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A study of pili on *Pseudomonas aeruginosa*

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SUMMARY

Pseudomonas aeruginosa carries polar pili which act as receptors for RNA-containing bacteriophages. In order to confirm that these pili were not involved in the transfer of the sex factor FP 2, eleven bacterial strains, both FP 2^+ and FP 2^-, were examined in the electron microscope for the presence of pili and tested for sensitivity to the RNA phage PP 7. Pili were found on all strains save one which was resistant to phage PP 7. It was also found by electron microscopy that about 25 times more pili per cell were present after PP 7 adsorption than before it. This result is discussed with reference to the pilus retraction theory, providing further evidence that some kinds of pili retract instead of acting as simple tubes for the transfer of genetic material. The strong supporting evidence provided by the infective processes of male-specific coliphages is discussed and compared to current knowledge of *P. aeruginosa* RNA phages.

It was also found that pili were present on the host strain for the *P. aeruginosa* filamentous phage Pf. Although similar in appearance to RNA phage pili, these differed in that they did not adsorb phage PP 7. However, it seemed likely that they were receptors for Pf. A structural comparison is made between *P. aeruginosa* pili and *Escherichia coli* F-pili. It is possible that *P. aeruginosa* pili could be coded for by a plasmid other than FP 2.

1. INTRODUCTION

The peripheral F-pili (Brinton, Gemski & Carnahan, 1964) and R-pili (Datta, Lawn & Meynell, 1966) of *Escherichia coli* are involved not only in conjugation, but also as receptors for RNA and filamentous bacteriophages. Similar thin filaments have been observed on the poles of *Pseudomonas aeruginosa* and they too act as receptors for RNA phages (Bradley, 1966; Fuerst & Hayward, 1969; Weppelman & Brinton, 1971). Bradley (1966) originally suggested that these pili could be involved in the transfer of the *P. aeruginosa* sex factor FP 2 (Holloway & Jennings, 1958), in which case the RNA phages should be specific for FP 2^+ bacteria. However, Holloway (1969) stated in his review that they are not and that the presence of pili is not linked with FP 2. The object of this report is to clarify the relationship between pili and the *P. aeruginosa* plasmids FP 2 and FP 39 (Holloway, personal communication), and to relate piliation to RNA phage sensitivity over a range of FP 2^+ and FP 2^- strains.

The exact function of both *P. aeruginosa* pili and F-pili is still uncertain. The

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essential role of F-pili and R-pili in conjugation and as coliphage receptors is indisputable, but currently there are two theories regarding their mode of operation. The first (Brinton, 1965) suggests that they act as tubes within which a nucleic acid molecule can travel during conjugation and phage infection. The objection to this is that these thin filaments must be capable of transporting different kinds of nucleic acids in different directions, and an alternative, the 'F-pili retraction model', has been proposed by Marvin & Hohn (1969). They suggest that, on contact with a recipient cell or bacteriophage, the pilus would retract, being depolymerized by a mechanism at its base; this would be activated by a signal transmitted from the tip or the side. Since *P. aeruginosa* pili act as receptors for RNA phages, it is reasonable to suppose that they too might retract. However, they are not necessarily functionally identical to F- and R-pili in that there is no evidence that they transfer genetic material. One of the most important results shown here is that pili are more numerous after RNA phage adsorption than before. The inference is that the adsorbed virions prevent retraction. The validity of this proposal is discussed together with other observations which include a study of pili on the host strain for the *P. aeruginosa* filamentous phage Pf (Takeya & Amako, 1966).

2. METHODS

(i) *Bacteria and bacteriophages*

The following strains of *P. aeruginosa* were kindly supplied by Professor B. W. Holloway: PTO 13 (Stanisich & Holloway, 1969), PAO 67, PAO 2, PAO 68, PAO 1264, PAO 41 (Holloway, 1969), PAO 38 and PAO 381 (Holloway, 1969). Details of their genotypes are shown in Table 1 (Results). PAO 1, the *P. aeruginosa* strain 1 wild type (Holloway, 1955) or ATCC 15692 was originally a gift from Dr T. Feary, and has been redeposited as phage PP 7 host (ATCC 25247). Strain 1/7 is a mutant of Holloway strain 1 resistant to phage PP 7 (Bradley, 1966). An FP 2^{+} derivative of Holloway strain 1 (PAO 1), designated strain 1 FP 2^{+}, was given by Dr M. G. Marinus (see Table 1 for genotype). The host for the *P. aeruginosa* filamentous phage Pf (ATCC 25102) was donated by the American Type Culture Collection, likewise the phage (ATCC 25102B). The small (25 nm) icosahedral RNA-containing phage PP 7 was isolated on *P. aeruginosa* Holloway strain 1 (ATCC 25247) from sewage located at Pangbourne, England (Bradley, 1966), and is deposited as ATCC 25247 B. The contractile phage PB 1 (Bradley & Robertson, 1968) and PB 8^{+} (short non-contractile tail) were isolated on strains 1 (PAO 1) and 1 FP 2^{+}, respectively, from sewage located at Basingstoke, England.

(ii) *Culturing media and methods*

Oxoid nutrient broth was used for plate and broth cultures, the former containing 2% (w/v) agar. Bacteria were grown for electron microscopy in 25 ml of broth in a 250 ml conical flask shaken at 37° at about 2 oscillations/sec. Bacteriophage stocks were grown by lysing 15 cm diameter plates of confluent bacterial

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growth using the double agar layer method (Adams, 1959) with 0·5 % (w/v) agar in the top layer. Phages were harvested by extracting each plate with 10 ml of broth for several hours. Partial purification was achieved by differential centrifugation.

