ADSORPTION OF BACTERIOPHAGE PBS 1

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By

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ABSTRACT

The adsorption of *Bacillus subtilis* bacteriophage PBS 1 was studied by electron microscopy. It was demonstrated that the site of final adsorption and DNA injection is the flagellar base. A multistage process of adsorption is proposed. It was also shown that primary adsorption is not species-specific as PBS 1 adsorbed to the flagella of various PBS 1-resistant *Bacilli*. The role of motility in adsorption is discussed.

PREFACE

The experiments described in this thesis were conducted in the Department of Biology, McMaster University, from September 1975 to March 1977. Except where others are specifically mentioned, this thesis consists of original research. To my knowledge, no similar thesis has been submitted to any other university.

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INTRODUCTION

The initial event of infection by a bacteriophage is the adsorption to its bacterial host. This process is generally a specific event requiring correct orientation of the bacteriophage particle on the specific receptor site. The actual mechanisms involved in adsorption and the location of adsorption sites vary considerably among phage-host systems. These variations are a direct consequence of the ultrastructure of both phage and host bacterium.

Bacteriophages have been classified according to morphology and genetic composition (9,63). Table 1 summarizes the system proposed by Bradley (9).

Specific receptor sites for various bacteriophages are found on the bacterial cell wall. Studies have shown that both Gram-negative and Gram-positive bacteria are susceptible to infection by phages with contractile (group A) and noncontractile (group B and C) tails despite the differences in cell wall structure (1,2,6,7,8,9,23,24).

A number of workers have found that the bacterial cell wall is not the only site of phage adsorption (3,11,24,25,32,37,42,51). Pili are protein

Table 1. Classification of bacteriophages

Group	Description	Nucleic acid[a]
A	Contractile tail	dsDNA
B	Long noncontractile tail	dsDNA
C	Short noncontractile tail	dsDNA
D	No tail, large capsomeres	ssDNA
E	No tail, small capsomeres	ssRNA
F	No head, flexible filament	ssDNA

[a] ds = double stranded; ss = single stranded

fibres which project from the cell wall of some Gram-negative bacteria. These fibres are often associated with substrate attachment, cell clumping, and sexual conjugation. In addition, certain phages such as f1 (31), R17 (17) and M13 (45) can adsorb specifically to the pilus. The actual mechanism of phage genome injection by these pilus-dependent bacteriophages remains unclear.

Brinton (15) proposed that the F-pilus, which mediates bacterial conjugation, may consist of two parallel protein filaments, each consisting of an assembly of F-pilin monomers. The transfer of DNA from donor to recipient cells could occur by the following three ways. One of them is a conduction mechanism in which the DNA strand moves between stationary F-pilus filaments. In the conveyor belt mechanism, the DNA strand is bound to one of the filaments of the F pilus and F-pilus filaments move with respect to each other as a continuous conveyor belt. In the carrier mechanism, DNA strand is bound to both filaments of the F-pilus. Assembly of the F-pilus at the membrane of the donor cell and depolymerization at the membrane of the recipient cell results in the movement of the F pilus towards the recipient (47). A similar transfer of the phage genome could occur by one of these proposals, namely the conduction mechanism (15,45,46,48)).

Marvin and Hohn (41) and Curtiss (18) suggested that during conjugation the F-pilus retracts into the donor cell after receiving an appropriate stimulus. According to this model, the adsorption of an F^- (recipient) cell to the tip of the pilus or an RNA phage to the side or filamentous DNA phage to the tip would trigger the retraction of the pilus through a process of sequential depolymerization of pili subunits within or at the cell membrane. The phage, after initial attachment to the F pilus, is pulled to the cell surface by the pilus retraction and then injects its genome. Many workers using the coliphage system have reported findings which support the pilus-retraction theory (18,31,41). The pilus-specific coliphages f1, f2, M13, MS2 and R17 have been shown to attach only to the F-pilus found on male strains (donors in conjugation) of *E. coli* (9,47). Group F (filamentous) phages appear to be specific for attachment at the tip of the F-pilus (16).

