ANTIGENS OF THE TYPE-2 FIMBRIAE OF SALMONELLAE: "CROSS-REACTING MATERIAL" (CRM) OF TYPE-1 FIMBRIAE

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THE filamentous bacterial appendages called fimbriae have been found in many enterobacteria and those studied serologically have been shown to contain distinctive agglutinogens (Gillies and Duguid, 1958; Duguid and Campbell, 1969).

Common fimbriae, determined by genes on the chromosome and present in large numbers on the bacterium, have been classified into six types (Duguid, Anderson and Campbell, 1966). Type-1 fimbriae, which are characterised by the mannose-sensitive haemagglutinating, adhesive and pellicle-forming properties they confer on the bacteria, are found in most strains of *Escherichia coli*, *Shigella flexneri*, *Klebsiella aerogenes* and *Salmonella* (Duguid and Gillies, 1957; Duguid, 1968; Old *et al.*, 1968). Type-2 fimbriae resemble type-1 fimbriae in number and dimensions, but differ from them in not conferring haemagglutinating, adhesive and pellicle-forming properties. They have been described so far only in *Salmonella* and have been found in only a few serotypes of this genus. Duguid *et al.* (1966) found them in 19 out of 135 strains of *S. paratyphi* B (106 strains had type-1 fimbriae and 10 were non-fimbriate), 11 out of 30 strains of *S. pullorum* and all of 14 strains of *S. gallinarum*. Type-1 fimbriae have not been reported in *S. pullorum* or *S. gallinarum*.

Serological analysis of the type-1 fimbriae of salmonellae has shown that they bear agglutinating antigens distinct from the O, H and Vi antigens and that there is wide sharing of fimbrial antigens among strains of *Salmonella* from different serotypes (Campbell, 1961). This sharing does not extend to *Sh. flexneri*, *E. coli* and *K. aerogenes*, whose type-1 fimbriae are distinct antigenically from those of salmonellae (Duguid and Campbell). Fimbrial antigen F1 is found in all serotypes of *Salmonella* with at least one or more of the other four salmonella fimbrial antigens in a more limited range of serotypes. Type-2 fimbriae resemble type-1 fimbriae morphologically, but observations on their antigenic properties have not yet been reported. We have, therefore, studied the antigens of type-2 fimbriae with a particular view to discovering any relationship between them and the antigens of type-1 fimbriae.

MATERIALS AND METHODS

Strains

The strains recorded in table I were from the collection of Professor J. P. Duguid. They had been examined with the electron microscope for the presence of fimbriae and flagella and in tests with guinea-pig red cells for the presence of haemagglutinating activity by Duguid *et al.* (1966).

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Culture media

The broth used was Oxoid nutrient broth no. 2 and the glucose broth was the same with 1 per cent. glucose added. The nutrient agar was Oxoid nutrient agar CM3.

Bacterial suspensions

Phenotypically fimbriate bacteria. Genotypically fimbriate (*fim*⁺) strains were subcultured serially in tubes containing 10 ml nutrient broth incubated aerobically and statically for two or three 48-hr periods at 37°C until the broth cultures were richly fimbriate, i.e., in the "fimbriate phase". Volumes of 0.1 ml from these fimbriate-phase cultures were used to inoculate flat bottles containing 20 ml nutrient broth. These were incubated on their sides to expose a large air-broth surface area favouring the production of fimbriate bacteria. The bacteria were killed by the addition of formaldehyde to 0.1 per cent., and

TABLE I

Fimbriation (fim) and flagellation (fla) genotypes of strains of Salmonella paratyphi B and S. pullorum tested for fimbrial antigens