(iii) *The isolation of phage-resistant bacteria*

P. aeruginosa strain 1/7 and a mutant of *P. aeruginosa* ATCC 25102 resistant to phage Pf (designated 25102/12Pf) were isolated by placing a loopful of high-titre phage suspension on the surface of a double agar layer plate of the host bacterium. After incubation, soft agar from the centre of the resulting 'plaque' was streaked and isolated colonies were subcultured a minimum of three times. Phage-resistant clones were retained and tested for possible lysogeny by placing a loopful of bacteria on a double agar layer plate of the original sensitive host. Clearing around the resulting colony indicated the presence of phage in the culture. Distinction between lysogeny and a form of carrier state was determined as follows. The clone was grown in the presence of anti-phage serum in broth for 3 days, during which it was subcultured three times. It was finally streaked three times and re-tested against the sensitive host. A 'cure' with no clearing around the colony indicated a carrier state.

(iv) *Determination of phage-sensitivity patterns*

The sensitivity of the various strains of *P. aeruginosa* to representative phage types was tested by the spot-test method described above using high-titre (about 1×10^{10} p.f.u./ml) phage suspensions. In addition to phages PP7 and Pf, PB 1 (Bradley & Robertson, 1968) and PB 8^{+} (Bradley, unpublished) were included to determine whether any strains exhibited a markedly different pattern from the remainder.

(v) *Electron microscopy*

Pili were counted on negatively stained preparations as follows. Shake cultures were grown from overnight plates for 4½–6 h, at which time a carbon-coated specimen grid was held in contact with the surface for a few seconds then immediately transferred to the surface of a 0·1 M ammonium acetate solution. After about 30 sec it was transferred to another ammonium acetate bath and finally touched on to a 1 % (w/v) solution of sodium phosphotungstate. After excess liquid had been removed with a filter paper the grid was dried. If too many bacteria were present, the culture was diluted with broth. Bacteria prepared thus appeared very clean in the electron microscope and were not subjected to physical disturbances such as centrifugation. Such a preparation will be referred to as 'unlabelled'.

'Labelling', or treatment with phage PP7 or Pf was carried out by diluting a sample of the same culture with broth to an optical density corresponding to a viable count of about $1·5 \times 10^{8}$ cells/ml. One ml of this was mixed with an equal amount of phage suspension in broth (titre about 1×10^{10} p.f.u./ml) to give a multiplicity of about 670 PP 7 phages/bacterium and about 67 Pf phages/bacterium. The mixture was then incubated at 37° in a shaking water bath in a 250 ml flask for 10 min, at which time it was removed and a carbon-coated grid floated on its

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surface for about 5 min. Finally, the grid was washed and negatively stained as above.

In the electron microscope, pili were counted on about 50 reasonably isolated cells in a number of different grid squares. The percentage of piliated cells and the average number of pili per cell (including unpiliated ones) was calculated. In addition, several experiments were carried out to determine whether or not there was any gross change in the numbers of pili present during the growth cycle of a culture. The organism chosen was *P. aeruginosa* strain 1 (PAO 1): 200 ml of broth in a 1 l conical flask (base diameter 17·5 cm) was inoculated with an overnight plate culture to give a final concentration of about 1×10^8 cells/ml. Incubation at 37 °C was carried out in a water bath with shaking at 1 oscillation/sec. Samples were taken every 45 min for optical absorbance measurements and for the preparation of PP7-labelled and unlabelled organisms for counting pili in the electron microscope.

3. RESULTS

(i) *Tests for lysogeny on strains 1/7 and 25102/12Pf*

When *P. aeruginosa* strain 1/7 was spotted on a double agar layer plate of the parent strain (PAO 1), a wide clear area, found to be produced by phage PP7, surrounded the colony. However, this indication of apparent lysogeny was 'cured' after growth in the presence of phage antiserum. Thus strain 1/7 was not truly lysogenic and phage PP7 existed in some form of carrier state rather than as prophage, which was suggested for phage 7s, an RNA phage described by Feary, Fisher & Fisher (1964). Strain 25102/12Pf did not give clearing when spotted on its Pf-sensitive parent and was therefore merely resistant to and not lysogenic for Pf.

(ii) *Phage-sensitivity patterns*

The results of spot tests together with the genotypes of the strains of *P. aeruginosa* are given in Table 1. According to Takeya & Amako (1966), Pf-sensitive strains are rare. The one tested here differed from PAO 1 derivatives in its pattern of sensitivity to other phages.

(iii) *Changes in piliation during the growth cycle of a culture of* Pseudomonas aeruginosa *strain 1 (PAO 1)*

Three experiments were carried out in which the percentage of piliated cells (Text-fig. 1) and the average number of pili per cell (Text-fig. 2) were recorded for each sample taken during the growth cycle. The preparations examined are detailed in the figure legends, all three experiments being included to indicate the degree of reproducibility attained. In each case the bacterial growth curve (absorbance at 620 nm) was similar, and the doubling time was 50 min. Text-fig. 1 shows that in the labelled samples 100 % of the cells are piliated throughout while piliation in unlabelled ones falls to a more or less constant value (particularly in Expt 1) after an initial rise. Text-fig. 2 shows the average number of pili per cell

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based on total cell count. The changes for the unlabelled samples follow the same trend as those in Text-fig. 1. However, the much higher numbers of pili in labelled samples show a steady decrease from early log. phase. Considering the obvious inaccuracies involved in the methods, the results in Text-fig. 2 are reasonably reproducible.

Table 1. *Phage-sensitivity patterns and genotypes of strains of* Pseudomonas aeruginosa

		Bacteriophages			
Strain	Genotype	PB1	PB8+	PP7	Pf
PTO 13	trp-6, FP 2+	+	(+)	(+)	−
PAO 67	his-67, ese-r, FP″−	+	+	+	−
PAO 2	ser-3, FP 2−	+	(+)	+	−
PAO 68	trp-54, str-r, chl-r, FP 39−	+	+	−	−
PAO 1264	trp-54, str-r, chl-r, FP 39+	+	+	−	−
PAO 41	trp-54, str-r, chl-r, FP 2+	+	(+)	+	−
PAO 38	leu-38, FP 2−	+	(+)	+	−
PAO 381	leu-38, str-r, FP 2+	+	+	+	−
1 (PAO 1)	Wild type, FP 2−	+	(+)	+	−
1/7	PP 7-r, FP 2−	+	(+)	−	−
1 FP 2+	str-r, ese-s, FP 2+	+	+	(+)	−
ATCC 25102	Not known	(+)	−	−	+
25102/12 Pf	Not known	(+)	−	−	−

NOTE. + signifies a strong reaction (a clear area), (+) weak activity (a veiled area), and − no activity. Strain PAO 68 is the parent of PAO 1264 but not PAO 41. FP 39 is a new sex factor with a different point of origin to that of FP 2.

