbriae on bacterium from MR/K-HA\textsuperscript{+} culture of \textit{Prov. stuarti}. 2. Thin (4–5 nm) fimbriae and thicker fimbriae (arrowed) on bacterium from a multiply haemagglutinating (MR/K-HA\textsuperscript{+}, MR/P-HA\textsuperscript{+}) broth culture of \textit{P. mirabilis}. 3. Thick (8–9 nm), channelled fimbriae on bacterium from a multiply haemagglutinating (MS-HA\textsuperscript{+}, MR/K-HA\textsuperscript{+}) broth culture of \textit{Prov. stuarti}. 4. Thick (8–9 nm) fimbriae on bacterium from broth culture of \textit{Prov. stuarti} strain NCTC10834 which formed weak MR-HA (see text). EM. All stained with methylamine tungstate. The bar marker represents 200 nm.}
\end{figure}
seen along with the thin fimbriae (fig. 2). Methods were not available for the isolation of MR/K-HA- variant or mutant bacteria from MR/K+ strains and so it was difficult, in view of the rarity of MR/K-HA- strains in this series, to acquire further evidence that might indicate a correlation between the presence of these thin fimbriae and the production of MR/K-HA. However, these fimbriae were not observed in cultures of the two MR/K- strains of *Providencia stuartii* that did not form MR/K-HA in any of the cultural conditions used. Furthermore, examination of strains in which production of MR/K-HA was favoured by cultivation at 20° or 30°C rather than at 37°C showed that thin fimbriae were present on only c. 1–2% of the bacterial cells from MR/K-HA- cultures grown at 37°C, but on c. 60–80% of the bacterial cells from MR/K-HA+ cultures grown at 20° or 30°C.

(b). Bacteria from haemagglutinating cultures of strains of *Providencia* that produced both MS-HA and MR/K-HA bore thin fimbriae, similar to those found on bacteria from MR/K-HA+ cultures (see fig. 1), and thicker, channelled fimbriae of external diameter of 8–9 nm (fig. 3). The latter were similar to the channelled fimbriae described on bacterial cells from MS-HA+ cultures of *Providencia* (Old and Scott, 1981) and considered to be associated with the presence of MS-HA. We observed fimbriae of this kind on bacteria from MS-HA+ cultures of all MS-HA+ strains in this series, but fimbriae morphologically similar to those shown in fig. 3 were observed also on bacteria from MS-HA- cultures of strains that did not produce MS-HA in any condition of culture.

(c). Bacteria from cultures of the strain of *Providencia stuartii* (NCTC10834), which gave weak mannose-resistant (MR) agglutination of fowl and guinea-pig erythrocytes but only in the settling tests, were richly fimbriated; the fimbriae were thick, 8–9 nm external diameter and probably channelled (fig. 4), i.e., similar to those fimbriae described in (b). Bacteria from cultures of other strains of *Providencia stuartii*, which in agglutination tests had given only MR/K-HA reactions, were found on electron-microscopy to possess, in addition to the thin fimbriae (fig. 1) present on most of the cells and associated with MR/K-HA+ bacteria, fimbriae similar to those shown in fig. 4. The latter thick fimbriae were found on c. 10–20% of the bacteria from those MR/K-HA+ cultures of *Providencia stuartii*. Antiserum prepared against *Providencia stuartii* strain NCTC10834 coated the thick fimbriae of the same strain (fig. 5) and also those fimbriae on bacteria of the latter strains of *Providencia stuartii* which apparently did not produce the weak MR-HA (type FG) produced by strain NCTC10834. The fimbriae described in (a) and (b) above were not coated with this antiserum.

(d). On very few bacteria from cultures of the only strain of *Providencia stuartii* that was non-haemagglutinating in agglutination and in settling tests, were very thin fimbriae of diameter c. 2–3 nm in numbers from 30 to 100 per bacterial cell (fig. 6). The finding, however, that only c. 1% of the bacteria were fimbriate might explain why that strain was non-haemagglutinating even in settling tests.

