Characterisation of a fimbrial, mannose-resistant and eluting haemagglutinin (MREHA) produced by strains of Salmonella of serotype Sendai

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Summary. Strains of Salmonella of serotype Sendai, producing a mannose-resistant and eluting haemagglutinin (MREHA) when cultured at 37°C but not at 18°C, were examined by electronmicroscopy after negative staining. Production of this MREHA, previously thought to be nonfimbrial, was correlated with the presence of thick fimbriae with an external diameter of 13.6 nm. These fimbriae were readily fragmented and, when purified, had an estimated Mₐ of 28 Kda. Production of fimbrial MREHA by Sendai strains was associated with the ability to adhere to a wide range of substrates and to form a fimbrial pellicle at the surface of liquid media incubated statically in air. The origin of this unusual Sendai fimbrial MREHA is unknown. Thin filamentous structures produced independently of fimbrial MREHA by Sendai strains were also described. Fimbrial MREHA was not produced by strains of the antigenically similar serotype Miami which, however, and unlike Sendai strains, formed mannose-sensitive haemagglutinin and type-1 fimbriae. The ability to differentiate strains of Miami and Sendai (serotype 1,9,12:a:13) by means of their fimbriae is noted.

Introduction

Most strains (80%) of Salmonella possess type-1 fimbriae (Duguid et al., 1966; Duguid and Old, 1980; Duguid, 1985) which are polymers of fimbrellin, a protein of 21 Kda with a high proportion (40%) of hydrophobic amino acids (Korhonen et al., 1980). Type-1 fimbriae of salmonellae of different serotypes are antigenically diverse, share a major fimbrial antigen and carry a common adhesin which recognises mannosyl-containing residues (Duguid et al., 1966; Duguid and Campbell, 1969, Old, 1972; Duguid, 1985). The adhesive properties of type-1 fimbriate salmonellae can be inhibited by D-mannose, its analogues and a few, highly specific oligosaccharides (Duguid et al., 1966; Old, 1972; Firon et al., 1983). Receptors for type-1 fimbriae are distributed in a wide range of cells including erythrocytes of many animal species, and detection of mannose-sensitive haemagglutinating activity (MSHA) in salmonella cultures affords good presumptive evidence of type-1 fimbriation (Duguid et al., 1966; Old, 1985).

Other kinds of haemagglutinating activity not inhibited by D-mannose have also been described in salmonellae. Some of these mannose-resistant haemagglutinating activities (MRHAs) are associated with fimbriae, others with non-fimbrial material (Duguid and Old, 1980; Old, 1985). Thus, rare strains of Salmonella of subspecies 1–3 possess thin fimbriae (of external diameter ≤ 4 nm) which render cultures inagglutinable in all available O antisera (Rohde et al., 1975; Aleksić et al., 1978). The thin fimbriae of subspecies-1 salmonellae are serologically related to the type-3 fimbriae of strains of Enterobacter, Klebsiella and Yersinia (Old and Adegbola, 1985) and, like them, are associated with a mannose-resistant and klebsiella-like haemagglutinin (MR/K-HA, see Duguid and Old, 1980) which agglutinates tannic acid-treated red-blood cells (Adegbola et al., 1983). The thin fimbriae of salmonellae of subspecies 2 and 3 are also associated with a "tanned red-cell" haemagglutinin (Adegbola et al., 1983; Old, 1985) but they are serologically unrelated to the type-3 fimbriae of other Enterobacteriaceae (Old and Adegbola, 1985).

Non-fimbrial MRHAs are produced by salmonellae of many different serotypes, mostly from subspecies 1 (Jones and Richardson, 1981; Tavendale et al., 1983; Manning et al., 1986; Old and
plates (25 cm², Mast Laboratories). Manipulation of containment in a category 3 laboratory.

sent more than 24 h on phosphate-buffered agar (pH 7.0, PBA) at 37°C. For the isolation of fimbriae from strains 187.K, CNS 2.84, CNS 7.84 and 71.K and CNS 3666.51 [Hanoi] were received as serotype Sendai and four strains (187.K, CNS 2.84, CNS 7.84 and CNS 1.85) as serotype Miami. Strain NCTC 5772 was obtained from the National Collection of Type Cultures, Colindale Avenue, London; the other six strains were kindly donated by Professor L. Le Minor, Service des Entérobactéries, Institut Pasteur, Paris, France.