Pilus-specific phages of groups B, C, E and F for *Pseudomonas aeruginosa* have been reported (9,13). Bradley (13) has shown that the pilus-specific bacteriophages PP7, PO4, M6 and PE69 adsorb only to the polar pili. It appears that *P. aeruginosa* pilus-specific phages do not eject their nucleic acid until they reach the pilus base, presumably by means of

retracting pili. From electron micrographs, it has been shown that only those phages at the base of pili have empty heads and thus appear "ghosted", whereas phages adsorbed to non-contractile pili have full heads and hence still possess their nucleic acid (9,12,47).

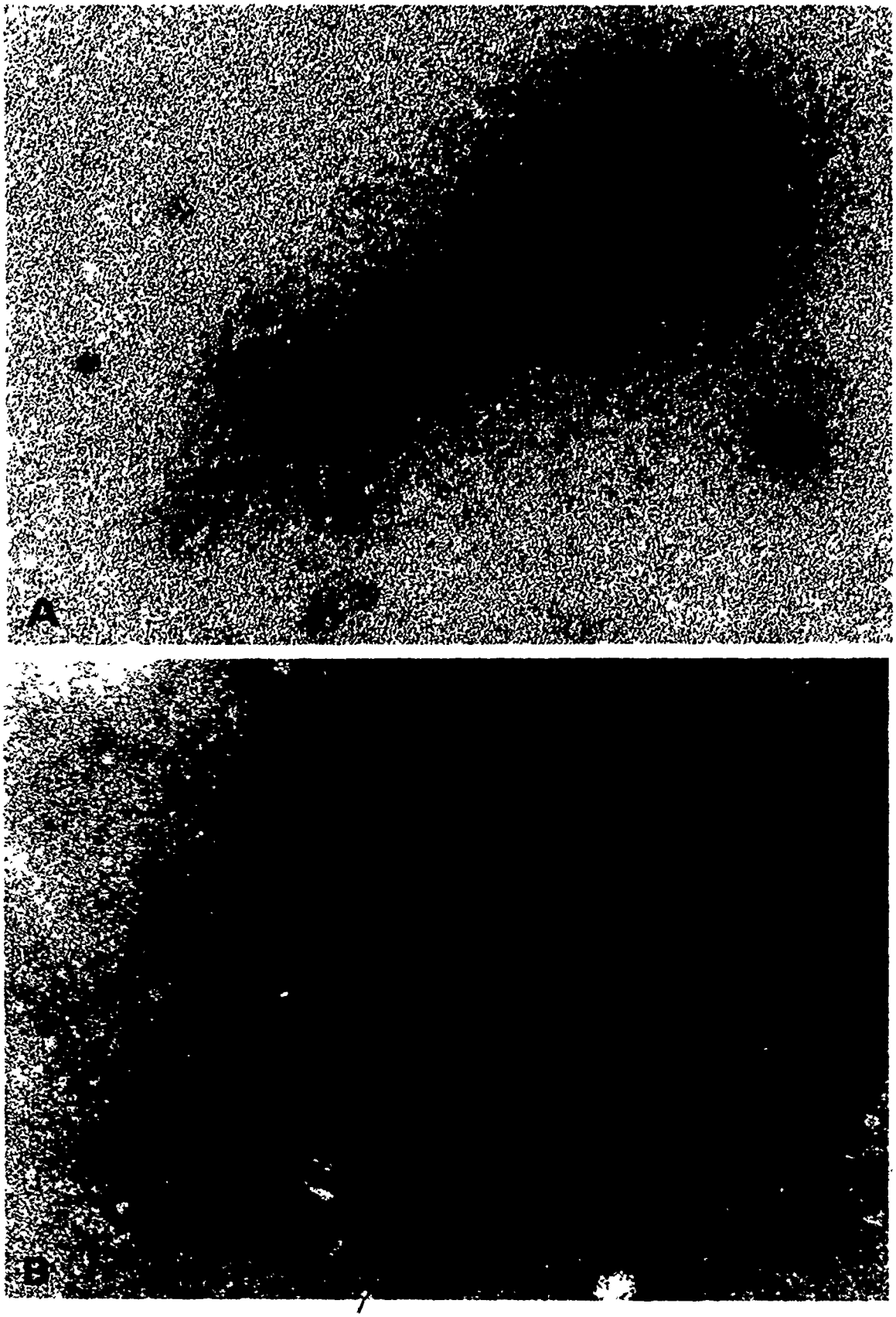
The other possible adsorption site is the bacterial flagellum. Numerous reports of flagella-specific, or flagellotropic, bacteriophages for both Gram-positive and Gram-negative bacteria appear in the literature (3,25,32,33,36,37,42). Most of these belong to the group B phages including phage χ for *E. coli* and *Salmonella* spp. (42,56), ϕCP34 and ϕ6 for *Caulobacter vibrioides* (25,32), $\phi\chi_7$ for *Proteus mirabilis* (3), PBP1 for *Bacillus pumilus* (37) and SP15 for *B. subtilis* (65). Only a few group A flagellotropic phages have been reported and most of these infect *B. subtilis*. These include PBS 1 and a clear plaque derivative, PBS 2 (59,60), AR9 (4), 3NT (30) and I10 (54). All of these phages appear to be morphologically similar to PBS 1, each possessing the characteristic contraction fibres and helical tail fibres. Rima and van Kleeff (54) compared PBS 1, 3NT and their own isolate, I10, with respect to ultrastructure, host range, serological identity, and buoyant density of the DNA and found them identical. The similarity in host range and

