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# Serotypes of sex pili

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SUMMARY

There are situations in which direct observation of the attachment of antibody molecules may be the simplest method of detecting antigen-antibody reactions. Applied to the study of the sex pili determined by a number of transmissible bacterial plasmids, the method has distinguished four serotypes in the F-like class and two in the I-like class. Antibody was usually attached haphazardly to the pili; however, in a few cases a regular periodicity could be observed. When few antibody molecules were attached, they could frequently be individually resolved and in certain antibody-pilus combinations large antibody molecules, tentatively identified as IgM, could be seen to predominate.

## INTRODUCTION

The ability of *Escherichia coli* K12 to conjugate and act as a genetic donor is due to the presence of an extrachromosomal genetic element named F, for 'fertility' (Cavalli, Lederberg & Lederberg, 1953; Hayes, 1953) or 'sex factor'. The first indication that F determined the synthesis of a structural component of the donor cell was the discovery by Ørskov & Ørskov (1960) of an F-specific surface antigen, which made $F^+$ bacteria agglutinable by homologous antiserum from which all agglutinins for $F^-$ bacteria had been removed. When F was later shown to determine the special type of filamentous appendage named F-pilus (see Brinton, 1965), it was here the F-specific antigen detected by agglutination was seen by electron microscopy to be located (Ishibashi, 1967; Lawn, Meynell, Meynell & Datta, 1967).

Since the discovery of F, a number of analogous genetic elements have been found in plasmids such as the Col factors responsible for transmissible colicin production (see Fredericq, 1963) or R factors responsible for transmissible resistance to antibiotics (see Watanabe, 1963). These sex factors also determine sex pili as can be demonstrated with derepressed mutants, pilus production being regulated by a repressor in the wild-type (see Meynell, Meynell & Datta, 1968; Meynell & Cooke, 1969). The majority of sex factors belong to one or other of two major classes related either to F or to the sex factor of ColI (Lawn *et al.* 1967). F-like and I-like sex pili can be distinguished morphologically, by the existence of donor-specific phages specific for each and by serological relationships within, but not between, each class (Sekijima

& Iseki, 1966; Lawn *et al.* 1967; Nishimura, Ishibashi, Meynell & Hirota, 1967; Kétyi & Ørskov, 1969). There are, however, minor differences between sex piil belonging to the same class, and the present paper reports a serological analysis which has distinguished four serotypes in the F-like class and at least two in the I-like class. The tests were made by observing antibody bound to the sex pili by electron microscopy; in this way, difficulties of agglutination tests with unstable bacterial suspensions were avoided. Bacteria carrying F or ColV form uniform suspensions in a medium of suitable pH, but the pili of bacteria carrying the other sex factors caused the bacteria to aggregate spontaneously. Moreover, agglutination tests for sex pili with whole organisms require antisera free from antibodies to other bacterial components such as cell wall or flagella. Such antibodies do not interfere with electron microscopical observation where the reactions of the serum with the sex pili can be distinguished morphologically from reactions with flagella or bacterial surface.

## METHODS

### *Plasmids*

The plasmids examined are listed in Table 1 with references to their isolation, and their classification into the two major groups, F-like and I-like (Lawn *et al.* 1967). Only cultures in which a large proportion of the bacteria produce sex pili could be tested. Wild type F and ColV produce sex pili constitutively but it was

Table 1. *Plasmids*

| Plasmid | Sex factor class | References |
|---|---|---|
| F | F-like | Hayes, 1953; see Brinton, 1965 |
| ColV–K94 | F-like | Kahn & Helinski, 1964; MacFarren & Clowes, 1967 |
| R100 | F-like | Egawa & Hirota, 1962; Nishimura *et al.* 1967 |
| R1 | F-like | see Meynell *et al.* 1968 |
| R136 | F-like | see Meynell *et al.* 1968 |
| R192 | F-like | see Meynell *et al.* 1968 |
| R538–1 | F-like | Romero & Meynell, 1969 |
| R64 | I-like | see Meynell *et al.* 1968 |
| R144 | I-like | see Meynell *et al.* 1968 |
| R163 | I-like | see Meynell *et al.* 1968 |
| R538–2 | I-like | Romero & Meynell, 1969 |
| ColI–P9 | I-like | Ozeki, Stocker & Smith, 1962; Meynell & Lawn, 1967*a* |
| ColEla | I-like | Lewis & Stocker, 1965; Meynell & Lawn, 1967*b* |

necessary to use derepressed mutants of the other factors. There is no reason to believe that the structure of the sex pilus in these mutants differs from the wild-type, for no difference between individual mutants could be detected in cases where more than one derepressed mutant of the same factor was available for examination. Reference to any particular sex factor other than F and ColV implies a bacterium carrying a derepressed mutant of that factor. The sex factors

R538–1 and R538–2 are given suffixes because they are two separate R factors which together account for the infectious drug resistance of a clinical isolate, R538 (Romero & Meynell, 1969).