Culture methods

Tryptone Soya Agar (CM 131) was from Oxoid. Otherwise, the media and methods of culture used were essentially as described before (Duguid et al., 1979; Old et al., 1987). Thus, in tests for the production of haemagglutinins, bacteria were grown serially for 48-h periods in static phosphate-buffered broth (pH 7.0, PBB) or for 24 h on phosphate-buffered agar (pH 7.0, PBA) at 18°C or 37°C. For the isolation of fimbrae from strains NCTC 5772 and 3666.51, bacteria were grown for 24 h at 37°C on PBA (500 ml) in glass-bottomed bioassay plates (25 cm², Mast Laboratories). Manipulation of Sendai cultures was performed in appropriate conditions of containment in a category 3 laboratory.

Materials and methods

Bacteria

Seven strains of S. enterica subsp. enterica (see Le Minor and Popoff, 1987) with the antigenic formula 1,9,12:a:1,5 were examined: three strains (NCTC 5772, 71.K and CNS 3666.51 [Hanoi]) were received as serotype Sendai and four strains (187.K, CNS 2.84, CNS 7.84 and CNS 1.85) as serotype Miami. Strain NCTC 5772 was obtained from the National Collection of Type Cultures, Colindale Avenue, London; the other six strains were kindly donated by Professor L. Le Minor, Service des Entérobactéries, Institut Pasteur, Paris, France.

Haemagglutination tests

Bacteria were harvested and tested for their haemagglutination titres as before (Adegbola and Old, 1982). MREHA titres were expressed as the haemagglutinating power (HP) estimated in tests with the most sensitive species of red-blood cell and MSHA titres similarly in tests with guinea-pig cells (Old, 1985).

Improved salt-aggregation test

Different concentrations of ammonium sulphate (0.02–4.0 M) were prepared in 0.02 M sodium-phosphate buffer (pH 6.8) containing methylene blue. Equal volumes of bacterial suspension (c. 5 x 10⁹ cells/ml) and solutions of ammonium sulphate at different concentrations were mixed with tooth picks on hydrophobic paper (Rozgonyi et al., 1985). The concentration of ammonium sulphate at which bacterial aggregation occurred was scored immediately after mixing and confirmed next day by examination of the dried-up mixtures.

HEp2 cell-adhesion tests

HEp2 cells obtained from Flow Laboratories (Irvine, Scotland) were maintained according to the procedures detailed by Tavendale et al. (1983). Bacterial cultures, grown in PBB or on PBA at 18°C or 37°C, were diluted in Eagle’s Minimal Essential Medium (Gibco Europe Ltd, Paisley, Scotland) supplemented with fetal bovine serum 0.5% v/v, 20 mM glutamine and HEPES buffer (pH 7.3) (Gibco) 2% v/v, (1 x 10⁹ bacteria/ml). Static overlay tests performed in the absence or presence of α-methyl-D-mannoside (α MM) 0.2% w/v were incubated for 1 h; washing and staining procedures followed the methods of Tavendale et al. (1983). The numbers of bacteria attached per epithelial cell and the percentages of epithelial cells showing adherent bacteria were estimated from counts of c. 200 cells.

Other adhesion tests

Preparation of, and adherence to, other mammalian cell types followed previously published methods: human buccal epithelial cells (Burke and Axon, 1987); mouse duodenal epithelial cells (Sato and Okinaga, 1987); and mouse enterocytes (Lindquist et al., 1987).

Electronmicroscopy

PBB- or PBA-grown bacteria suspended in water were placed on clean dental-wax sheets (EMscope Laboratories Ltd, Ashford, Kent). A copper grid (3.05 mm diameter), coated with formvar and carbon, was placed on, and allowed to remain in contact with, the culture drop for c. 20 min. Bacteria were washed through 2 or 3 drops of glass-distilled water and stained for 1 min with uranyl acetate (pH 4.0) 0.3% w/v, ammonium molydate (pH 7.0) 2% w/v or phosphotungstic acid (pH 6.8) 0.3% w/v.
After removal of excess stain, grids were dried and each side of the grid was exposed to ultraviolet light for 2 min before examination with a Jeol 100CX transmission electron microscope.