Various unsuccessful attempts were made to preserve the high pilus numbers of labelled cells by changing the support films to plastic, coating them with evaporated platinum, using fixatives, etc. Only in the case of 0·1% (w/v) OsO_4 was any degree of preservation achieved; seven times the normal number of unlabelled pili were visible after treatment as opposed to 25 times as many after labelling with PP7 and no fixation. These results indicated that the best time to take samples from other strains for counting pili was between 4½ and 6 h.

(iv) *Numbers of pili on strains of* Pseudomonas aeruginosa

Two sets of results are shown in Table 2: the percentage of cells with pili and the average number of pili per cell (including those with no pili) for labelled and unlabelled preparations. Additional columns indicate whether or not phage PP7 adsorbs to the pili, and the susceptibility to PP7. It will be noted (Table 2, note ‡) that strain PAO 67 has two kinds of filament – one which labels with PP7 and one which does not. Table 2 includes both with a breakdown in Table 3.

(v) *The appearance of pili in the electron microscope*

The pili observed on each of the different strains appeared more or less similar in the electron microscope. Such variations as could be discerned from sample to sample were slight and attributed to variations in negative staining. To demon-

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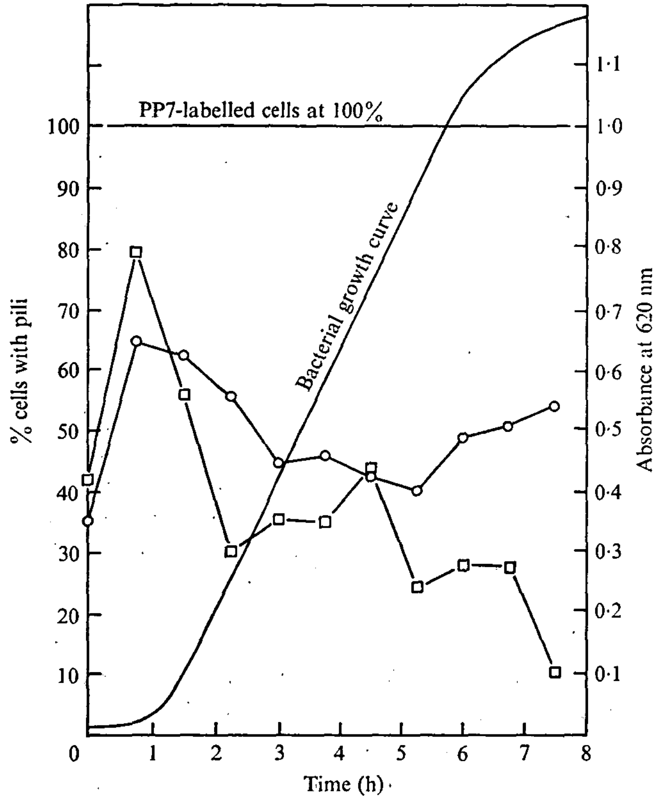
strate the conditions of counting, an example of labelled pili, which is exceptional only in the numbers present, is shown in Plate 1*a*. The filaments arise at or near the pole of the *P. aeruginosa* strain 1 (PAO 1) organism, and with the multiplicity of infection used each one is clearly distinguishable, not being obscured by too many phage particles. There are about 50 pili on both poles, the largest number observed



Text-fig. 1. Changes in the percentage of cells with pili during the growth cycle in liquid culture for *Pseudomonas aeruginosa* strain 1 (PAO 1). Expts 2 and 3 phage PP 7-labelled samples contained 100 % cells with pili throughout. Expt 1, unlabelled, ○—○; Expt 2, unlabelled, □—□. Absorbance at 620 nm refers to the bacterial growth curve.

in the present study. The points of entry into the cell of many of the pili can be clearly discerned despite the fact that they are well back from the pole (Plate 1*b*); they are not obscured by the body of the cell. It was notable in both labelled and unlabelled cells that it was comparatively rare for a pilus to be other than at the poles of a cell, and to be folded back across the body.

The average pilus length was not measured since it was so variable even on a single organism, but on *P. aerigunosa* strain 1 (PAO 1) it was usually 1–3·5 μm. Thickness measurements of parallel arrays of four or more pili in negatively stained preparations gave a value of 6·0 nm per pilus. There were no terminal appendages of any kind attached to the pili of the strains examined.