Serotype	Strain no.	Fimbriation genotype (and type of fimbriae)	Flagellation genotype
<i>S. paratyphi B</i>	S66	+(2)	+
	S543	+(1)	—
	S859	+(1)	+
	S867	+(1)	+
	S957	+(2)	+
	S1076	+(1)	+
	S2416	—	+
<i>S. pullorum</i>	S298	+(2)	—
	S318	—	—
	S1662	+(2)	—

harvested by centrifugation, and the deposited bacteria were resuspended to a concentration of 4×10^8 bacteria per ml in formaldehyde-saline (0.1 per cent. formaldehyde in 0.85 per cent. NaCl), and stored at 4°C. Such suspensions were used for inoculation into rabbits, in tube agglutination tests and, when concentrated by centrifugation, for the absorption of antiserum. Type-2 fimbriate bacteria do not form a pellicle under these conditions, but, grown similarly, gave cultures containing a significant proportion, e.g., 10–50 per cent., of fimbriate bacteria as shown by electron microscopy.

Phenotypically non-fimbriate bacteria. These were obtained from *fim*⁺ strains by two methods: (i) The non-fimbriate phase of *fim*⁺ bacteria was selected by culture for 24-hr periods on nutrient agar. Several colonies were then suspended in sterile saline to $c. 4 \times 10^8$ bacteria per ml. Volumes of 0.1 ml were spread on well-dried nutrient agar plates and incubated for 48 hr at 37°C. The bacteria harvested from these plates were nearly non-fimbriate and non-flagellate but were made into O-suspensions by boiling at 100°C for 30 min., by which treatment they were deflagellated, if flagellate, and defimbriated. The boiled bacteria were resuspended in fresh formaldehyde-saline as described above. (ii) A suspension of live non-fimbriate-phase bacteria from agar was used to inoculate flasks containing 20 ml glucose broth and these cultures were incubated for 6 hr at 37°C aerobically and statically. This method of culture produced bacteria that were only poorly fimbriate but, in the case of genotypically flagellate (*fla*⁺) bacteria, richly flagellate. They were harvested and stored as above.

Phenotypically non-fimbriate bacteria were also obtained, without need for special treatment, from genotypically non-fimbriate (*fim*⁻) strains. Single colonies were inoculated into each of several tubes of 10 ml nutrient broth and incubated aerobically and statically for 48 hr at 37°C. This method gave cultures of biphasic salmonellae that were richly flagellate. Harvesting and storage were as described above.

Non-fimbriate suspensions of bacteria from either a *fim*⁺ or *fim*⁻ strain prepared by any of these three methods were used as both agglutinable bacterial suspensions for tube tests and, after concentration by centrifugation, for the absorption of antibodies from serum. Both H phases of biphasic salmonellae were used for the absorption of flagellar antibodies from antiserum.

Crude fimbrial antiserum

Antisera were prepared in rabbits against fimbriate-phase *S. paratyphi* B strains S66, S543, S859, S957 and S1076 and against *S. pullorum* strain S1662. Each rabbit was bled before immunisation and the serum examined for the presence of natural agglutinins to the immunising strain. In no case were such agglutinins found in a 1 in 25 dilution of the serum. Smooth fimbriate-phase bacteria (10⁹ per ml) prepared as above were used as immunising vaccine. Two subcutaneous (0.25 ml) followed by four intravenous injections (0.25, 0.5, 0.5 and 1.0 ml) were administered at 3-4 day intervals. The rabbits were bled 6 wk after the final injection and the separated serum clarified by centrifugation. Sodium azide (0.02 per cent.) was added as preservative.