Isolation and purification of fimbriae

Bacteria from an overnight PBA culture grown on a bioassay plate (vide supra) were harvested in nutrient broth (10 ml) and the resultant dense suspension (≥10^12 bacteria/ml) was heated (60°C for 20 min) to detach fimbriae. Bacteria were deposited by centrifugation (3600g for 20 min) and the fimbriae in the supernatant were concentrated either by centrifugation at 150 000g for 2 h or by precipitation with saturated ammonium sulphate 50% w/v overnight at 4°C. After dissolving the deposit in 50 mM Tris-HCl buffer (pH 8.0) or by precipitation against water overnight at 4°C, the material was applied to an anion-exchange Mono Q column and chromatographed by a fast protein liquid chromatography (FPLC) system (Pharmacia Ltd). Material was eluted from the column with a linear gradient of 0-0.5 M NaCl and 1 M NaCl in 50 mM Tris-HCl buffer (pH 8.0). Column fractions were examined by electronmicroscopy and electrophoresis.

Polyacrylamide-gel electrophoresis (PAGE)

Column fractions and protein markers of known M, (14.4-97.4 KDa) were analysed by sodium dodecyl sulphate (SDS)-discontinuous PAGE after the method of Laemmli (1970). Samples in disruption buffer (62.5 mM Tris-HCl, pH 6.8, containing SDS 2% w/v, glycerol 10% v/v, 2-mercaptoethanol 5% v/v, and a trace of bromophenol blue dye) were boiled for 2 min and applied to slabs of acrylamide 4% w/v in 125 mM Tris-HCl (pH 6.8) containing SDS 0.1% w/v over a resolving gel of acrylamide 15% w/v in 375 mM Tris-HCl (pH 8.8) containing SDS 0.1% w/v. Electrophoresis was carried out in the cold at 20 mA/gel in 25 mM Tris-HCl (pH 8.3) containing 192 mM glycine and SDS 0.1% w/v until the dye front reached the bottom of the gel. Gels were stained overnight in Coomassie Brilliant Blue R250 0.1% w/v in methanol/glacial acetic acid : water (50:10:40), destained in several changes of the same solvent and further washed in methanol : acetic acid : water (10:10:80). The stained gel was dried under vacuum.

Results

Haemagglutinins

When cultured in PBB or on PBA at 37°C for 24 h, two of the Sendai strains (NCTC 5772 and 71.K) produced a haemagglutinin which in the presence of αMM agglutinated the red blood cells of fowl (F), guinea pig (G), man (M) and ox (O) (i.e., pattern FGMO) but not those of horse, pig and sheep of the seven red cell species routinely used by us. This Sendai MRHA possessed the unusual property of “elution” whereby there was dispersion of the agglutinated red cell-bacteria mixture as the test was warmed from 4°C to 45°C; hence, it was a haemagglutinin of the mannose-resistant and eluting (MRE) kind. Furthermore, Sendai MREHA was not produced by either of these two strains when grown in PBB or on PBA at 18°C for 2 days. Sendai strain 3666.51 did not form this MREHA in either of the phosphate-buffered media even after eight serial 2-day subcultures at 18°C or 37°C. None of the three Sendai strains produced MSHA on serial subculture in nutrient broth or PBB at 18°C or 37°C.

When the four strains of serotype Miami were cultured similarly, they produced MSHA in serially-grown broth cultures at 18°C and 37°C but none formed MREHA in PBB or on PBA.