(e). Many (c. 75%) strains of *Morganella*, *Proteus* and *Providencia* that were multiply haemagglutinating produced MR/K-HA and MR/P-HA together in cultures, especially when grown at 30°C. Because there were no methods for obtaining phenotypically MR/K-HA-, MR/P-HA+ cultures they were examined only when present together. Many thin fimbriae (fig. 1) were usually present on most of the bacterial cells from these cultures as well as 100–200 thick fimbriae of external diameter 7–8 nm and probably channelled (fig. 7). Thick fimbriae were observed on bacteria
from MR/P-HA+ cultures of strains of *Morganella* and *P. mirabilis* that produced MR/P-HA with broad-spectrum activity, i.e., type FGHMS, and of strains of *P. vulgaris* and *Providencia* that produced MR/P-HA with narrow-spectrum activity (e.g., type F). However, on bacteria from MR/P-HA+, MR/K-HA+ cultures from some other strains in this series, thin fimbriae only were observed.

(f). On c. 10–20% of the bacteria from cultures of a strain of *Prou. alcalifaciens* which, when cultured at 30°C, produced MR/K-HA only, yet another type of thick fimbria was observed, 6–7 nm in diameter and non-channelled (fig. 8) along with the

**Figs. 5–8.** 5. Fimbriae of *Prou. stuarti* strain NCTC10834 coated with antiserum prepared against the same strain. 6. Very thin (2–3 nm) fimbriae on bacterium of non-haemagglutinating strain of *Prou. stuarti* (see text). 7. Thick (7–8 nm) fimbriae on bacterium from multiply haemagglutinating (MR/K-HA+, MR/P-HA+) broth culture of *P. mirabilis*. 8. Thick (6–7 nm), non-channelled fimbriae on bacterium from MR/K-HA+ broth culture of *Prou. alcalifaciens*. EM. All stained with methylamine tungstate. The bar marker represents 200 nm.
thin fimbriae usually found on MR/K-HA+ bacteria. This strain, however, gave no demonstrable MR/P-HA reactions even in settling tests, and was MS-HA-.

**DISCUSSION**

Results from the present study with strains of *Morganella*, *Proteus* and *Providencia* confirmed the earlier findings obtained for a few strains (Duguid and Old, 1980; Old and Scott, 1981), viz., that strains produced one or more of the haemagglutinins MS, MR/K or MR/P—as defined by Duguid and Old (1980)—and that most strains formed more than one. Thus, the distribution of types of HAS in *Proteus* species is much more complex than indicated from earlier studies, which demonstrated only the MR/P-HA (Duguid and Gillies, 1958; Coetzee et al., 1962; Shedden, 1962).

In the study of bacterial strains that form more than one type of HA with different but overlapping affinities, it is often difficult to identify the separate presence of each HA and to determine the full range of its properties. Some activities of the one HA may mask or mimic some of those of the other.

Previously, in *E. coli* strains that produced MS-HA or an MRE-HA, or both or neither, and in *K. aerogenes* strains that produced MS-HA or MR/K-HA, or both or neither, the range of properties of each HA was first determined in strains forming only the one kind, and then the presence of each HA in strains forming both kinds was determined in tests made in conditions such that only the one or other kind was present and active (Duguid et al., 1955, 1979; Duguid, 1959). Thus, the MR/K-HA of MS+MR/K+ klebsiellas was demonstrated in tests with tannic acid-treated ox erythrocytes, against which the MS-HA is inactive even in the absence of D-mannose, and the MS-HA was demonstrated in tests with the fresh red cells of guinea-pig, fowl, horse and other species against which, when not treated with tannic acid, the MR/K-HA is inactive.

With strains such as those of *Proteus* and *Providencia*, in cultures of which at least three different HAS (MS, MR/K and MR/P) may be present simultaneously, the task of separately identifying and characterising each HA is even more difficult. Though it has been possible to identify the presence of each of the HAS in strains that produced all three HAS when each was present in large amount, it has not been possible to determine the full range of their properties. We may, moreover, have failed to detect the presence of a small amount of one kind of HA—e.g., MS—in a strain in which its activity was masked by that of a large amount of another HA—e.g., MR/P—active on the same species of red cells.