Pure fimbrial antiserum

The unabsorbed "crude" fimbrial antiserum contained antibodies against all the surface antigens of the bacteria and it was therefore absorbed with non-fimbriate bacteria to free it from non-fimbrial agglutinins and so yield "pure" fimbrial antiserum. Repeated absorptions with flagellate non-fimbriate suspensions of *fim*⁺ strains were considered likely to cause some loss of fimbrial antibody because it was noted that the "non-fimbriate phase" cultures of the *fim*⁺ bacteria prepared as described above were not always entirely non-fimbriate and richly flagellate. Thus, when grown in glucose broth for 6 hr, the type-1 strains used were still weakly haemagglutinating, and when grown on nutrient agar for 24 hr they were only poorly flagellate. Therefore, H-antibodies were removed from the crude fimbrial antiserum by repeated absorptions with richly flagellate suspensions of a heterologous *fim*⁻ strain, e.g., *S. paratyphi* B, S2416, or with *S. pullorum*, S318. These absorptions also removed shared O-antibodies. Any remaining strain-specific antibodies were removed by a single absorption with a non-fimbriate-phase suspension of the homologous immunising bacteria grown in glucose broth for 6 hr or on nutrient agar for 24 hr. The serum, either undiluted or diluted 1 in 6.25 in saline (NaCl, 0.85 per cent.) was added to a dense suspension of the absorbing bacteria (i.e., the centrifuged deposit from a saline suspension of washed bacteria) in a 100-ml flat bottle and incubated at 37°C for 1 hr. The serum-bacteria mixture was centrifuged twice to remove bacteria from the serum. Removal of all O- and H-antibodies was confirmed by the absence of agglutination at any dilution from 1 in 25 and more with suspensions that were phenotypically non-fimbriate and either flagellate or non-flagellate. Since only such pure fimbrial antisera were used in this study, they will be referred to hereafter simply as "antisera".

Absorption of fimbrial agglutinins

Fimbriate bacteria of various strains were tested for their ability to absorb fimbrial agglutinins from antisera prepared against other strains. Absorption, by the method just described for the preparation of pure fimbrial antisera, was repeated with such fimbriate-phase suspensions until there was no detectable titre (i.e., <25) with the absorbing strain. Each treated (absorbed) antiserum was then used in tests with the original immunising strain and other heterologous strains.

Agglutination tests

Bacterial suspensions in 0.4-ml volumes in agglutination tubes were mixed with equal volumes of serum at final dilutions of from 1 in 25 to 1 in 25,600. The final bacterial concentration in each tube was 2×10^8 per ml. A control tube containing bacteria plus saline was included to eliminate false-positive results attributable to auto-agglutinability of the test bacteria. The tubes were incubated in a waterbath at 37°C for 6 hr and allowed to develop overnight at room temperature or at 4°C. The tubes were shaken slightly to disperse the agglutinated bacteria and viewed by transverse illumination with a hand lens (magnification $\times 3$). Degrees of agglutination were assessed in arbitrary units from — to + + + +. The agglutinin titre of the serum was calculated from the highest dilution giving definite + + agglutination.

Detection of fimbriae

Type-1 fimbriae were detected by haemagglutination tests performed on tiles with 3 per cent. guinea-pig red cells in saline according to the method of Duguid *et al.* (1955) and, where necessary, the degree of fimbriation was estimated by the calculation of the haemagglutinating power of a culture by the method of Duguid (1959). Type-2 fimbriae were detected by slide or tube tests with pure fimbrial antiserum prepared against type-2 fimbriate bacteria. Suspensions of both type-1 and type-2 fimbriate bacteria in distilled water were examined in metal-shadowed films with the electron microscope (Duguid *et al.*, 1955).

RESULTS

Demonstration of fimbrial agglutination

Agglutinations dependent on the reaction of fimbrial antigens with fimbrial antibodies were demonstrated for both type-1 and type-2 fimbriate bacteria in tests with pure fimbrial antiserum from which non-fimbrial agglutinins had been absorbed. The results of typical experiments with fimbrial antisera against the type-1 fimbriate strains of *Salmonella paratyphi* B, S543, S859 and S1076, the type-2 fimbriate strains of *S. paratyphi* B, S66 and S957, and the type-2 fimbriate strain of *S. pullorum* S1662 are given in table II. Each type-1 fimbrial antiserum was shown to agglutinate fimbriate-phase bacteria both of the homologous strain and of the heterologous strains with type-1 fimbriae at moderate or high titres (100–25,600). Similarly, each of the three type-2 fimbrial antisera agglutinated fimbriate-phase bacteria both of the homologous strain and of a heterologous strain with type-2 fimbriae (titres from 100–6400).