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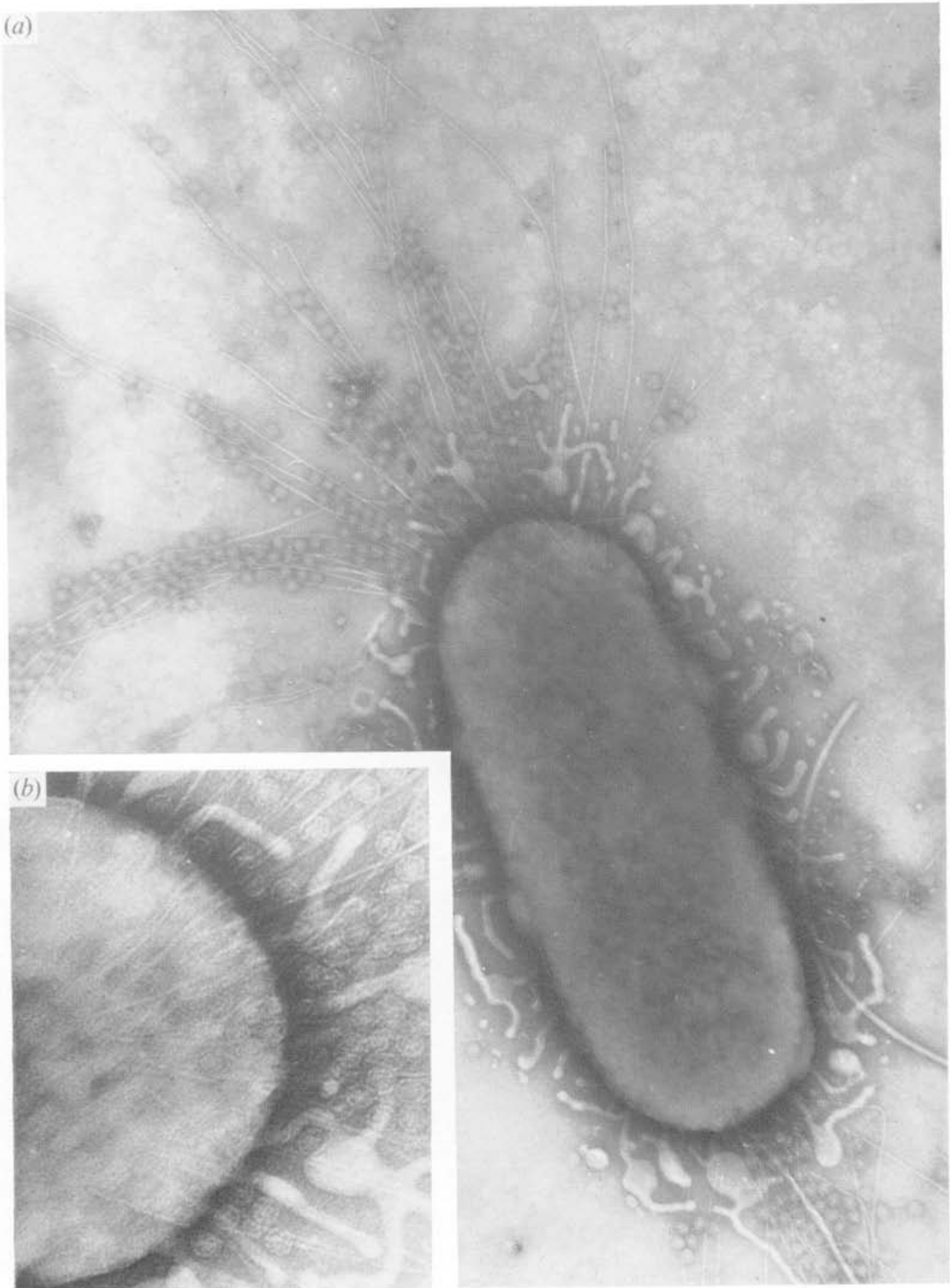

(*a*) *Pseudomonas aeruginosa* strain 1 (PAO 1) with PP7 phage virions adsorbed to polar pili (× 60000). (*b*) The upper pole enlarged to show pili entering the cell (× 130000).

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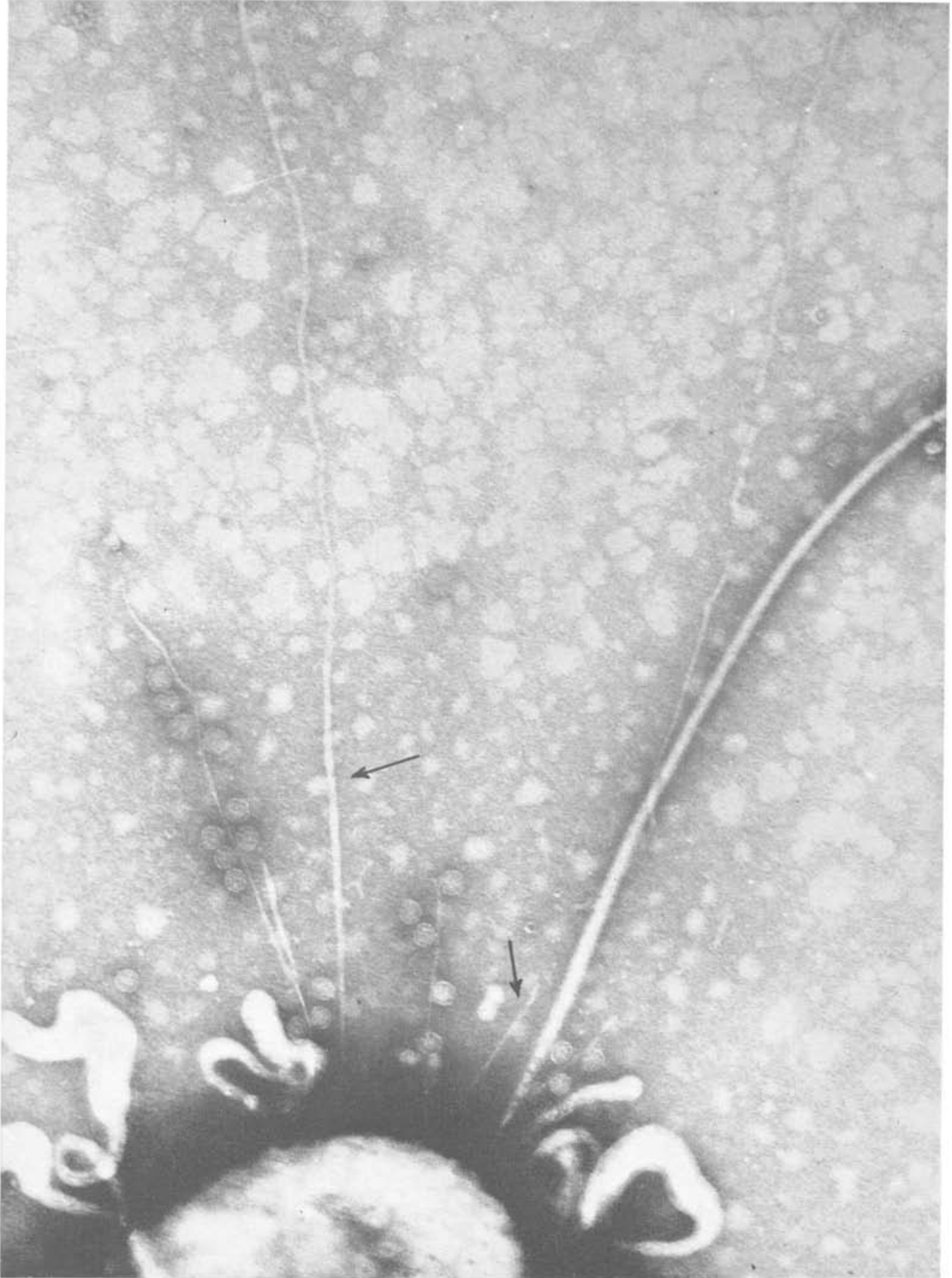

The pole of a cell of *Pseudomonas aeruginosa* strain PAO 67 after labelling with phage PP 7, showing pili with and without adsorbed virions (without arrowed) (× 120 000).