morphology indicates that these phages probably adsorb to *B. subtilis* and inject their DNA in the same manner.

Of these phages, PBS 1 (Fig. 1) has been studied most extensively. Since it is a transducing phage it has been used in many genetic studies on *B. subtilis* (21,60,65,68,70). It is one of the largest phages isolated to date and has been the subject of an ultrastructural study by Eiserling (22). Unlike the other group A phages, the contractile tail of PBS 1 is unusually complex. In addition to the tail sheath there are several thin fibres, about 2 nm by 80 nm, which project outwards from the base of the sheath when it is contracted. As these are not seen on uncontracted sheaths, Eiserling has termed them "contraction fibres" to distinguish them from the tail fibres of the other phages. There are also three helical tail fibres found at the base of the tail. These measure about 8 nm by 125 nm and appear to wrap around the flagellum during primary adsorption (22,51). In contrast, phage T4 for *E. coli* has six ridged tail fibres that serve to anchor the phage to the cell wall (34). The large icosahedral head of PBS 1, with a diameter of 120 nm, corresponds to the large amount of DNA carried by this phage. The molecular weight of PBS 1 DNA has been determined to

Fig. 1. Bacteriophage PBS 1.

(A) Uncontracted conformation showing three helical tail fibres. X 221,000. (B) Ghosted conformation showing contracted tail sheath with contraction fibres. X 221,000. Negatively stained with 2% uranyl acetate.


A
B

be 1.9 x 10^8 (27) and each DNA molecule has two single strand interruptions along its length (67). Another peculiarity of PBS 1 DNA is that thymine is completely replaced by uracil (62).

The capacity of PBS 1 to hold such a large amount of DNA enables it to transduce relatively large segments of *B. subtilis* DNA. The molecular weight of the host DNA extracted from transducing particles is heterogeneous and ranges from 4.0 x 10^6 to 1.3 x 10^8 (68). Because of its ability to transduce, PBS 1 has been used by many workers in chromosomal mapping in *B. subtilis* (21,70) and *B. pumilus* (38,39,52,69).

The interaction between PBS 1 and its host is known as a pseudo-lysogenic or carrier state (60). It was found by Takahashi (60) that "lysogenic" cultures of *B. subtilis* with PBS 1 and PBS 2 were unstable and reverted to the sensitive state when grown in the presence of phage antiserum. He also showed that phage DNA in spores carrying PBS 1 was not physically attached to the host chromosome (61). Therefore, PBS 1 and PBS 2 are not true temperate phages as is the coliphage λ (40) or ϕ3T of *B. subtilis* (64), and as such do not exhibit classical lysogeny with their host.

PBS 1 and the related phages AR9, I10 and 3NT of *B. subtilis* are the only flagellotropic group A

phages that have been reported for Gram-positive bacteria. Recently, phage 7-7-1 for the Gram-negative bacterium, *Rhizobium lupini* has been reported to have a slightly contractile tail (36). No other flagellotropic phages possess the unique contraction fibres of PBS 1. One or more helical tail fibres are found on the group B phage PBP1 which is specific for the flagella of *B. pumilus* (37). Lovett found that some strains of *B. pumilus* are sensitive to PBS 1 infection (at a reduced efficiency) as well as to PBP1 (37). Recently, Bramucci and Lovett (14) reported the ability of PBS 1 to transduce, at low frequency, a plasmid in *B. pumilus*.