**MR/K-HA.** In the single strain of *P. vulgaris* and the 11 strains of *Providencia* that we identified as forming only MR/K-HA (table I), the properties of the HA resembled those of the MR/K-HA of *K. aerogenes* described by Duguid (1959). The bacteria strongly agglutinated tannic acid-treated ox red cells in the presence and in the absence of D-mannose, and did not agglutinate the untreated red cells of fowl, guinea-pig, horse, man, ox and sheep.

**MS-HA.** None of our strains possessed only the MS-HA, although that HA could be partly characterised in strains of *Providencia* that possessed also the MR/K-HA, but not an MR/P-HA. It was assumed that in these strains the MR/K-HA, like that in the strains with it alone, would be inactive with red cells not treated with tannic acid. In tests with such untreated cells, the MS-HA of strains of *Providencia* was shown to have
a pattern of specific activities similar to those of the classical MS-HA of *E. coli*. It agglutinated fowl, guinea-pig and horse cells strongly, human cells moderately, sheep cells weakly and ox cells not at all; all these activities could be inhibited by D-mannose.

**MR/P-HA.** None of our strains possessed only an MR/P-HA, but it was possible to characterise partly such HAs in the strains of *Morganella*, *Proteus* and *Providencia* that possessed also the MR/K-HA but not the MS-HA. On the assumption that the MR/K-HA behaved only as described above, the properties of the MR/P-HAs were demonstrated in tests made with untanned red cells. Because their haemagglutinating activities with the cells of any species were unaffected by the addition of D-mannose, it could be concluded that none of them was attributable to the presence of MS-HA. There appeared, however, to be several kinds of MR/P-HA having specific affinities for the various species of red cells (table II). At this stage, these MR/P-HAs have been classed together, for convenience, on the basis of their mannose resistance and their activity with fresh erythrocytes, although future studies may well reveal subgroups. MR/P+ cultures of the more common strains agglutinated fowl red cells very strongly, guinea-pig, horse and sheep cells less strongly, and human cells (and those of oxen; data not shown) weakly, or more often, not at all. MR/P+ cultures of the less common strains agglutinated only one of the several species of red cells tested, those of fowl, guinea-pig, horse or man. MR/P-HAs which agglutinated the untreated red cells of any species also agglutinated the cells after treatment with tannic acid.

Although, as shown in table II, we distinguished 18 patterns of MR/P activity, we do not conclude that there are as many as 18 distinct HAs because some of our observed differences in HA patterns may reflect merely differences in the amount of the same HA in different strains. Thus, some of the patterns in which fowl red cells and only one or two other species of cells were agglutinated may have been given by cultures producing small amounts of HA which, if it had been produced in larger amount, would have agglutinated also other cells of less agglutinable species. That the MR/P-HAs, however, were different from the MRE-HAs of *E. coli* that also give diverse patterns with fresh erythrocyte species, was shown by their different properties. Thus, whereas the MRE-HAs are produced best in cultures grown on phosphate-buffered agar (PBA) at 37°C but not at 18°C (Duguid *et al.*, 1979), and their HA activity shows the phenomenon of “elution” (Duguid *et al.*, 1955), the MR/P-HAs are not produced in cultures grown on PBA, and are formed well at temperatures (c. 20°C) at which MRE-HAs are not produced. Furthermore, the HA activity of the MR/P-HAs could not be “eluted”. Whether there are yet other kinds of MR/P-HA active only on species of red cells different from those we tested is unknown.