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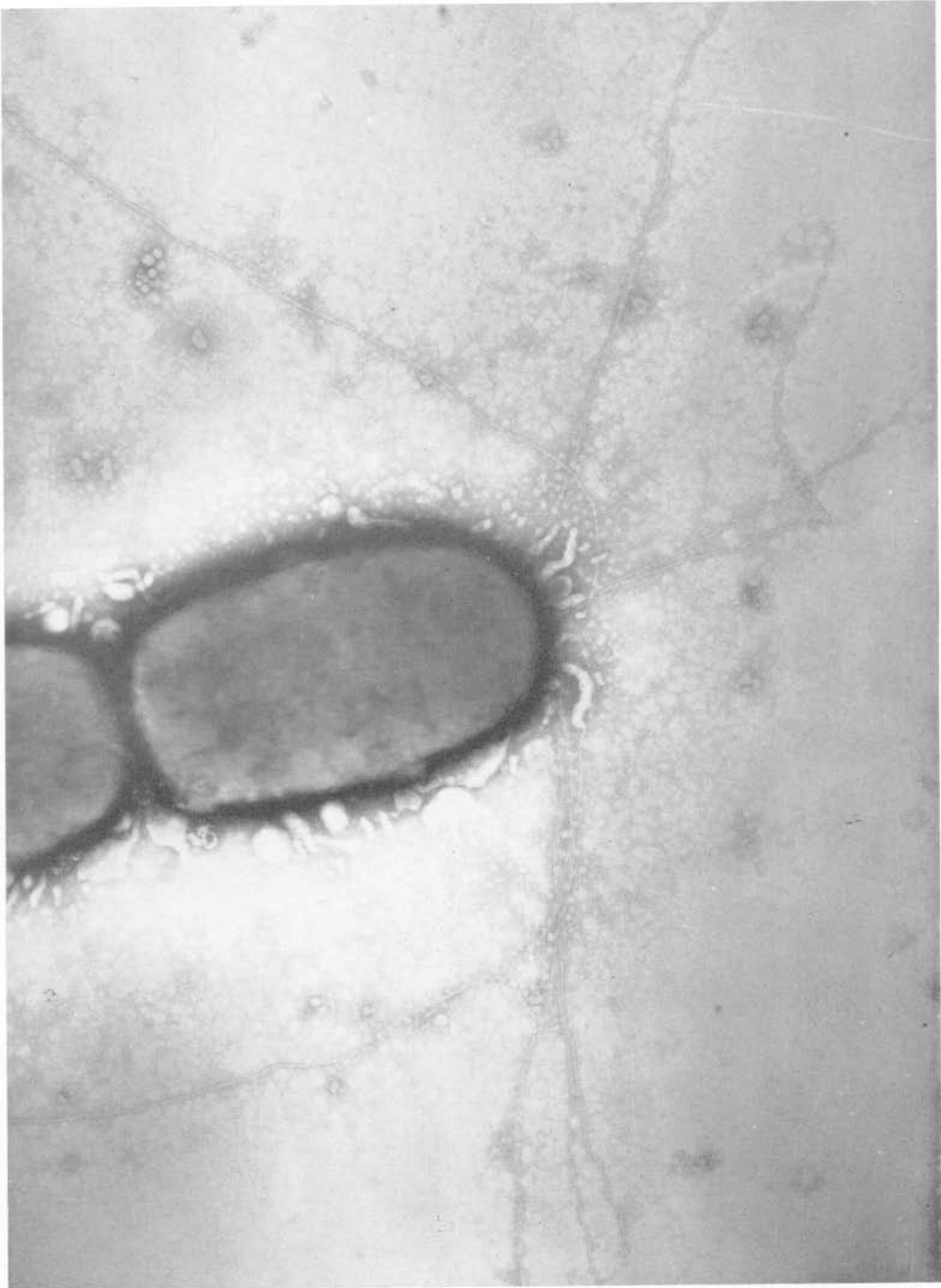

Pseudomonas aeruginosa strain ATCC 25102 (host for filamentous phage Pf) after labelling with phage PP7. The phage does not adsorb to the pili ($\times$ 50000).