That the agglutination of the fimbriate-phase bacteria by the fimbrial antiserum depended on the interaction of fimbrial antigens and antibodies was demonstrated by the absence or low titre (e.g., 25) of agglutination in tests of the antiserum against non-fimbriate-phase suspensions of the same *fim*⁺ bacteria, either richly or poorly flagellate. Further confirmation that the agglutination was fimbrial in nature came from tests with flagellate and non-flagellate suspensions of the *fim*[–] strain S2416 of *S. paratyphi* B, which showed titres of <25 in all ten instances in which they were tested against the five antisera to *S. paratyphi* B fimbriae.

Comparable findings were obtained with the *fla*[–] *S. pullorum* organisms. When the fimbrial antiserum to *S. pullorum* strain S1662 was tested against fimbriate suspensions of the homologous strain or of the heterologous strain

TABLE II

Agglutination of strains of Salmonella paratyphi B and S. pullorum by pure (absorbed) fimbrial antisera to type-1 and type-2 fimbriae

Bacteria in agglutinable suspension				Agglutination titre in test of bacterial suspension with fimbrial antiserum to						
Species and strain no.	Method of culture and preparation*	Phenotype		<i>S. paratyphi</i> B strain†					<i>S. pullorum</i> strain†	
		fimbriae (type)	flagella	S543 (type 1)	S859 (type 1)	S1076 (type 1)	S66 (type 2)	S957 (type 2)	S1662 (type 2)	
<i>S. paratyphi</i> B	S543	A	+(1)	—	100	100	1600	400	1600	400
		C	—	—	<25	<25	<25	<25	<25	<25
	S859	A	+(1)	+	400	1600	6400	400	1600	...
		B	—	+	<25	<25	<25	<25	<25	...
		C	—	—	<25	<25	<25	<25	<25	...
	S867	A	+(1)	+	100	400	6400	400	1600	...
		B	—	+	<25	<25	25	<25	25	...
		C	—	—	<25	<25	<25	<25	<25	...
	S1076	A	+(1)	+	1600	400	25600	1600	1600	1600
		B	—	+	25	25	25	25	25	...
		C	—	—	<25	<25	<25	<25	<25	<25
	S66	A	+(2)	+	400	100	1600	1600	1600	400
		B	—	+	<25	<25	<25	25	25	...
		C	—	—	<25	<25	<25	<25	<25	<25
	S957	A	+(2)	+	400	400	1600	6400	6400	400
		B	—	+	<25	25	<25	<25	25	...
		C	—	—	<25	<25	<25	<25	<25	<25
	S2416	D	—	+	<25	<25	<25	<25	<25	...
		C	—	—	<25	<25	<25	<25	<25	...
<i>S. pullorum</i>	S1662	A	+(2)	—	100	400	100	100	1600	1600
		C	—	—	<25	25	<25	<25	<25	25
	S298	A	+(2)	—	...	...	...	...	...	100
		C	—	—	...	...	...	...	...	<25
	S318	D	—	—	...	...	...	...	...	<25

* A = Two or three serial 48-hr subcultures were grown in aerobic static broths.

B = Two or more 24-hr subcultures were grown on nutrient agar until, in the case of type-1 strains, individually tested colonies showed a low haemagglutinating power (HP of 1-10). A subculture of such a colony was then grown aerobically and statically for 6 hr in glucose broth.

C = Two or more 24-hr subcultures were grown on nutrient agar until, in the case of type-1 strains, individually tested colonies showed a low HP. Single colonies were then spread on nutrient agar plates. The bacteria from such cultures were weakly haemagglutinating, or non-haemagglutinating, and poorly flagellate, and after harvesting were boiled at 100°C for 30 min. to remove flagella, if any, and fimbriae, and then resuspended in fresh saline.

D = A culture was grown in aerobic static broth for one period of 48 hr.