Adherence to epithelial cells

Bacteria from cultures of Sendai strain NCTC 5772 grown in PBB or on PBA at 37°C for 24 h formed moderate amounts of MREHA (titres of 835–875) (table I); when examined in the static overlay test in the absence of αMM, many (48–71%) of the HEp2 cells showed adherent bacteria (2–3 bacteria/epithelial cell). The levels of adherence by this strain were similar when tests were made in the presence of αMM (52–77% of the cells showed adhesive bacteria/epithelial cell, table I). Thus, αMM did not inhibit the adherence of strain NCTC 5772, as would be expected for a strain that is type-1 non-fimbriate and does not form MSHA. Tests with the other two Sendai strains were made, therefore, only in the presence of αMM, i.e., only their MR adherence to HEp2 cells was assessed. MREHA+ cultures of strain 71.K showed the same overall pattern of adherence to HEp2 cells as had strain NCTC 5772, although its adherence was slightly less (0.7–0.9 bacteria/epithelial cell) than that of strain NCTC 5772 despite the fact that its MREHA titre was greater than that of strain NCTC 5772. Accurate titration of MREHA, however, is difficult (see Old, 1985).

Motility seemed to play no important role in the adherence of bacteria from MREHA+ cultures, because poorly motile bacteria from PBA cultures adhered as well as, if not better than, actively motile bacteria from PBB cultures. Neither strain NCTC 5772 nor strain 71.K was adherent after growth on PBA or in PBB at 18°C, i.e., conditions in which they were phenotypically MREHA− and their MREHA titres were 0 (table I).
Table I. MREHA and adhesion to HEp2 cells by broth- and agar-grown cultures of salmonellae of serotype Sendai

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Phosphate-buffered medium (pH 7.0)</th>
<th>Temperature of incubation (°C)</th>
<th>MREHA titre of culture</th>
<th>Percentage of HEp2 cells with adherent bacteria (and average number of attached bacteria/HEp2 cell) in static overlay tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- αMM</td>
</tr>
<tr>
<td>NCTC 5772</td>
<td>Agar</td>
<td>37</td>
<td>875</td>
<td>71 (2)</td>
</tr>
<tr>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>2 (&lt;0.1)</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>37</td>
<td>835</td>
<td>48 (3)</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>2 (&lt;0.1)</td>
</tr>
<tr>
<td>71.K</td>
<td>Agar</td>
<td>37</td>
<td>4040</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>37</td>
<td>3005</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>3666.51</td>
<td>Agar</td>
<td>37</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>37</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>NT</td>
</tr>
</tbody>
</table>

* None of the Sendai strains was type-1 fimbriate; thus, the MSHA titres of their cultures were 0.
† <0.1 = 0-9 adherent bacteria/100 epithelial cells.
NT = not tested.

By contrast, bacteria from PBB- and PBA-grown cultures of strain 3666.51, which did not form MREHA at 18°C or 37°C, adhered poorly (table I). Indeed, bacteria from 37°C-grown cultures of this strain were as poorly adherent as the phenotypically MREHA− bacteria of the two Sendai strains grown at 18°C (table I).

Other adhesive properties

When the MREHA+ strains NCTC 5772 and 71.K were grown statically in air in tubes of PBB (10 ml) at 37°C, MREHA activity was first detected after 12 h; a pellicle of growth at the broth-air interface first appeared between 18 and 24 h, and was observed in all static PBB cultures incubated for ≥ 24 h. The MREHA− strain 3666.51 grown similarly did not form a pellicle. Measurement of the amount of growth (as estimated by turbidity values at 540 nm) of cultures of the MREHA+ and MREHA− strains showed that the amounts of growth of MREHA+ strains incubated for 24–72 h were consistently 1.65–2.35 times greater than those of the MREHA− strain grown similarly (data not shown). No such growth advantage for the MREHA+ strains was observed in PBB cultures grown at 37°C for <24 h (i.e., before pellicle formation appeared) when the strains were cultured in PBB at 18°C.

Both MREHA+ strains also bound to human buccal epithelial cells (100% of cells, 33 bacteria/cell), mouse duodenal epithelial cells (95% of cells, five bacteria/cell) and mouse colonocytes (94% of cells, three bacteria/cell) in the presence of αMM. The MREHA− strain, tested similarly, showed negligible adherence to these same mammalian cell types (data not shown). Yet both the MREHA+ and MREHA− strains were equally hydrophobic, aggregating in 0-02 M ammonium sulphate. Thus, hydrophobicity alone did not account for adherence to epithelial cells.