**Multiple HAs.** Accepting the characterisation of the three main kinds of HA just described, we identified the presence of different HAs in strains with multiple HAs (table II) on the following bases. If any culture of a strain agglutinated, in the presence of D-mannose, any species of red cells untreated with tannic acid, an MR/P-HA was judged to be present; if any culture of a strain, when tested in the presence of D-mannose, agglutinated tannic acid-treated red cells of any species not agglutinated when untreated, an MR/K-HA was judged to be present. Because the MR/P-HA in most strains failed to agglutinate untreated ox red cells, the MR/K-HA in these strains could be identified by its reaction with treated ox cells. In the few (< 10%) strains with an MR/P-HA active on untreated and treated ox cells (data not shown), the presence of the MR/K-HA was identified by its reactions with the treated cells of another species,
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e.g., sheep or human, against which that MR/P-HA was inactive, or by its reaction
with treated ox cells in a culture grown in conditions, e.g., PBA at 37°C, in which the
strain failed to form sufficient MR/P-HA to agglutinate ox cells. If any culture of a
strain agglutinated in the absence, but not in the presence, of D-mannose the
erythrocytes of fowl, guinea-pig, horse, sheep or man, the MS-HA was judged to be
present. Because the tests were done with cells untreated with tannic acid, difficulty in
detection of the MS-HA arose only from the simultaneous presence of an MR/P-HA in
the strain. In many such strains, the MR/P-HA was inactive with guinea-pig or horse
red cells and the MS-HA could be identified by its reactions with these cells. In strains
forming an MR/P-HA active on those species of red cells most susceptible to the
activity of MS-HA, the presence of the latter HA could sometimes be detected by tests
with cultures grown in conditions, e.g., in broth at 37°C, in which the MR/P-HA was
produced in smaller amounts, or not at all.

HAS and fimbriae. The 12 strains of Proteus and Providencia that formed
MR/K-HA only (table I) possessed fimbriae, 4–5 nm in width, which resembled the
type-3 fimbriae associated with the MR/K-HA in K. aerogenes (Duguid, 1959); and
morphologically similar fimbriae were present on all other MR/K+ strains and absent
from the only two MR/K− strains in the series. The observation by Adegbola (1981)
that bacteria from MR/K-HA+ cultures of many strains generally bore more thin
fimbriae after staining with methylamine tungstate (pH 6-5) than with uranyl acetate
(pH 4-6) prompted us to determine the effect of acid on these fimbriae. Exposure to
acid (0.1M HCl for 5 min at 37°C) resulted in the loss of MR/K-HA activity and thin
fimbriae from cultures of all MR/K+ strains except P. vulgaris (unpublished data).
These demonstrations of the extreme acid lability of the thin fimbriae help explain our
previous failure to show large numbers of thin fimbriae in many MR/K+ cultures of
Providencia (Old and Scott, 1981) and provide additional evidence of a probable
correlation between the presence of MR/K-HA and that of thin fimbriae.

All strains that formed MR/P-HA also formed fimbriae but it was difficult to
associate a particular kind of MR/P-HA with fimbriae of a particular form, a problem
that was exacerbated by the absence of strains that formed MR/P-HA only.
Nevertheless, bacteria from MR/K-HA+, MR/P-HA+ strains of M. morgani and P.
mirabilis, that produced a broad-spectrum MR/P-HA (type FGHMS), bore thin
fimbriae, probably associated with MR/K-HA, and thicker, 7–8 nm fimbriae, possibly
associated with that MR/P-HA. Similar kinds of fimbriae were described on a single
isolate of P. mirabilis by Silverblatt (1974) who reported that the fimbriae on bacteria
from log-phase or stationary-phase broth cultures were, respectively, predominantly
thin or thick. Had we been able to reproduce these observations with our larger series
of strains, our suggestion that the presence of the 7–8 nm fimbriae is associated with the
FGHMS-type of MR/P-HA might have been more strongly made.

The MR/P-HAs in species other than M. morgani and P. mirabilis were diverse
(table II). Bacteria from MR/K-HA+, MR/P-HA+ cultures of some of these strains
bore thin and thick kinds of fimbriae whereas those of other strains bore thin fimbriae
only. We have not yet established for the former strains that the thick fimbriae are the
agents of their MR/P-HA activity. In the latter strains, the MR/P-HAs may be
associated with either a thin kind of fimbria indistinguishable from the type found on
MR/K+ strains, or, like the narrow-spectrum MRE-HAs of E. coli, with non-fimbrial
materials (Duguid et al., 1979; Duguid and Old, 1980; Ip et al., 1981). Furthermore, in
view of the diversity of MR/P-HA patterns, similar diversity among their fimbrial or non-fimbrial determinants should not be unexpected.