*Bacteria*

The numbers of sex pili produced with a given sex factor depend to a substantial extent on the bacterial host. Wherever possible, the sex pili were examined in the *fim*$^-$ (*pil*$^-$) mutants, RC22 or RC24, of *E. coli* K12 strain 945 which are unable to produce common pili (Maccacaro, Colombo & Nardo, 1959). Derepressed R100 (R100–1: Egawa & Hirota, 1962) produced very few sex pili in either RC22 or RC24 and was therefore examined in another K12 strain, J5–3, which was *pil*$^+$. Since the bacteria were generally grown on solid medium, which discourages production of common pili (Duguid & Wilkinson, 1961), these interfered very little with the tests. In general, derepressed R factors lead to the formation of many more sex pili than either F or ColV and when F pili were scarce in RC22 or RC24 they were sometimes more abundant in a *pil*$^+$ strain of *Salmonella typhimurium* carrying an F-*lac* factor (SA 197, kindly provided by Dr K. Sanderson). ColI and ColEla sex pili were examined in both J5–3 and *S. typhimurium* LT2.

Both for the production of antisera and for the serological tests, the bacteria were grown on nutrient agar plates incubated at 37° C. for 18–24 hr. Culture on agar improved the yield of R pili and as many F pili were obtained on agar as in broth. Reactions with common pili were examined in broth cultures of strain J5–3 incubated at 37° C. for 24 hr. without shaking (Duguid & Wilkinson, 1961).

*Antisera*

For all the antisera except EM159, EM168, EM157, EM165 and EM169, which were produced with formalinized cultures and kindly provided by Dr Naomi Datta, rabbits were inoculated intravenously with live cultures containing about $5 \times 10^8$ bacteria/ml. Two, or in a few cases more, rabbits were used for each plasmid. The numbers in the tables refer to individual rabbits. Antibacterial antibodies were removed by absorption with the host bacterium, and sex pilus antibody with bacteria carrying the appropriate plasmid. The absorbed sera were Seitz filtered, which was effective in removing residual fragments of sex pili of the absorbing strain but slightly decreased the antibody titre.

*Serological tests*

The serological test entailed the direct observation of unlabelled antibodies bound to the antigen (sex pilus), according to the method of Lawn (1967). The bacteria were suspended in distilled water at a concentration of about $2 \times 10^8$/ml. and one drop of suspension was added either to one drop of unabsorbed antiserum diluted 1/20 in distilled water, to give a final concentration of 1/40, or to one drop of absorbed antiserum whose effective concentration after dilution and Seitz filtration was nearly twofold higher. The mixtures, on sheets of 'Parafilm', were left at room temperature for $\frac{1}{2}$–3 hr. The amount of antibody bound increased considerably during the first 10 min. after mixing, but changed little after the

first ½ hr. About 0·005 ml. of the mixture was then transferred to a 5 mm. diameter disk of membrane filter ('Sartorius', pore size 0·05 mμ) and unbound antibody was removed by suction. The organisms were then washed by dialysed broth followed by distilled water; transferred to an electron microscopy specimen grid (coated with formvar and carbon) by floating the grid on the final drop of water and sucking it down on the filter; and, finally, stained with a 1 % solution of uranyl acetate in distilled water. Hydrophobic grids (coated with carbon in an evaporation unit lacking a liquid nitrogen trap to the diffusion pump) adsorbed fewer bacteria but gave a cleaner background because they adsorbed less unbound antibody. For investigating a non-random form of antibody attachment observed in certain antigen–antiserum combinations, other negative stains and methods of fixation were tested, which are mentioned in the relevant section.

*Grading the antigen–antibody reaction*

The degree of antibody binding after exposure to a fixed dilution of serum was assessed according to an arbitrary grading system. An end-point method with graded dilutions of serum proved to be impracticable because of the difficulty of establishing a precise end-point (see Non-uniform attachment of antibody). Five grades ranging from 0 to + + + + (maximal reaction) were recognized (Pl. 1, fig. 1). These grades have not been related to titres obtainable by standard immunological procedures such as agglutination; however, in electron micrographs of comparable tests with flagella (Feinstein & Munn, 1966), the reaction shown at the agglutination end-point would have been graded 0 to + in the scheme used here. The reliability of the method was established by repeated tests of the same serum and antigen, for the results agreed within one grade even if they were separated by intervals of several months. Many of the antisera were sufficiently potent to give a + + to + + + reaction at a dilution of 1/8000.