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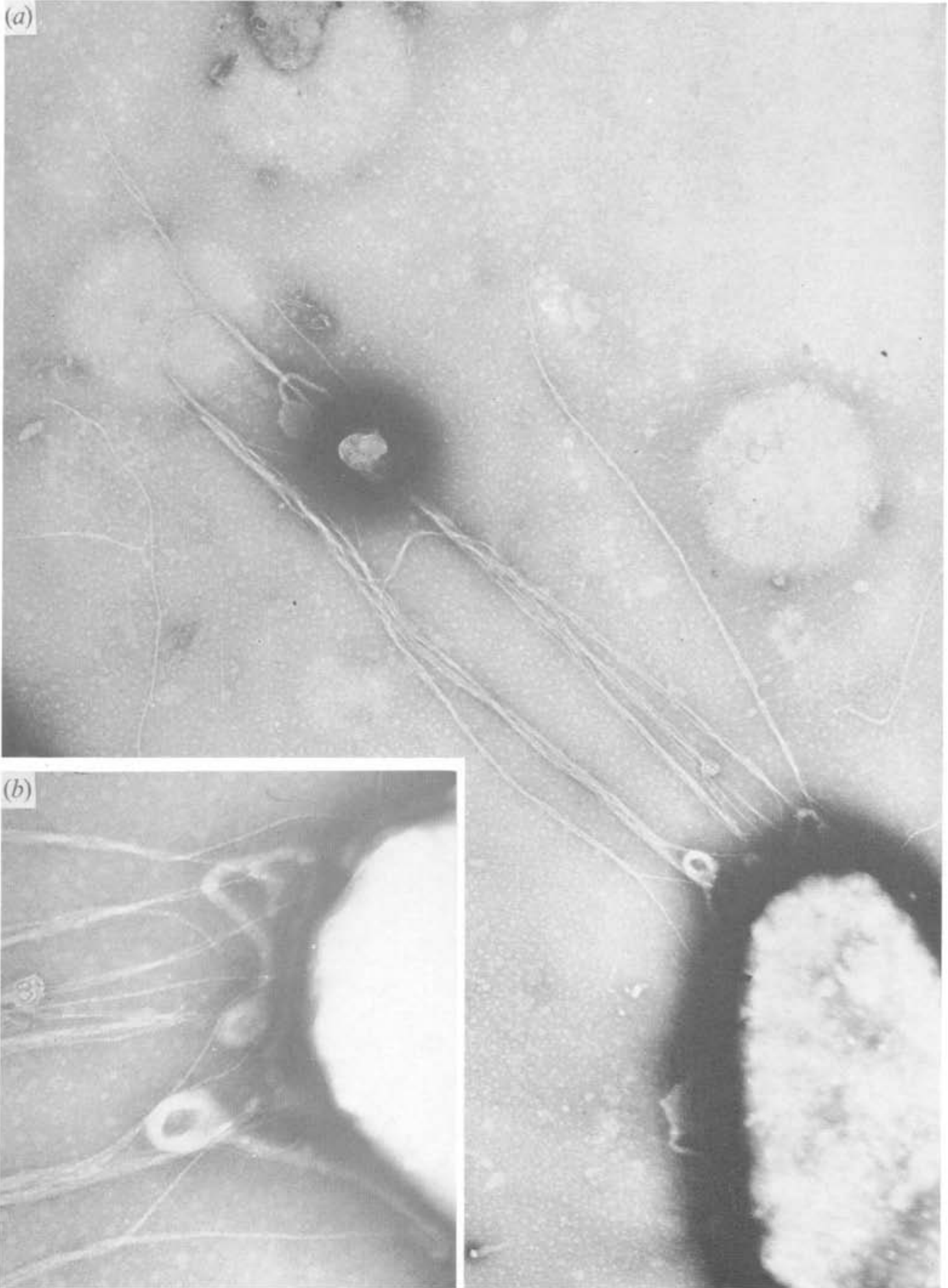

(*a*) *Pseudomonas aeruginosa* strain ATCC 25102 after adsorption with filamentous phage Pf (× 50000). (*b*) The pole enlarged to show the points of attachment of the filaments to the cell (× 100000).

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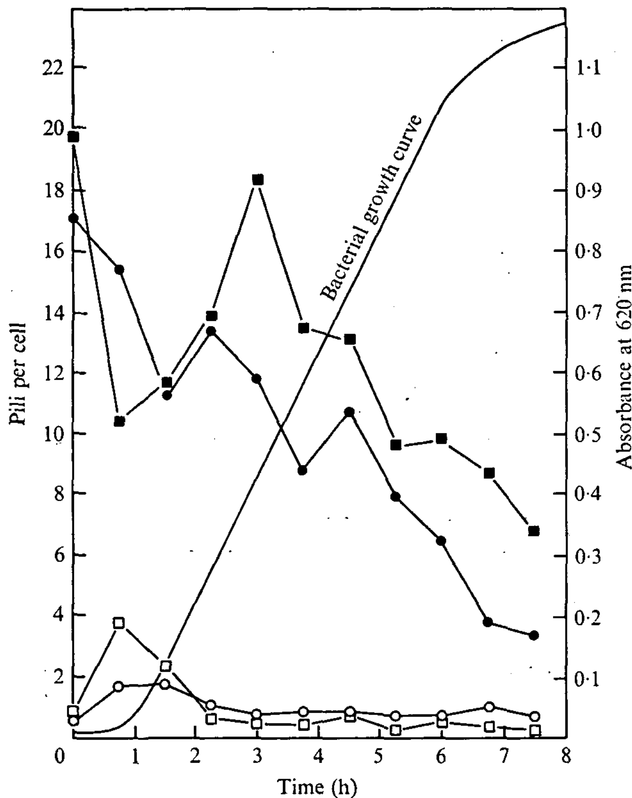
As shown in Table 2, *P. aeruginosa* strains PAO 68 and PAO 1264 had pili to which phage PP7 adsorbed, but it did not multiply within the cells of either

present on the same cell (Plate 2), the unlabelled filaments (arrowed) invariably appearing slightly thicker than the labelled ones.



Text-fig. 2. Changes in the average number of pili per cell (including non-piliated cells) during the growth cycle in liquid culture for *Pseudomonas aeruginosa* strain 1 (PAO 1). Expt 1, unlabelled, ○—○; Expt 2, unlabelled, □—□; Expt 2, phage PP7-labelled, ■—■; Expt 3, labelled, ●—●. Absorbance at 620 nm refers to the bacterial growth curve.

The appearance of the pili on *P. aeruginosa* strain ATCC 25102 (host for filamentous phage Pf), to which phage PP7 did not adsorb, is shown in Plate 3. They seemed generally longer than the strain 1 (PAO 1) pili: 1–4·5 μm. Their thickness was the same (6·0 nm). In this typical micrograph the pili do not show any tendency to become tangled. After allowing phage Pf to adsorb for 5–10 min, the appearance of the cells changed (Plate 4*a*). The polar filaments became a tangled mass attached to the cell at well-separated points (Plate 4*b*). They closely resemble phage Pf in appearance, their tangled condition being typical (Takeya & Amako,

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1966; Minamishima *et al.* 1968), suggesting that the original pili have been replaced with Pf filaments.