The phage χ attaches to the flagella of *E. coli* by means of a single tail fibre, 200-220 nm by 20-25 nm, which forms kinks, twisted loops, and figure eights (56). The flagellotropic phage, $\phi\chi_7$, of *Proteus mirabilis* has a similar tail fibre, approximately 185 nm in length (3). Jollick and Wright (32) reported that phage ϕ6 for *Caulobacter vibrioides* appears to have three short spikes and occasionally what appears to be very fine fibrils emerging from the central spike.

That the flagellum is the primary adsorption site of PBS 1 has been well documented (24,51) but the mechanism by which the phage DNA is injected into the host is still obscure. Many theories have been

considered and these include (i) direct injection of the phage DNA into the flagellum, (ii) a modified transfection mechanism, and (iii) flagellar attachment as a primer for cell wall adsorption by the phage. The first mechanism can be eliminated, as it would require that the flagellum possess a central canal of sufficient dimension to allow the phage DNA to pass along the length of the flagellum to the cell body. No such central canal has been observed in the flagellum of *B. subtilis* (51). Kerridge *et al.* (35) found hollow cores in the flagella of *Salmonella typhimurium*, but the hollow cores were only demonstrable after disruption of the flagella by ultrasonic treatment or by some other means. In native flagella, the hollow may be filled with a substance or substances easily removable upon disruption (29). As flagella have been shown to grow at the flagellar tip and not at the base (28), it is possible that the central canal is filled with the flagellin subunits en route to the flagellar tip. Only a few ghosted PBS 1 particles appear on the flagella where DNA injection would presumably have taken place by this model. In addition, Raimondo *et al.* (51) suggested that the DNA which is injected into the flagellum might cause a morphological alteration of the flagellum at the site of injection. However, no such changes have been observed so far.

The second mechanism, a modified transfection using the flagellum as a vector for phage DNA entry, can be ruled out, since it has been shown that the infectious process of PBS 1 is completely deoxyribonuclease-resistant (W.B. Pritikin, Ph.D. Thesis, Univ. of California, Los Angeles, 1967). Here again, the absence of ghosted phage particles on the flagella conflict with this proposed mechanism.

In the third mechanism, it was thought that initial adsorption to the flagellum might alter the properties of PBS 1 so that adsorption to the cell wall could then follow. Raimondo *et al.* (51) eliminated this possibility by showing that PBS 1, released from flagella after initial adsorption, could not infect nonflagellated cells which were also present in the same infection mixture. This, however, does not rule out the possibility that phage having attached to the flagella could then attach to the cell wall of the same cell (51).

A mechanism for the adsorption of PBS 1 and subsequent DNA injection into the host bacterium is proposed as a result of our present investigation. This mechanism is similar to that proposed by Schade, Adler and Ris in 1967 (56) for χ phage adsorption and DNA transfer in *E. coli*. These workers suggested the χ phage attaches to the flagellum of *E. coli* and then

moves down the length of the flagellum to its base, where DNA injection occurs. They also suggested that the flagellar movement is responsible for, or aids in, the sliding of the phage down the length of the flagellum. This mechanism is, in essence, the same as that proposed by Bradley (12,13) for the pilus-specific phages, except that the requirement for pilus retraction is replaced by a requirement for flagellar movement. It appears that DNA injection does not occur at sites other than the cell wall except when phage heads are ruptured or defective particles eject their DNA prematurely.

The mechanism of adsorption of PBS 1 to *B. subtilis* which is proposed in this thesis has been divided into four stages based on the analysis of electron micrographs. These stages will be referred to further in the text. The four stages are:

Stage I	Attachment of phage to flagella
Stage II	Attachment of phage at the base of a flagellum
Stage III	Contraction of the phage tail sheath
Stage IV	DNA injection (phage ghosting).