† The type of fimbriae is indicated in brackets after the strain number.

S298, the titres (100–1600) were much higher than those shown when it was tested against phenotypically non-fimbriate suspensions of these strains (titres <25 or 25) or against the *fim*⁻ strain S318 (titre <25).

The agglutinating titre found in the serum appeared to vary both with the degree of fimbriation of the suspension used in the agglutination tests and with that of the suspension used as immunising vaccine. For example, the *fla*⁻ strain *S. paratyphi* B S543 in its fimbriate phase was relatively poorly fimbriate, and the fimbrial antiserum to it showed a titre of only 100 in a test against the homologous strain, but the same serum had a titre of 1600 in tests with the richly fimbriate heterologous strain S1076. Correspondingly, the fimbrial antiserum to strain S1076 showed a higher content of fimbrial agglutinins for the homologous and heterologous strains than the fimbrial antisera raised with vaccines containing less richly fimbriate organisms.

Cross-reaction between type-1 and type-2 fimbriae

The main purpose of this work was to determine the relationship between the antigens of type-1 and type-2 fimbriae, and the closeness of this relationship was first shown by tests in which bacteria with one type of fimbriae were agglutinated by antiserum raised against the other type (table II). The pure fimbrial antiserum prepared against the type-1 fimbriate strains S543, S859 and S1076 of *S. paratyphi* B agglutinated fimbriate-phase bacteria of the type-2 fimbriate strains S66 and S957 of *S. paratyphi* B and S1662 of *S. pullorum* to titres (100–1600) generally similar in magnitude to those (100–25,000) to which they agglutinated fimbriate suspensions of the type-1 strains. Conversely, the fimbrial antisera prepared against the type-2 fimbriate strains S66 and S957 of *S. paratyphi* B and S1662 of *S. pullorum* agglutinated fimbriate-phase bacteria of the type-1 fimbriate strains S543, S859, S867 and S1076 of *S. paratyphi* B to titres (400–1600) similar to those (100–6400) to which they agglutinated the type-2 fimbriate bacteria. That these cross-reactions were dependent on fimbrial antigens and antibodies was shown by the absence of reaction, or very low titre of agglutination (<25–25), obtained when the sera were tested against the same bacteria in the non-fimbriate phase. The results indicate the presence of a common antigen in the type-1 and type-2 fimbriae of *S. paratyphi* B and the type-2 fimbriae of *S. pullorum*.

Absorption of fimbrial agglutinins

Absorption tests were done in order to determine whether the sharing of antigens between type-1 and type-2 fimbriae was complete or only partial. Antiserum against fimbriae of one type was absorbed with fimbriate bacteria of the other type and the serum after each absorption was tested against the immunising strain, the absorbing strain and another heterologous strain. The results are shown in table III. When the type-1 fimbrial antisera to strains S859 and S1076 of *S. paratyphi* B were absorbed with type-2 fimbriate bacteria of strains S957 and S66 of *S. paratyphi* B respectively, until they contained no

fimbrial agglutinins (titre <25) against the absorbing strain (respectively, two and one absorptions), they were then found to have become nearly as deficient in fimbrial agglutinins for the type-1 immunising strain (titres 25 and 25). Similarly, when the type-2 fimbrial antisera to strain S66 of *S. paratyphi* B and *S. pullorum* strain S1662 were absorbed with type-1 fimbriate bacteria of,

TABLE III

Agglutination reactions of fimbrial antisera to two strains of S. paratyphi B with type-1 fimbriae and one strain of S. paratyphi B and one strain of S. pullorum with type-2 fimbriae before and after absorption with type-1 or type-2 fimbriate S. paratyphi B bacteria