## RESULTS

### *F-like factors*

The results with unabsorbed antisera confirmed the previous conclusion that F-like sex factors are serologically related to one another (Lawn *et al.* 1967). Nevertheless, there was such a variety of reactions that the pili determined by different plasmids could in many cases be distinguished (Table 2). The differences between the pili were emphasized when cross-absorbed sera were used (Table 3). The results indicated four serotypes in the F-like class: the first is the F serotype, represented by F and ColV–K94; the second and third are the R538–1 and R1 serotypes, each represented by only one member; and the fourth is the R100 serotype, represented by R100, R136 and R192. Cross-absorbed sera did not distinguish between the pili determined by F and ColV–K94, nor between those determined by R100, R136 and R192. The R538–1 and R1 serotypes were more closely related to one another than were any other pair of serotypes because absorption of anti-R1 serum with R538–1 pili removed all antibody reacting with R1 pili. However, the two factors were not identical because absorption of anti-

Table 2. *Reactions of F-like sex pili with unabsorbed antisera*

| Antiserum to ... | F Number | | | | ColV-K94 Number | | R1 Number | | | R538-1 Number | | R192 Number | | R136 Number | | R100 Number | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EM 159 | EM 168 | 265 | 266 | EM 157 | EM 165 | EM 169 | 257 | 264 | 97 | 100 | 96 | 99 | 214 | 215 | 216 | 217 |
| Antigen | | | | | | | | | | | | | | | | | |
| F | 4* | 3–4 | 4 | 4 | 4 | 4 | 1 | 2–3 | 3 | 3 | 3–4 | 1–2 | 2–3 | 1–2 | 2–3 | 2–3 | 1–2 |
| ColV-K94 | 4 | 3–4 | 4 | 4 | 4 | 3–4 | 0–1 | 2–3 | 2–3 | 3 | 4 | 1–2 | 2–3 | 1–2 | 3 | 2–3 | 1–2 |
| R1 | 2–3 | 1–3 | 0 | 0 | 0–1 | 0–1 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0–1 | 0 | 0 |
| R538-1 | 2–3 | 0–3 | 1–2 | 0–2 | 2–3 | 0–3 | 4 | 4 | 4 | 4 | 4 | 0 | 0–2 | 0 | 0–2 | 1–2 | 0–1 |
| R192 | 0–1 | 0 | 1–2 | 1–3 | 1–2 | 1–2 | 0 | 0 | 1–2 | 2–3 | 1–3 | 4 | 4 | 4 | 2–4 | 2–4 | 4 |
| R136 | 0–2 | 0 | 0–2 | 1–2 | 0–2 | 0–2 | 0 | 0 | 0–1 | 1–3 | 1–3 | 4 | 4 | 4 | 3–4 | 3–4 | 4 |
| R100 | 0–2 | 0 | 0–2 | 1–2 | 0–2 | 1–2 | 0 | 0 | 0 | 1–3 | 1–3 | 4 | 4 | 4 | 2–4 | 3–4 | 4 |

* For the system of grading, see Methods (the + symbols have been omitted).

Table 3. *Reactions of F-like sex pili with cross-absorbed antisera*

| Antiserum to ... | F Number | | | | | ColV-K94 Number EM 165 | | | R1 Number EM 169 | | | R538-1 Number 97 | | | | R192 Number 99 | | | | R136 Number 214 | | R100 Number 217 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Absorbed with ... | EM 168 ColV | EM 168 R1 | EM 168 R538-1 | 265 R538-1 | 265 R136 | F | R1 | R538-1 | F | ColV | R538-1 | F | ColV | R1 | R192 | F | R538-1 | R136 | R100 | R192 | R100 | R192 | R136 |
| Antigen | | | | | | | | | | | | | | | | | | | | | | | |
| F. | 0 | 4 | 1–3 | 4 | 4 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 1–2 | 4 | 0 | 1–2 | — | — | — | — | — | — |
| ColV-K94 | 0 | 4 | 2–4 | — | — | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0–2 | — | — | — | — | — | — | — | — | — |
| R1 | 0 | 0 | 0 | — | — | 0 | 0 | 0 | 4 | 4 | 0 | 4 | 1–3 | 0 | 4 | — | — | — | — | — | — | — | — |
| R538-1 | 0 | 0 | 0 | 0 | 0 | 0 | 2–3 | 0 | 4 | 4 | 0 | 4 | 2–3 | 0–3 | 4 | 0 | 0 | — | — | — | — | — | — |
| R192 | — | — | — | 0 | 0 | — | 0 | 0 | — | — | — | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| R136 | — | — | — | — | 0 | — | — | — | — | — | — | — | — | — | — | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| R100 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0 | 0 | 0 | 0 | 0 | 0 |

Table 4. *Reactions of I-like sex pili with unabsorbed antisera*

| Antiserum to ... | R64 Number | | R144 Number | | R163 Number | | R538–2 Number | |
|---|---|---|---|---|---|---|---|---|
| | 113 | 114 | 160 | 172 | 116 | 117 | 108 | 110 |
| R64 | 4 | 3–4 | 3–4 | 3–4 | 3–4 | 1–2 | 4 | 3–4 |
| R144 | 3–4 | 3–4 | 3–4 | 3–4 | 3–4 | 1–2 | 4 | 2–3 |
| R163 | 3–4 | 3–4 | 3–4 | 3–4 | 3–4 | 2–3 | 4 | 3–4 |
| R538–2 | 3–4 | 3–4 | 3–4 | 3–4 | 3–4 | 1–3 | 4 | 3 |
| ColI | 4 | 3–4 | 3–4 | 3–4 | 3–4 | 2–3 | 4 | 3–4 |