MATERIALS AND METHODS

1. Symbols and abbreviations

The units of length, weight, volume, and time were abbreviated according to the format of the Journal of Bacteriology. Other abbreviations frequently used were:

AD	adsorption medium
ETY	enriched, tryptone-yeast extract medium
MM	minimal medium (Spizizen's (58))
MMP	minimal medium (Pritikin and Romig (50))
PA	Penassay Broth (Difco)
TBB	Tryptose Blood Agar Base
TY	tryptone-yeast extract medium
SM	suspension medium
SSC	standard saline citrate
CFU	colony forming unit
PFU	plaque forming unit
MOI	multiplicity of infection

Table 2. List of bacterial strains

Bacillus	Source of strains
B. subtilis SB19E	spontaneous mutant of SB19 (44)
168	C. Anagnostopoulos
MS14	T. M. Joys
SB108	T. M. Joys
B. megaterium	laboratory stock
B. cereus 819	Microbiol. Res. Inst., Ottawa
B. thuringiensis var. *thuringiensis*	D. Kushner
B. thuringiensis var. *alesti*	D. Kushner
B. amyloliquifaciens H (RUB 500)	G. A. Wilson

2. Bacteriophage and bacterial strains

Bacteriophage PBS 1 was obtained from a single plaque isolate from our laboratory stock.

The bacterial strains used in this study are listed in Table 2.

3. Media

(a) Difco antibiotic medium 3 (Penassay Broth) (PA)

(b) Difco Tryptose Blood Agar Base (TBB).

(c) Minimal medium (MM) was described by Spizizen (58).

$(NH_4)_2SO_4$	2.0 g
K_2HPO_4	14.0 g
KH_2PO_4	6.0 g
Sodium citrate	1.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
Distilled water	1.0 l

The pH was adjusted to 7.0 and after autoclaving, 50 ml of 10% glucose were added per litre of medium.

(d) Adsorption medium (AD) (68) was used for all phage dilution unless otherwise stated.

NaCl	4.0	g
K_2SO_4	5.0	g
KH_2PO_4	1.5	g
$Na_2HPO_4 \cdot 7H_2O$	5.68	g
$MgSO_4 \cdot 7H_2O$	0.12	g
CaCl solution (1.0 g/100 ml)	1.0	ml
$FeCl_3$ solution (0.5 g/100 ml)	2.0	ml
Difco yeast extract	1.0	g
Distilled water	1.0	l

(e) Tryptone-yeast extract broth (TY) (55)

Bacto tryptone (Difco)	10.0	g
Bacto yeast extract (Difco)	5.0	g
NaCl	10.0	g
$MnCl_2 \cdot 4H_2O$	2.0	mg
Distilled water	1.0	l

(f) Enriched tryptone-yeast extract both (ETY) (50)

Bacto tryptone (Difco)	10.0	g
Bacto yeast extract (Difco)	5.0	g
NaCl	10.0	g

$MnCl_2$ (126 mg/100 ml)	1.0	ml
$FeCl_3 \cdot 6H_2O$ (25 mg/100 ml)	1.0	ml
$CaCl_2$ (1.0 g/100 ml)	10.0	ml
Distilled water	988	ml

(g) Minimal medium described by Pritikin and Romig (MMP) (50). Used for dilutions from TY and ETY.

$(NH_4)_2SO_4$	2.0	g
KH_2PO_4	6.0	g
K_2HPO_4	14.0	g
Sodium citrate $\cdot 5\frac{1}{2}H_2O$	1.0	g
L-glutamic acid	1.0	g
dextrose (after autoclaving) (50 g/100 ml)	10	ml
$MgSO_4 \cdot 7H_2O$	0.2	g
$MnCl_2$ (126 mg/100 ml)	1.0	ml
$FeCl_3 \cdot 6H_2O$ (25 mg/100 ml)	1.0	ml
$CaCl_2$ (1.0 g/100 ml)	10.0	ml
Distilled water (to make 1.0 l total)	978	ml

(h) Suspension medium (SM) (66)

Tris buffer (1 M pH 7.5)	6.0	ml
$MgSO_4 \cdot 7H_2O$	0.12	g
NaCl	4.0	g

gelatin	0.05	g
Distilled water	1.0	l

(i) Phosphate buffer (0.1 M)