Serotype and strain no. (and fimbrial type) to which antiserum was raised	Strain (and fimbrial type) of <i>S. paratyphi</i> B used to absorb fimbrial agglutinins from antiserum	Serotype and strain no. (and fimbrial type) of bacteria in agglutinable suspension	Agglutination titre in test of bacteria with fimbrial antiserum				
			before absorption	after absorption no.*			
				1	2	3	4
<i>S. paratyphi</i> B S859 (1)	S957 (2)	<i>S. paratyphi</i> B S859 (1) S957 (2)	1600	...	25	...	...
			400	25	<25	...	...
<i>S. paratyphi</i> B S1076 (1)	S66 (2)	<i>S. paratyphi</i> B S1076 (1) S66 (2)	25600	25	...	...	...
			1600	<25	...	...	...
<i>S. paratyphi</i> B S66 (2)	S1076 (1)	<i>S. paratyphi</i> B S66 (2) S1076 (1)	1600	...	400	...	<25
			1600	1600	400	100	<25
<i>S. pullorum</i> S1662 (2)	S867 (1)	<i>S. pullorum</i> S1662 (2)	400	25	...	...	...
		<i>S. paratyphi</i> B S867 (1)	400	<25	...	...	...
		<i>S. typhi</i> S30 (1)	1600	<25	...	...	...

* The absorption procedure was repeated up to four times; ... = not done.

respectively, strains S1076 and S867 of *S. paratyphi* B until they contained no fimbrial agglutinins (titre <25) against the absorbing strain, they were found to have become nearly as deficient in agglutinins for the type-2 immunising strain (titres <25 and 25).

These complete or nearly complete absorptions of type-1 fimbrial agglutinins by type-2 fimbriate bacteria and of type-2 fimbrial agglutinins by type-1 fimbriate bacteria suggest that there is a complete or at least a very close sharing of antigens between the type-1 fimbriae of *S. paratyphi* B, the type-2 fimbriae of *S. paratyphi* B and the type-2 fimbriae of *S. pullorum*.

Cross-reactions with type-1 fimbriae in other serotypes

The three type-2 fimbrial antisera were tested against agglutinable suspensions of fimbriate-phase and non-fimbriate-phase bacteria of eight *fim*⁺ strains

of various salmonella serotypes whose somatic (O), flagellar (H) and fimbrial (F) antigens were different from those of the immunising strains. These strains were: *S. adelaide* S291 (35/f, g: -/1, 2, 3), *S. cubana* S211 (1, 13, 23/z₂₉: -/1, 2), *S. montevideo* S1002 (6, 7/g, m, s: -/1), *S. oranienburg* S17 (6, 7/m, t: -/1, 2), *S. saintpaul* S209 (1, 4, 5, 12/e, h: 1, 2/1), *S. typhi* S30 (9, 12/d: -/1), *S. typhimurium* S206 (1, 4, 5, 12/i: 1, 2/1, 2, 3) and *S. waycross* S292 (41/z₄, z₂₃: -/1). Fimbriate suspensions of all eight strains were agglutinated to high titres (400-6400) by the type-2 fimbrial antisera to *S. paratyphi* B strains S66 and S957, and to moderate or high titres (100-1600) by the type-2 antiserum to *S. pullorum* strain S1662. Non-fimbriate suspensions of the same strains, either flagellate or non-flagellate, were not agglutinated or were agglutinated only to low titre (25). The only fimbrial antigen common to all eight strains tested is fimbrial antigen F1, an antigen known to occur in all type-1 fimbriate salmonellae. Thus, the type-2 fimbriae of salmonellae also must contain this antigen.