All strains with MS-HA possessed channelled, 8–9 nm-wide fimbriae resembling the type-1 fimbriae associated with the MS-HA in *E. coli* (Duguid et al., 1955, 1979); but similar fimbriae were present on the strain of *Prov. stuarti* that was MR-HA+ in settling tests only, and on some strains of *Prov. stuarti* that were MR/K+ and formed also characteristic thin fimbriae. There may, therefore, be several types of channelled fimbriae about 8–9 nm in width, morphologically indistinguishable by electronmicroscopy, some or none of which may be associated with the distinct HAs of the different species. Difficulty in interpreting the possible role of this fimbrial type in HA activity in other strains of *Providencia* was encountered by Old and Scott (1981).

Other fimbrial types, not apparently associated with HA activity, were the 2–3 nm-wide fimbriae in the only HA− strain in the series; and the 6–7 nm-wide, non-channelled fimbriae present along with thin fimbriae in some MR/K+ strains of *Prov. alcalifaciens*. Identification of the cultural conditions for the optimal production of these two types of fimbria may enable us to perform in-vitro adhesion tests with substrates other than erythrocytes.

It is of interest to examine our findings in the light of recent taxonomic changes proposed for the protei, based primarily on results from DNA-relatedness studies (Brenner et al., 1978). In general terms, the distribution of types of HAs among *Proteus* and *Providencia* was quite characteristic for each genus. Thus MS-HA was more common in *Providencia* and MR/P-HA was more regularly found in *Proteus*. Furthermore, “Rettgeri” strains behaved more like *Providencia* than *Proteus*, i.e., in agreement with the results of studies on DNA homology (Brenner et al., 1978). In the light of studies on DNA-base composition and homology, it was not surprising to find that *Morganella* showed a distribution of HA types and fimbriae very like that of *P. mirabilis*. In each of the species considered by Brenner et al. (1978) to be genetically homogeneous, most isolates formed a single type of MR/P-HA—i.e., *M. morganii* and *P. mirabilis*—or two MR/P-HAs with similar patterns—*Prov. stuarti*. In species in which heterogeneity of DNA was evident (Brenner et al., 1978), the diversity of MR/P-HAs was also great—*P. vulgaris*, *Prov. alcalifaciens* and *Prov. rettgeri*.

Enterobacteria have recently received much attention, particularly in studies that attempted to establish whether adhesins and fimbriae were involved in the initiation of infection by aiding colonisation of epithelial cells. Nevertheless, many in-vitro studies have insufficiently characterised the adhesins and fimbriae of enterobacteria, and yet their results have been extrapolated to provide explanations of in-vivo colonisation processes. If the present comprehensive study, which has attempted no more than to identify the adhesins and fimbriae produced in vitro by *Proteus* strains, has highlighted only some of the pitfalls, future studies purporting to relate to in-vivo colonisation should prove worthwhile.

From this survey, it is apparent that the distribution of HAs and fimbriae in *Proteus* species is much more complex than that described so far for other genera of Enterobacteriaceae (Duguid and Old, 1980), and it is obvious that the establishment of any correlation between different HAs and different types of fimbriae will be troublesome and for the future. Because we found it difficult to distinguish accurately, by direct electronmicroscopy, different kinds of morphologically similar fimbriae, our future studies are being directed towards the use of immunoelectronmicroscopy with
specific antisera prepared against the different types of fimbriae. Furthermore, our search for naturally occurring strains producing one kind of HA (or fimbria) continues.

We thank Professor J. P. Duguid for invaluable discussions and continued encouragement in the course of this work. Dr Sheila Scott provided much useful guidance in the techniques of electronmicroscopy.

R.A.A. is an Oyo State Government scholar on study leave from Lagos University Teaching Hospital, Lagos, Nigeria.

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