Fimbriae

The approximate percentages of fimbriate bacterial cells detected in cultures of the three Sendai strains after growth in phosphate-buffered media at 18°C or 37°C are shown in table II. Three different kinds of fimbrial (or fibrillar) structures were noted. First, thick fimbriae with an external diameter of 13-6 nm were present on a high percentage of bacteria in MREHA+ cultures of strains NCTC 5772 and 71.K. When these thick fimbriae were attached to the bacterial cells, they appeared long and straight (fig. 1a), but they fragmented readily even in the course of the gentle in-situ washings with water required for electron-microscopical examination. Thus, most preparations of MREHA− cultures contained large amounts of thick fimbrial fragments detached from the bacterial cells (fig. 1b). The observation that thick fimbriae were more readily observed on
**Table II.** Fimbriation of salmonellae of serotype Sendai grown in phosphate-buffered medium

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>MREHA production</th>
<th>Phosphate-buffered medium</th>
<th>Growth temperature (°C)</th>
<th>Percentage of fimbriate bacteria</th>
<th>Kinds of fimbriae* present</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCTC 5772</td>
<td>+</td>
<td>Agar</td>
<td>37</td>
<td>63</td>
<td>Thick and thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>37</td>
<td>55</td>
<td>Thick and thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>71.K</td>
<td>+</td>
<td>Agar</td>
<td>37</td>
<td>65</td>
<td>Thick and thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>37</td>
<td>61</td>
<td>Thick and thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>3666.51</td>
<td>–</td>
<td>Agar</td>
<td>37</td>
<td>17</td>
<td>Thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>37</td>
<td>16</td>
<td>Thin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broth</td>
<td>18</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

* Fimbriae and fibrillar material described are shown in figs. 1–4.

bacteria of Sendai cultures grown in static liquid PBB rather than on solid PBA may have been associated with that lability. Furthermore, the finding that thick fimbriae were readily seen in preparations stained with neutral stains such as ammonium molybdate or phosphotungstic acid, but not after staining with uranyl acetate (pH 4-0), suggests that the thick fimbriae may also be acid-labile.

In MREHA+ Sendai cultures, there was a tendency for the cells to aggregate, e.g., as in the formation of fimbrial pellicles (vide supra). Examination of these cultures indicated that the clumping of bacteria was mediated by tangled masses of thick fimbriae which, unlike the straight form shown in fig. 1a, had a distinct curly appearance reminiscent of flagellar structures (fig. 2a). However, the flagella of Sendai strains, like those of other salmonellae (MacNab, 1987), have an external diameter of c. 19 nm, i.e., about 50% thicker than the Sendai thick fimbriae. Furthermore, immuno-electronmicroscopy tests with flagellar antiserum of Ha specificity (Wellcome Reagents Ltd, Beckenham) showed that Sendai flagella were stained strongly with flagellar antibody whereas the thick curly fimbriae remained uncoated (fig. 2b). Thick fimbriae, straight or curly, were not detected on bacteria from cultures of the MREHA− strain 3666.51 nor from phenotypically MREHA− cultures of the other two Sendai strains grown at 18°C.

Two other kinds of fimbrial (or fibrillar) structures were observed, each of which was much thinner than the thick 13.6 nm fimbriae. First, thin fibrillae of c. 3 nm external diameter were present.

![Fig. 1. Ammonium molybdate-stained preparations of MREHA+ Sendai strain NCTC 5772 showing thick fimbriae (diameter 13.6 nm); (a) attached to the bacterial cell; (b) fragmented and detached from the bacterial cell. Bar = 100 nm.](image-url)
on bacteria of all three Sendai strains grown at 37°C, and were more readily seen on bacteria grown in PBB than on PBA (fig. 3). A second kind of fibrillar material with an external diameter of c. 2 nm was found on all three Sendai strains grown at 37°C; it was detected equally well on bacteria from PBB- and PBA-grown cultures (fig. 4). Although we estimated that c. 16–17% of the bacteria from 37°C-grown cultures of Sendai strain 3666.51 possessed one or other, or both, of the thin kinds of fibrillae, we experienced considerable difficulty in seeing these structures and it may be that we have underestimated the actual percentages of bacteria bearing fibrillae. We made no attempt to score the numbers of individual bacteria possessing each of the different kinds of fibrillae and fimbriae.