DISCUSSION

Our results show that the type-2 fimbriae found in a minority of salmonellae, like the type-1 fimbriae found in the majority, carry antigens that are distinct from the O and H and other non-fimbrial antigens and react with fimbrial antibodies in serological agglutination tests. Pure fimbrial antisera to three strains of *Salmonella paratyphi* B with type-1 fimbriae, and two strains of *S. paratyphi* B and one strain of *S. pullorum* with type-2 fimbriae, which had been absorbed free from O- and H-antibodies, agglutinated fimbriate but not non-fimbriate suspensions of both the type-1 and type-2 bacteria. Reciprocal mirror absorption testing revealed that virtually all the fimbrial agglutinins in the antisera to two strains of *S. paratyphi* B with type-1 fimbriae were removed by absorption with *S. paratyphi* B bacteria with type-2 fimbriae and that virtually all the fimbrial agglutinins in the antisera to strains of *S. paratyphi* B and *S. pullorum* with type-2 fimbriae were removed by absorption with *S. paratyphi* B bacteria with type-1 fimbriae. More absorptions were required for the exhaustion of fimbrial agglutinins from type-2 antisera by type-1 fimbriate bacteria than for the exhaustion of fimbrial agglutinins from type-1 antisera by type-2 fimbriate bacteria. However, no significance can be attached to this difference because the sera differed in agglutinin contents and the bacteria in the various absorbing and agglutinable suspensions differed in degree of fimbriation. The conclusion drawn from our results is that the type-1 and type-2 fimbriae of *S. paratyphi* B and *S. pullorum* contain qualitatively the same range of fimbrial antigens though they may not contain these different antigens in the same proportions.

Type-1 fimbriae have been shown to consist mainly or wholly of protein (Brinton and Stone, 1961; Old, 1963; Brinton, 1965) which has been called "pilin" or "fimbriin". The composition of this protein presumably determines the morphological, adhesive and antigenic properties of the fimbriae, and it seems possible that a small change in the composition might lead to the production of fimbriae similar in morphology and antigenic specificity to type-1

fimbriae but lacking their adhesive, haemagglutinating and pellicle-forming properties. Type-2 fimbriae may represent such altered forms of type-1 fimbriae.

Mis-sense mutations that lead to the production of a functionally abnormal protein differing slightly from the functionally normal wild-type protein in amino acid composition, but showing an immunological cross-reaction with antiserum to the wild-type protein, have been well documented for many enzymes. Because of their immunological relationship with the wild-type protein, these mutant proteins have been called "cross-reacting material", or CRM. CRM-like proteins have also been reported for the products of the structural H genes that determine the composition of flagellar proteins and these mutational productions of abnormal flagella may serve as models for the possible mode of origin of type-2 fimbriae. Various *Salmonella* species produce "flagella-shape" mutants, e.g., "curly" flagella whose wavelength is less than normal and whose motility is impaired (Leifson and Hugh, 1953). The curly flagellar shape is H-phase-specific and either phase may be affected (Iino, 1962). Enomoto and Iino (1966) finger-printed the peptides of the flagellins from normal and curly flagella of *S. abortus-equi* and showed that these differed in only one among 35 of their component peptides. They proposed that the change of a single amino acid leads to changes in the secondary structure of the flagellin so that the peptide monomers polymerise to abnormal "curly" flagella. Absorption and agglutination tests revealed no differences in antigenic specificity between the wild-type and mutant flagella.

Other *S. typhimurium* strains are non-motile because they produce abnormal, straight flagella that are antigenically homologous with the normal flagella (Iino and Mitani, 1967). Similarly, Martinez and co-workers (Martinez *et al.*, 1968) showed by detailed amino acid analysis of normal and straight flagella from *Bacillus subtilis* that the substitution of valine for alanine present in wild-type flagellin led to the aggregation of the monomers into straight flagella. Both types of shape mutants thus satisfy the definition of CRM proteins.

The fact that type-2 fimbriae are immunologically similar to or identical with type-1 fimbriae, but lack the latter's "functional" properties of adhesiveness to animal and plant cells and ability to promote early pellicular growth of the bacteria on the surface of static liquid media (Duguid *et al.*, 1966; Old *et al.*, 1968), qualifies them for consideration as mutational CRM forms of type-1 fimbriae. The probability that type-2 fimbriate strains of *S. paratyphi* B originated by mutation from an ancestral type-1 fimbriate strain is further suggested by the finding of one of us (D. C. O., unpublished) that type-1 fimbriate back-mutants could be isolated from some type-2 strains, e.g., *S. paratyphi* B, S66. The type-1 mutants originated spontaneously in some experiments and after induction with ethyl methane sulphonate or nitrous acid in others, and they were isolated by serial culture in aerobic static broth, a procedure that is powerfully selective for type-1 fimbriate bacteria (Old and Duguid, 1970).