Table 5. *Reactions of I-like sex pili with cross-absorbed antisera*

| Antiserum to ... | R64 Number 113 | | | R144 Number 172 | | | R163 Number 117 | | | R538–2 Number 110 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Absorbed with ... | R144 | R 163 | R 538–2 | R64 | R163 | R538–2 | R64 | R144 | R538–2 | R64 | R144 | R163 | I |
| R64 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| R144 | 0 | 0 | 0 | 1–3 | 0 | 0 | 2–3 | 0 | 0 | 4 | 0 | 0 | 0 |
| R163 | 0–1 | 0 | 0 | 2–3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 0 |
| R538–2 | 0 | 0 | 0 | 2–3 | 0 | 0 | 3–4 | 0 | 0 | 4 | 0 | 0–1 | 0 |
| ColI | 0–1 | 0–1 | 0–1 | 3 | — | — | 3–4 | — | — | 4 | — | — | 0 |

R538–1 serum with R1 pili did not remove all the activity for R538–1 pili and, further, there was present in a serum prepared against ColV–K94 a small minority of antibody molecules which reacted with R538–1 pili and not with R1 pili.

### *I-like factors*

There was evidently less antigenic diversity among I-like than among F-like sex pili, for each reacted indistinguishably from all the others in tests with unabsorbed antisera (Table 4). The pili of ColI and the I-like sex factor of ColEla, which were also tested, reacted similarly. Even serum 117, prepared against R163, which had a relatively low titre of antibody and gave submaximal reactions at the standard dilution, did not distinguish between its homologous and heterologous antigens. With cross-absorbed sera, R64 pili could be distinguished from the pili of R144, R163 and R538–2, which, if one discounts minor reactions which may have been due to inadequate absorption of the sera, were probably similar to one another (Table 5). The reactions of ColI and ColEla pili placed them with the latter group. These I-like sex pili are therefore provisionally divided into two serotypes only, an R64 serotype and another which is called the ColI-R144 serotype (Table 6). The status of the second type may need revision when mirror tests are made with antisera to the Col factor pili.

Table 6. *Serotypes of sex pili*

| | F-like | | | |
|---|---|---|---|---|
| Serotypes | F | R1 | R538–1 | R100 |
| Members | F<br>ColV-K94 | R1 | R538–1 | R100<br>R136<br>R192 |

| | I-like | |
|---|---|---|
| Serotypes | R64 | ColI-R144 |
| Members | R64 | R144<br>R163<br>R538–2<br>ColI<br>ColEla |

### *Tests for cross-reactions between F-like and I-like sex pili: spurious heterologous reactions*

The lack of antigenic relationship between F-like and I-like sex pili (Lawn *et al.* 1967) was confirmed with a more extensive range of antisera. All the antisera prepared against F-like pili were tested with R64 and R144 and all those against I-like pili with F, R1 and R192.

The sera were tested at 1/10 after absorption of antibacterial antibodies. However, at this low dilution there was a certain amount of particulate material which could, by masking a weak positive reaction, make it indistinguishable from a negative reaction. Five sera, nevertheless, gave reactions that could be unambiguously graded as + + or more. Three of these (numbers 160, 116 and 108) had been prepared against bacteria carrying I-like pili (R144, R163 and R538–2,

respectively) but reacted also with F-like pili (F and either R1 or R192). The other two (EM159 and 100) were prepared against F-like pili (F and R538–1, respectively) but reacted also with the I-like pili of R64 and R144. When the sera were retested at the standard 1/40 dilution, the activities of numbers 160, 108 and 100 could no longer be detected, and those of numbers 116 and EM159 could be graded as only + to + +. Although this result suggested an antigenic relationship, even if only a minor one, it proved to be due to antibodies other than those directed against the immunizing antigens. In each case, absorption of the serum with the strain used for immunization removed all reactivity with pili of its own class (F-like or I-like) but not the other class.

To test whether rabbit sera contained 'natural' antibodies to sex pili, sera from 11 rabbits which had been used for purposes unconnected with the production of enterobacterial antibodies were tested against the same representative set of sex pili of both classes. Three of the 11 sera gave positive reactions with the F pili at 1/10 and two of these at 1/40 (graded 0 to + and + to + +).

All the sera, including these 11 as well as the 25 sex pilus antisera, were tested at 1/10 with J5–3F$^-$R$^-$ for antibody against *E. coli* common pili. The R100 antisera, numbers 216 and 217, necessarily had anti-common pilus activity because the animals had been immunized with the R factor in the *pil*$^+$ strain J5–3. Two of the 34 other sera, numbers EM168 and 214, also contained common pilus antibody, although in relatively smaller amounts.