The OD_{280} profile of the material eluted from the Mono Q column, after loading with supernate from a heated and centrifuged suspension of Sendai strain NCTC 5772, is shown in fig. 5. Three major peaks were present at fractions 54, 78 and 95. Samples of fractions 72–80, eluting at 0.4 M NaCl, were shown by electronmicroscopy to contain thick fimbriae (fig. 6). Furthermore, these thick fimbriae, when detached, gave the same pattern of MRE haemagglutination as the original suspension of MREHA⁺ bacteria, suggesting that MREHA was mediated by thick fimbriae. Strain 71.K behaved similarly. When fractions 72–80 from Sendai strain NCTC 5772 (or strain 71.K) were pooled and analysed by SDS-PAGE, the subunit M, of the thick fimbriae was estimated as being c. 28 Kda (fig. 7). Samples of fractions 54 and 95 from strain NCTC 5772, eluting at 0.25 M and 1 M NaCl, respectively, were found by electronmicroscopy to contain fibrillar material. Fig. 8a shows fibrillae from fraction 95; sometimes, however, as with material from fraction 54, the filaments were stacked together in sheets (fig. 8b).
The major difference in the OD_{280} profile of material from the MREHA^- strain 3666.51, treated and fractionated similarly, was the absence of the major peak corresponding to that of the thick fimbriae (data not shown). Because strain 3666.51 did not produce thick fimbriae, we tried to characterise the thin fimbrial type(s) from that strain. We have been unable, however, to obtain by SDS-PAGE analysis bands whereby the M_r of the thin fimbrial type(s) could be assessed.

Discussion

Considerable advances in the techniques of electronmicroscopy prompted us to seek an explanation of the "non-fimbrial" MREHAs of Salmonella (Duguid et al., 1966) and we have recently established that Salinatis MREHA, agglutinating the red cells of fowl, guinea pig, mouse and ox (Duguid et al., 1966), is associated with thin fimbriae which have an external diameter of 3-6 nm and a subunit M_r of 19 Kda (Yakubu et al., 1989). In the present study, by contrast, we have shown that Sendai MREHA, agglutinating the red cells of fowl, guinea-pig, man, monkey, ox and rabbit (Duguid et al., 1966), is associated with thick fimbriae which have an external diameter of 13-6 nm and a subunit M_r of 28 Kda. Furthermore, thick fimbriae in Sendai are extremely fragile, readily fragmented and the associated Sendai MREHA, unlike that of Salinatis (Yakubu et al., 1989), is destroyed by formaldehyde (Duguid et al., 1966). Formaldehyde sensitivity and mechanical fragility were the likely reasons why the thick Sendai fimbriae were not visualised in earlier studies in which formaldehyde-killed cultures were washed twice by centrifugation in distilled water before examination by electronmicroscopy; again, any short stumps of thick fimbriae remaining after these aggressive preparative techniques, would
have been difficult to detect by the relatively insensitive shadowing techniques then used (Duguid et al., 1966).

It was interesting that the unusual property of MREHA production by salmonellae should be found in two such infrequently occurring salmonellae as Salinatis and Sendai and that their MREHAs should be so different. These fimbrial MREHAs can be readily distinguished by several criteria from other salmonella MRHAs including both the widely distributed, diffusible, non-fimbrial MRHAs (Tavendale et al., 1983; Halula and Stocker, 1987) and the less common type-3 fimbrial MRHAs (Adegbola et al., 1983). Although a relationship between Salinatis and Sendai MREHAs and some of the many MREHAs found in human strains of E. coli might seem likely, none of the 10 important classes of fimbrial and "non-fimbrial" MREHAs of E. coli described by Duguid et al. (1979) had a spectrum of haemagglutinating activity like those of salmonellae.