Our conclusions, therefore, are firstly that the common wild-type structural gene *fim* codes for a polypeptide that is polymerised to form the structural

molecule of type-1 fimbriae, which happens to possess adhesive properties and, secondly, that its mutation to certain allelic forms determines the formation of polypeptides with amino acid substitutions that are polymerised to type-2 fimbriae, which lack adhesive properties. The final proof of this hypothesis would be the demonstration of different peptide sequences for the two types of fimbriae.

Type-2 fimbriae of *S. pullorum* showed close or complete antigenic similarity with both the type-1 and type-2 fimbriae of *S. paratyphi* B and it seems reasonable to suppose that the type-2 strains of *S. pullorum* originated from type-1 ancestral bacteria in a way similar to that envisaged for the origin of the type-2 strains of *S. paratyphi* B. However, type-1 fimbriae have never been detected in *S. pullorum* or *S. gallinarum* so that a parental type-1 form of these organisms cannot be demonstrated. If it ever existed, it appears now to have disappeared, and it may be concluded that the presence of type-1 fimbriae was not helpful for the colonisation by *S. pullorum* of its natural habitat in poultry.

Since type-2 fimbriae appear not to lack any of the agglutinating antigens present in type-1 fimbriae, it may be assumed that the haemagglutinin is not one of the detectable antigens, and, therefore, that the haemagglutinin is present only in relatively small amounts at the surface of the fimbriae. Gillies and Duguid (1958) showed with the electron microscope that the fimbrial agglutinins of *Shigella flexneri* became attached to the sides of the fimbriae. We think that the haemagglutinin may be located only on the tip of the fimbriae, and, hence, may occupy too small a proportion of the total surface area of the fimbriae to play an appreciable part in bacterial agglutination. Further evidence that the tip is the site of the haemagglutinin comes from the electron micrographs of Brinton (1965) that show polystyrene latex particles attached to the tips but not to the sides of mechanically detached fimbriae.

SUMMARY

Antisera were prepared against the type-1 fimbriae of three strains of *Salmonella paratyphi* B and against the type-2 fimbriae of two strains of *S. paratyphi* B and one strain of *S. pullorum*. The anti-type-1 and anti-type-2 sera agglutinated fimbriate-phase bacteria possessing either type-1 or type-2 fimbriae. Neither antiserum agglutinated non-fimbriate-phase bacteria of the same strains. Mirror absorption tests were performed and in each case the fimbrial agglutinin content of the serum of either type was absorbed by bacteria with fimbriae of the other type. The results showed that the type-1 and type-2 fimbriae of *S. paratyphi* B contain the same fimbrial antigens. It is suggested that the type-2 fimbriae of *S. paratyphi* B are mutational CRM forms of the type-1 fimbriae, because they are antigenically similar to type-1 fimbriae though they lack the "functional" adhesive properties of the latter type. A mutational origin of type-2 from type-1 bacteria is suggested by the observation that some strains of *S. paratyphi* B with type-2 fimbriae gave rise to mutants with type-1 fimbriae both spontaneously and after treatment with mutagens. The absence

of a distinctive antigen corresponding to the haemagglutinin in the type-1 fimbriae suggests that the haemagglutinin occupies only a small proportion of the surface of the fimbriae, possibly only on their tips.

The type-2 fimbriae of *S. pullorum* cross-reacted in agglutination and agglutinin-absorption tests with both the type-1 and the type-2 fimbriae of *S. paratyphi* B. No type-1 fimbriae have been found in any strain of *S. pullorum*, and it is thought that the type-2 strains have arisen by mutation from a parental *S. pullorum*-like organism with type-1 fimbriae that is now extinct.

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