### '*Terminal knobs*'

Lawn (1966) suggested that the knob sometimes seen at the tip of a sex pilus is cell-wall material. Detached cell-wall fragments evidently may become attached to the distal ends of sex pili, because they react with antibody to the cell-wall but not to the pilus (Pl. 2, fig. 6). Nevertheless, not all terminal knobs reacted in this way, for some were seen to adsorb pilus-specific antibody (Pl. 2, fig. 5). This observation, together with the fact that knobs are more frequent on pili with structural mutations (Meynell & Aufreiter, 1969) and can also be produced by treatment with low concentrations of the detergent sodium dodecyl sulphate (unpublished observations) suggests that many knobs are simply assemblies of pilus subunits less ordered than those forming the sex pili proper.

### *Type and distribution of antibody molecules*

#### *Non-uniform attachment of antibody*

When the concentration of antiserum was sufficiently high, all the pili were uniformly and maximally (+ + + +) coated with antibody molecules. With lower concentrations, the pattern of attachment of the fewer antibody molecules depended on the particular antigen-antibody system. At one extreme, the variation in distance between individual antibody molecules was not significantly greater than to be expected from random attachment to a uniform antigen. At the other, the density of packing varied considerably from one region of a pilus to another (Pl. 2, fig. 2) and to an even greater extent between different pili in the same

culture. This might have been due to attachment, not of individual antibody molecules, but of aggregates resulting, for instance, from the presence of free pili subunits in the preparations. Nevertheless, along the sections of pilus where the largest amounts of antibody were attached, the increase in diameter of the pilus due to the attached antibody was relatively constant and compatible with the estimated thickness of a monolayer of globulin molecules. Where, as occasionally occurred, there were recognizable aggregates of antibody molecules, these were associated with an increase in diameter which was both greater and more uneven.

Non-uniform distribution of antibody was characteristically most pronounced in weak reactions of antigen with heterologous antibody, but with certain of the antigens, particularly R538–1, it occurred with many antisera. It was generally absent in homologous reactions with unabsorbed antisera. Its occurrence in the tests at standard dilution is apparent from the way in which the reactions are recorded in Tables 2–5 where a range, such as 0 to + + or + to + + +, spanning more than one grade of reaction signifies greater or lesser degrees of non-uniformity. Reactions which were non-uniform on one occasion were always non-uniform when repeated, although the degree of non-uniformity might vary.

It is possible that the non-uniformity was due to the preparative procedures for electron microscopy. Thus, antibody might have been removed by the highly acid uranyl acetate stain. The preparations were therefore fixed with formalin or glutaraldehyde to produce cross-linking within antigen–antibody complexes before the uranyl acetate was applied. However, the distribution of antibody remained non-uniform, as before, and was equally so when uranyl acetate was replaced with neutral stains such as neutral solutions of potassium phosphotungstate or potassium silicotungstate. The image of free antibody molecules is not the same with uranyl acetate as with these other stains (Pl. 3, fig. 9) but the latter are greatly inferior to uranyl acetate as stains for bound antibody molecules. Non-uniformity was equally evident when broth, 0·85% saline or distilled water was used as diluent.

Another possible cause of non-uniformity is slow or inadequate penetration of antibody into clumps of pili, so that all the available antibody is bound to those parts of the pili that are exposed. Accordingly, the antigen concentration was varied over a tenfold range with a constant dilution of antiserum and antigen-antiserum mixtures were lightly sonicated to disperse bundles of pili before making a further addition of antiserum. None of these procedures altered the antibody distribution in any way.

*Types and arrangements of antibody molecules*

In maximal (+ + + +) reactions, the antibody molecules were usually attached haphazardly. However, in a few cases, a regular periodicity was observed which was sometimes transverse (Pl. 2, fig. 3) and sometimes diagonal (Pl. 2, fig. 4) to the long axis of the pilus. The reason for this occasional periodicity is not clear. It might reflect a repeating structural pattern of the antigen underneath, that cannot be resolved in the untreated pilus, or a regular arrangement of antibody molecules entailed by very close packing on the antigen surface.

In weak reactions, individual antibody molecules could sometimes be seen to resemble morphologically molecules of IgM or IgG (Green, 1969). For the most part, the antibody consisted of small molecules, like IgG (Pl. 3, fig. 7), as was to be expected in antisera raised by a prolonged course of immunization (Uhr & Finklestein, 1963). In a few antigen-antibody combinations, however, the predominant antibody molecule was large with the star- or crab-shaped appearance of IgM (Pl. 3, fig. 8, 9).

## DISCUSSION

Contrary to what was originally supposed, the ability to transfer genetic information by conjugation is relatively frequent among the enterobacteriaceae. Whatever the nature of sex factors and whether or not they constitute a single biological group in which all the members have evolved from one another, it is clear that they comprise at least two classes producing dissimilar sex pili subject to independent systems of regulation. In addition, four serotypes are distinguishable within the F-like class and two within the I-like class. Antigenic differences have also been reported between the sex pili determined by the ColB factor and both F and R 1 (Kétyi & Ørskov, 1969). Within each of the two classes, the genes determining the various pili serotypes probably bear the same relationship to one another as, for instance, the H 1 genes of different salmonellae, which have similarly been shown to share a common system of regulation (Lederberg & Iino, 1956; Pearce & Stocker, 1967).