Table 2. *The percentage of cells with pili and the average number per cell on different strains of* Pseudomonas aeruginosa

Strain	PP7*	PP7 adsorption†	PP7 labelled		Unlabelled	
			% piliated	Av./cell	% piliated	Av./cell
PTO 13 FP 2+	(+)	+	59	3·9	19·7	0·21
PAO 67 FP 2−‡	+	+	54	2·27	53	1·16
PAO 2 FP 2−	+	+	78	6·8	10·7	0·13
PAO 68 FP 2−	−	+	100	9·3	100	4·5
PAO 1264 FP 39+	−	+	100	12·2	100	10·1
PAO 41 FP 2+	+	+	64	2·8	12	0·12
PAO 38 FP 2−	+	+	91	4·35	39	0·7
PAO 381 FP 2+	+	+	30	1·87	20·4	0·24
1 (PAO 1) FP 2−	+	+	100	13·1	44	0·58
1/7 FP 2−	−	−	0	0	0	0
1 FP 2+	(+)	+	19·2	0·31	4·0	0·06
ATCC 25102	−	−	0	0	50	1·12
25102/12 Pf	−	−	0	0	0	0

* PP7 phage sensitivity (spot test).

† + indicates phage PP7 adsorbs to pili, − it does not or no pili present.

‡ PAO 67 had some pili which labelled and some which did not. Both kinds are included, see Table 3 for breakdown.

Table 3. *Distribution of pili on* Pseudomonas aeruginosa *strain PAO 67 labelled with phage PP7*

Type of pili present	% of cells	Average pili per cell*
None	48	0
Non-adsorbing for PP7 only	22	2·85
Adsorbing for PP7 only	19	3·9
Both kinds	11	6·1†

* Based on the number of cells bearing the type of piliation described only and not including those without pili.

† Based on the total number of pili. The average number of pili with PP7 adsorbed was 4·7 per cell, and without PP7 adsorbed was 1·4 per cell.

4. DISCUSSION

(i) *The comparative morphology of pili*

The structure of F-pili has been studied by Brinton (1965) and Lawn (1966), who give average diameters of 8·5 and 9·5 nm respectively. *P. aeruginosa* pili are thinner (6·0 nm). F-pili have a central dark line in negatively strained preparations (Lawn, 1966) probably representing a hollow core, but this is not obvious in *P. aeruginosa* pili. There are no terminal knobs on *P. aeruginosa* pili like those found on F-pili (Lawn, 1966). F-pili are longer: Lawn (1966) gives a maximum of 20 μm compared with 10 μm for *P. aeruginosa* pili (Weiss & Raj, 1970). Whether or not these structural differences reflect a functional difference remains to be seen.

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(ii) *Piliation, phage sensitivity and sex factors*

Tables 1 and 2 show that pili are present in both FP 2^+ and FP 2^- strains, indicating that, as stated by Holloway (1969), they play no part in the transfer of FP 2. However, this does not necessarily rule out the possibility that they are coded for by another transmissible plasmid such as a drug-resistance factor like R in *E. coli* (Datta *et al.* 1966). Alternatively the pili could be controlled by a chromosomal gene or a non-transmissible plasmid.

There is no relationship between the presence of FP 2 and sensitivity to RNA phage PP7, but sensitivity and piliation are related as follows:

(1) Some strains are PP7-sensitive and have pili to which PP7 adsorbs (PTO 13, PAO 2, PAO 41, PAO 38, PAO 381, PAO 1, 1FP 2^+). The suggestion that the pili on strain 1 (PAO 1) are RNA phage receptors (Bradley, 1966) has been confirmed for PP7 by Weppelman & Brinton (1971). It would seem that the situation is similar for the strains listed.

(2) Two strains are PP7-resistant but have pili to which PP7 adsorbs (PAO 1264 and parent PAO 68). In this case it seems likely that normal RNA phage pili are present, but that PP7 infection is blocked at some point after adsorption.

(3) Strain PAO 67 is PP7-sensitive and has pili to which PP7 adsorbs and additional filaments to which it does not. In this interesting situation, the normal RNA phage pilus coexists with another unknown filament. Since PAO 67 is Pf-resistant, the latter is not necessarily a receptor for the known filamentous phage. The possibility that it is itself a filamentous phage cannot be ruled out.

(4) Strain ATCC 25102 (Pf host) is PP7-resistant and has pili to which PP7 does not adsorb. These are thus distinct from RNA phage pili. After adsorption with Pf, the pili appear to be replaced with Pf virions attached to the poles of the cell (Plate 4*a*, *b*). A non-lysogenic resistant mutant (25102/12Pf) is pilus-free, suggesting that the pili are receptors for Pf.

(5) Strain 1/7 is PP7-resistant and has no pili. Weppelman & Brinton (1971) also isolated a pilus-free mutant of PAO 1 which was PP7-resistant. Thus a form of resistance to a pilus phage is the lack of its receptor.

(iii) *The effect of PP7-adsorption on the number of pili per cell*

The increase in piliation following PP7 labelling of strain 1 (PAO 1) is also found in PTO 13, PAO 2, PAO 41, PAO 38, PAO 381, 1FP 2^+, which are all PP7-sensitive. Strains which do not show an increase are different: PAO 67 has two kinds of filament, also PAO 1264 and its parent PAO 68 are PP7-resistant. It is therefore concluded that any normal PP7-sensitive strain has many more pili visible in the electron microscope after phage adsorption than before.