In their excellent electronmicroscopical study of the colonisation factors present on bovine, enterotoxigenic strains of E. coli, Duchet-Suchaux et al. (1988) reported that the structure of F41 colonisation factor was complex. Thus, although individual F41 filaments were thin and had a diameter of c. 3 nm, they were more generally present as thick, coil-like structures consisting of two or more filaments tightly wound on themselves to form dense spirals; again, large amounts of thick surface structures lay free in the medium. F41, therefore, had an appearance not unlike that of the thick fimbriae of Sendai (see figs. 1–4, Duchet-Suchaux et al., 1988) and their subunit Mₜₛ (29.5 Kda for F41, de Graaf and Roorda, 1982) were also similar. But F41 coils were much thicker (17–20 nm) than Sendai fimbriae and their associated MRHAs were different (Morris et al., 1982; Duchet-Suchaux et al., 1988). Furthermore, the finding that all three Sendai strains formed thin filaments, whereas thick fimbriae were formed by MREHA⁺ strains only, led us to conclude that Sendai thick fimbriae were neither related to F41 nor formed similarly.

The presence of type-1 fimbriae in salmonellae is associated not only with mannose-sensitive adhesive properties but also with the ability to form a type-1 fimbrial pellicle at the surface of liquid media incubated statically in air (Old and Duguid, 1970, 1971); pellicle formation by type-1 fimbriate salmonellae is also mannose-sensitive (Old et al., 1968). It was interesting, therefore, to find that type-1 non-fimbriate Sendai strains also formed fimbrial pellicles in liquid media but by a process that was mannose insensitive and restricted to MREHA⁺ strains of Sendai. Although pellicle formation by type-1 fimbriate salmonellae and MREHA⁺ fimbriate Sendai is associated in vitro with the attainment of growth yields greater than those achieved by non-fimbriate or MREHA⁻ cultures, it is not clear what, if any, function this property fulfils in vivo. The demonstration in this study and elsewhere (Duguid et al., 1966) that adherence by Sendai strains to a wide range of substrates is MREHA-dependent suggests that the thick fimbriae may contribute to the overall adhesiveness of Sendai strains; however, the finding that this fimbrial MREHA is not produced by all naturally occurring Sendai strains makes it unlikely that it is an essential virulence determinant.

Most of the salmonellae recovered from human infections belong to subspecies 1 (Le Minor, 1988) and to prototrophic, ubiquitous serotypes which inhabit a wide range of hosts; in man, they usually
cause a mild gastroenteritis. A few salmonellae, however, belong to serotypes—such as Paratyphi A and Typhi—which are auxotrophic and host-adapted to man in whom they cause systemic disease. Among salmonellae of group O9, two serotypes of subspecies I have the antigenic formula 1,9,12: a : 1,5, namely, Sendai and Miami (Le Minor and Popoff, 1988). Sendai, first isolated from cases of enteric fever in Japan (Aoki and Sakai, 1925), is another, albeit less-well known, example of a serotype adapted to man and its biochemical properties are remarkably similar to those of Paratyphi A (1,2,12:a: [1,5]) (Ewing, 1985); there are, however, few reports of its isolation in Japan, or elsewhere, in recent years (Professor L. Le Minor, personal communication). Miami, by contrast, is a prototrophic, ubiquitous serotype causing a mild gastroenteritis in man; it too is infrequently recovered, e.g., only 13 isolations of Miami among 67,767 strains of salmonellae in France in 1980–83 (Le Minor et al., 1985). Because strains of Sendai and Miami can be differentiated by biochemical properties only, the question has been raised as to whether both serotype names should be retained in the Kauffmann-White scheme (Le Minor et al., 1985). Sendai strains as fimbrial, additional problems have been raised by this study. Thus, all three strains of Sendai produced thin filamentous structures—perhaps even two kinds—which, though not associated with adherence to epithelial cells, may have contributed to the high hydrophobicity of these strains. Despite many attempts, we were unsuccessful in detecting bands on gels of the column-purified fibrillar structures seen by electronmicroscopy. We can only assume that, because of the highly hydrophobic nature of the molecule, significant amounts were lost during the procedures of purification, dialysis and PAGE analysis. Although we know that other salmonellae host-adapted to man or other animals do not form MREHA (Duguid et al., 1966), we have no information about how widely distributed these filamentous structures are in host-adapted or ubiquitous serotypes of Salmonella.

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