Most transmissible plasmids have been recognized because the sex factor is linked to other genes, collectively named 'somatic' (see Novick, 1969), determining bacterial characters such as colicin production or antibiotic resistance. The use of the names of the somatic genes to name the plasmid is a logical practice but experience indicates that these names do not reliably identify the sex factor itself, partly because of the readiness with which the somatic genes can be lost or exchanged by recombination between one plasmid and another (see Meynell *et al.* 1968). Even within the present relatively small sample, there were three cases where more than one plasmid determined a sex pilus of the same serotype. The simplest assumption is that in each case, different somatic genes were linked to the same sex factor. A difficulty arises therefore in referring to the sex pili serotypes but it seems nevertheless preferable, in the present state of knowledge, to retain terms such as 'R 100 serotype' rather than introduce entirely new letters or numbers. Ultimately it may prove desirable to define serotypes more accurately by combinations of symbols in the same way as these are used for flagellar and other bacterial antigens.

The 'natural' anti-pilus antibodies in some rabbit sera require comment. Most wild-type sex factors are normally repressed and therefore produce more than 1000-fold less pilus antigen than even the small amounts present with the derepressed mutants used for producing the antisera. Nevertheless, these amounts are evidently sufficient to elicit natural antibodies during the course of the rabbit's life, unless some other hitherto unrecognized antigen related to sex pili is implicated. 'Natural' antibodies to *E. coli* common pili were not any more frequent

than antibodies to sex pili, although the animals were likely to have been exposed to much greater numbers of common pili in the environment than to sex pili.

The non-uniform attachment of antibody molecules sometimes observed is difficult to explain if, as is believed, pili are composed of uniform subunits and if individual antibody molecules are attached independently of one another.

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## EXPLANATION OF PLATES

All are electron micrographs negatively stained with uranyl acetate. The calibration bars represent 100 nm.

### PLATE 1

Fig. 1. Grading of antigen–antibody reactions (see Methods).

### PLATE 2

Fig. 2. The non-uniform reaction between serum 97 (anti-R 538–1) and an R 538–1 pilus. There is a large variation in distance between individual antibody molecules along the pilus.

Fig. 3. Regular transverse periodicity shown when serum 97 (anti-R 538–1) reacts with R 100 pili.

Fig. 4. Regular diagonal periodicity shown when serum 113 (anti-R 64) absorbed with R 538–2 reacts with R 64 pili.

Fig. 5. Reaction of sex pili with anti-pilus serum containing no cell-wall antibody. The terminal knob (arrow) reacts in the same way as the pilus.

Fig. 6. Reactions of flagella and a free cell-wall fragment (single arrow) with antiserum. The sex pilus itself does not react with this antiserum but a positive reaction is shown by its terminal knob (double arrow).