(iv) *The pilus retraction theory applied to F-pili and* Pseudomonas aeruginosa *pili*

Details of the pilus retraction theory are illustrated for clarity. The mating process as suggested by Marvin & Hohn (1969) is shown in Text-fig. 3, RNA coliphage

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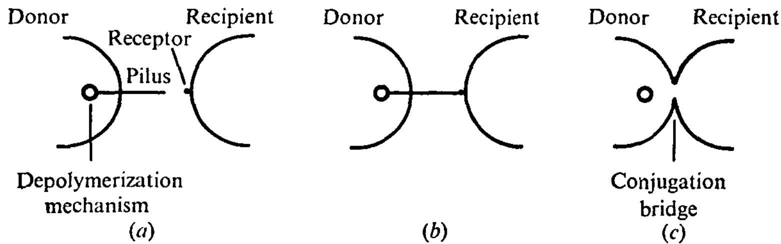
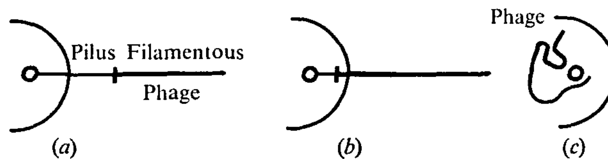
adsorption and penetration in Text-fig. 5(*a, b, c, e, f*) and filamentous coliphage infection in Text-fig. 4. Caro & Schnös (1966) demonstrated that filamentous phages attached to the tips of F-pili, and Trenkner, Bonhoeffer & Gierer (1967) showed that the capsid penetrated the cell completely, fitting perfectly with the model in Text-fig. 4.



Text-fig. 3. Hypothetical role of the *Escherichia coli* F-pilus during conjugation involving pilus retraction. (*a*) The cells approach one another; (*b*) the pilus tip adsorbs to a receptor site and a signal is sent to the depolymerization mechanism; (*c*) the pilus retracts and a classical conjugation bridge is formed (see Anderson, Wollman & Jacob, 1957; Anderson, 1958).



Text-fig. 4. The adsorption and penetration of a filamentous coliphage based on retraction and the results of Caro & Schnös (1966) and Trenkner, Bonhoeffer & Gierer (1967). (*a*) Adsorption to the pilus tip; (*b*) retraction after activation of the depolymerization mechanism; (*c*) complete penetration of the virion.

Retraction applied to RNA phage infection is particularly relevant to the present study. Valentine & Wedel (1965) and Silverman & Valentine (1969), studying f 2 infection, supported the conduction theory. They suggested that initially phage RNA was partially injected *into* the pilus where it was RNAse-sensitive (Loeb & Zinder, 1961; Paranchych, 1966). A second step consisted of the injection of the remainder of the RNA down the pilus followed by the desorption of the capsid, a small portion of its protein remaining on the pilus. This process can equally well be applied to the retraction model. The short length of f 2 RNA would be ejected on to, not into, the pilus (Text-fig. 5*b*), a condition more consistent with RNAse sensitivity. Ensuing pilus retraction would be halted by the f2 virion (Fig. 5*c*), allowing initial RNA penetration into the cell. The remaining steps would be as illustrated. Text-fig. 5 suggests that only the first virion adsorbed can infect; after it desorbs, the remainder (labelling the pilus) should all be full. Electron microscopy confirms this.

Evidence for the retraction of *P. aeruginosa* pili will now be considered. While it seems they are not involved in conjugation, they are receptors for RNA phages

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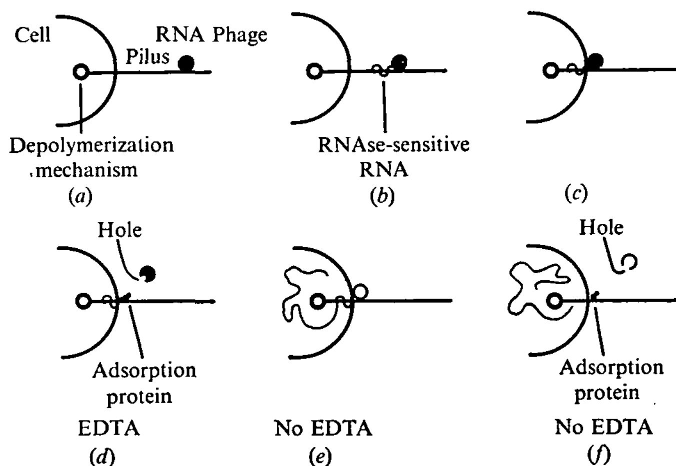
and like the morphologically similar RNA coliphages, the infective process is RNAse-sensitive (Bradley, 1966). It therefore seems certain that the injection process is similar.

It has been shown that the number of pili increases after PP7 adsorption. There are two possible explanations: either phage adsorption stimulates the synthesis of more pili, or more likely, processing for electron microscopy causes them to disappear. Disappearance could be caused by breaking off, winding round the cell, or retraction. Breaking off seems unlikely since the processing involves minimal disturbance. If pili were wrapped around the cell, one would expect to see numerous loose ends, but this is not the case. Retraction on contact with the carbon film seems most likely; it would be prevented by PP7 adsorption. The failure of some pili to retract can be explained by defective depolymerization mechanisms or cell death. The PP7-resistance of PAO 1264 and PAO 68 could well be that lack of retraction prevents RNA penetration (Text-fig. 5*c*). Indeed, PP7 labelling provides no significant increase in the number of pili per cell (Table 2).



Text-fig. 5. RNA coliphage adsorption and injection steps based on the pilus retraction model. (*a*) The virion adsorbs to the side of the pilus and activates the depolymerization mechanism; (*b*) simultaneously a short length of RNA is ejected on to the pilus surface; (*c*) the pilus retracts until stopped by the adsorbed virion and the RNA enters the cell; (*d*) if EDTA is present, the remainder of the RNA cannot be injected and the virion desorbs leaving some RNA and protein behind (Silverman & Valentine, 1969); (*e*) without EDTA, injection is completed; (*f*) the empty virion desorbs leaving some protein behind (Silverman & Valentine, 1969).

The pili on the host strain for Pf are different to RNA phage pili in that they do not adsorb RNA phages. However, it has been suggested that they could be receptors for Pf. The apparent disappearance of the pili after Pf adsorption is consistent with retraction. Clearly it is difficult to prove the retraction theory directly. It seems almost certain that it occurs in *E. coli*, and the evidence given here suggests that *P. aeruginosa* pili are similar in this respect.

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