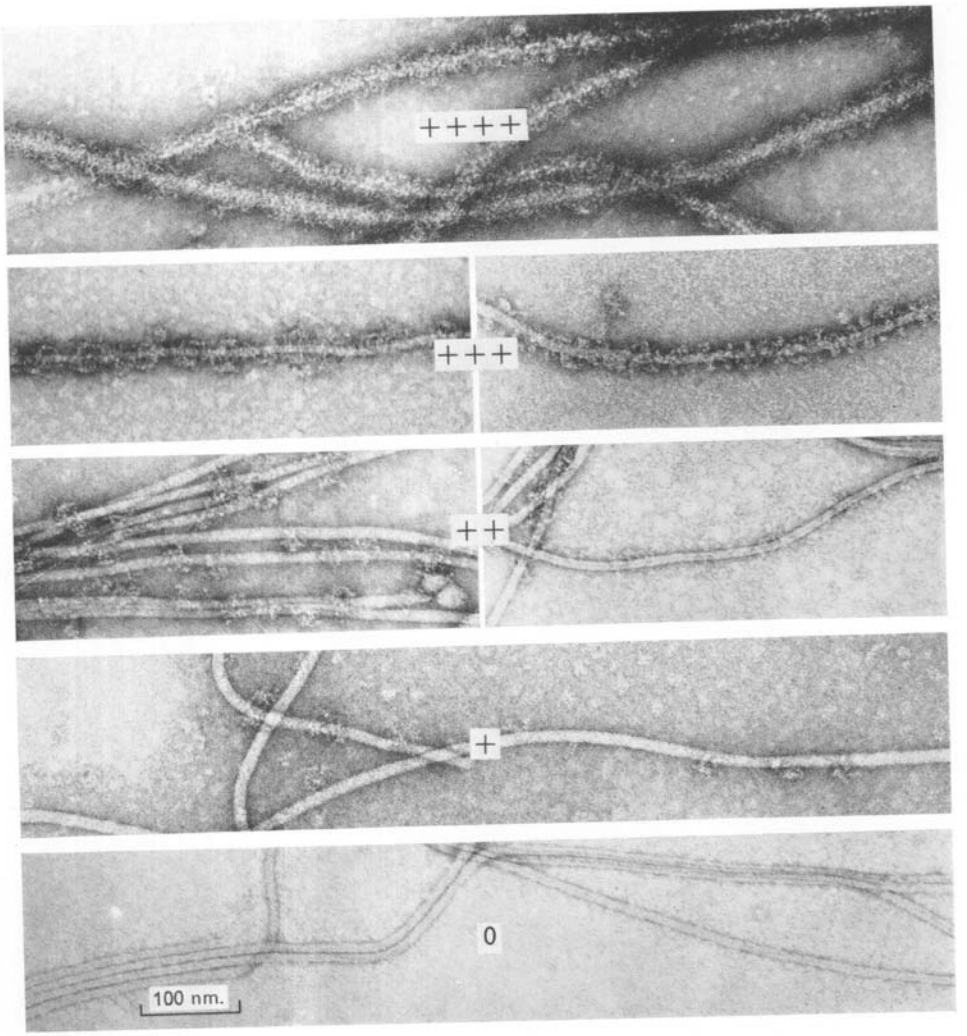
### PLATE 3

The calibration bar on Fig. 9 also refers to Figs. 7 and 8.

Fig. 7. Small immunoglobulin molecules attached to a sex pilus. Note the pairs of parallel rod-shaped structures (arrows).

Fig. 8. Large star- or crab-shaped immunoglobulin molecules (arrows) attached to sex pili.

Fig. 9. Human IgM immunoglobulin molecules negatively stained with uranyl acetate, for comparison with Fig. 8. (The sample of IgM immunoglobulin was kindly provided by Professor R. A. Kekwick.)

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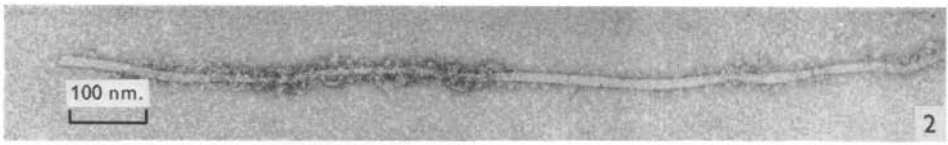


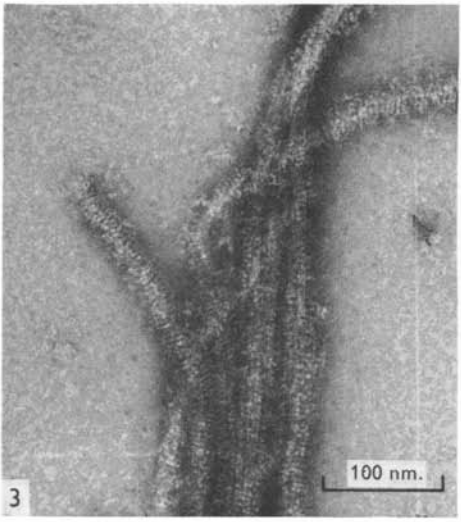


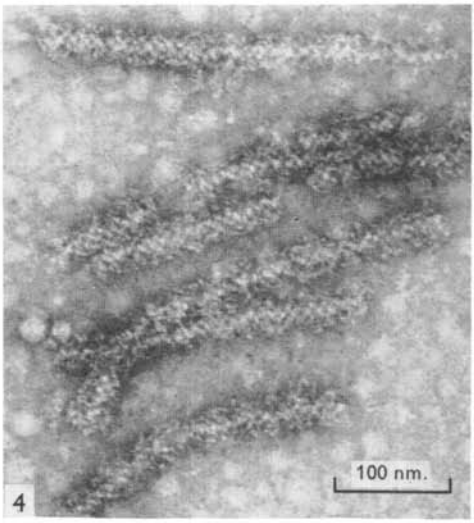


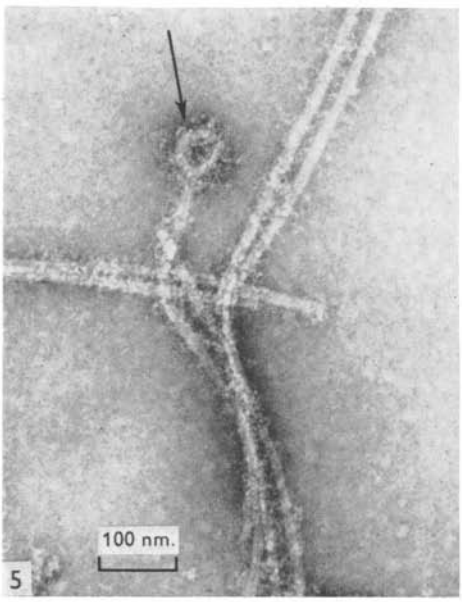


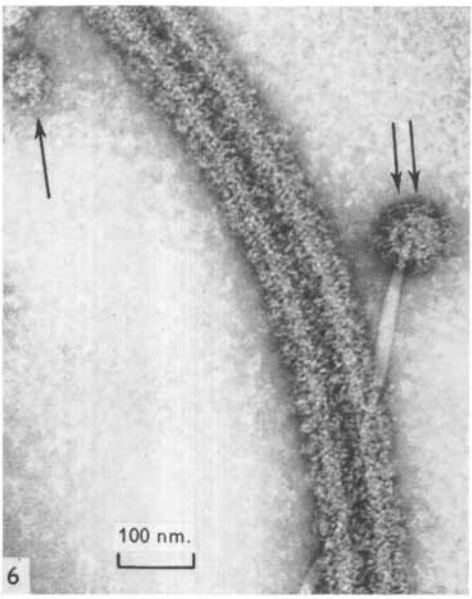




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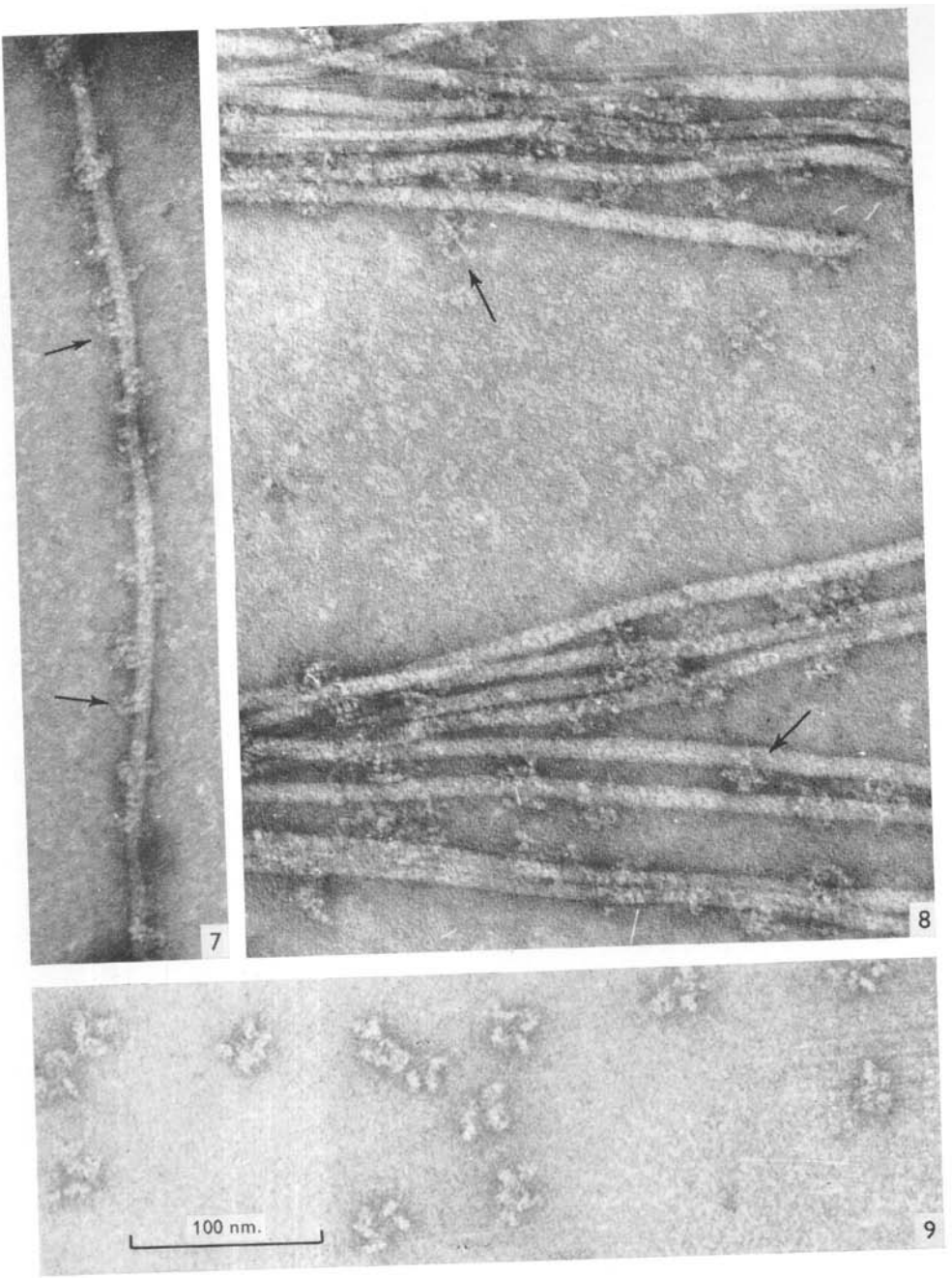


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