K_2HPO_4	14.0	g
KH_2PO_4	6.0	g
Distilled water	1.0	l

(j) Tris buffer (53)

Tris	0.2	M
NaCl	0.1	M
$MgSO_4$	0.01	M

(k) Standard saline citrate (1 x SSC)

NaCl	0.15	M
Sodium citrate	0.015	M

4. Colony forming units (CFU)

Cells grown overnight on TBB were used to inoculate PA (10 ml) in 250 ml flasks. Cultures were grown for 4 h at 37 C in a reciprocal shaker bath. Cultures were then diluted 1/10 in PA in Klett flasks and incubated as above. At required times or cell densities, 0.05 ml samples were removed from the Klett flask and diluted appropriately in MM, MMP, or phosphate buffer (0.1 M). The diluted

samples (0.1 M) were spread on TBB agar with a sterile glass spreader. Duplicate plates were incubated overnight.

All cultures were incubated at 37 C unless otherwise stated.

A Klett-Summerson colorimeter equipped with a #54 (green) filter was used to measure culture turbidity.

5. Plaque forming units (PFU)

The plaque assay method described by Takahashi (60) was used to determine the titre of phage suspensions.

6. Phage lysates

Lysates of PBS 1 (5.0×10^9 to 1.0×10^{10} PFU/ml) were obtained with strain SB19E as host by the method described by Takahashi (60). Cell free lysates were obtained by passing through a Millipore filter (HA 0.45 µm). Phage titre (PFU/ml) of each lysate was determined as described earlier (60,68). Sterility was checked by spotting 0.1 ml of lysate on a TBB plate and incubating overnight. Stock lysates were stored in glass vials at 4 C.

7. Phage purification by isopycnic CsCl gradient centrifugation

A 4 h culture of *B. subtilis* SB19E in PA (25 ml) was used to inoculate (5 ml each) four flasks of PA (20 ml each) which were then incubated for 1 h in a reciprocal shaker bath. Four flasks of PA (225 ml each) were inoculated with 25 ml each of the above preculture and shaken for 10 min. The phage was added to each flask at an MOI of 0.1. The infected cultures were shaken for 1 h on the rotating shaker and incubation continued without shaking overnight.

The lysate was centrifuged at 3,000 x g for 10 min at 0 C. The supernatant fluid was then centrifuged at 16,000 x g for 2 h. Pellets were resuspended in Tris buffer (20 ml) overnight and then centrifuged at 6,000 x g for 10 min. The supernatant fluid was centrifuged at 16,000 x g for 1 h and the pellet was resuspended in SM (3 ml) and left to stand overnight at 4 C.

CsCl was added to the phage suspension to a refractive index of 1.3753 to 1.3763 (ρ = 1.440 to 1.445 g cm^{-3}). This suspension was placed in three nitrocellulose tubes and centrifuged at 84,000 x g for 24 h at 20 C. A phage band was withdrawn with a hyperdermic syringe with a #25 needle. The purified phages were suspended in 1 ml of SM and dialyzed twice

in 100 x volume of SM over a 3 h period at 4 C. The lysates (~1.0 x 10^{11} PFU/ml) were stored at 4 C in glass vials.

8. Deflagellation and reflagellation

A modification of the procedure of Raimondo *et al.* (51) was used. Cells of strain SB19E were grown for 4 h in PA in a shaker bath. Motility was confirmed by examining cells under the phase-contrast microscope. Cells were deflagellated for 1.5 min at 4 C in a Sorval Omnimixer with a reostat setting of 100 V. Loss of motility was confirmed by phase-contrast microscopy. A 5 ml-sample was centrifuged at 6,000 x g for 10 min at 4 C then resuspended in 5 ml of cold PA. When the culture was incubated in a shaker bath, reflagellation was complete by 20 to 25 min (51).

9. Adsorption assay (phage titre reduction)

Samples (1 ml) of infection mixtures were centrifuged at 6,000 x g for 10 min at 4 C to sediment infected cells. The amount of unadsorbed phage was determined from the PFU in the supernatant fluid. Efficiency of adsorption could then be calculated as follows:

$$\text{Efficiency of adsorption} = \frac{\text{Initial phage titre} - \text{Unadsorbed phage titre}}{\text{Initial phage titre}} \times 100$$

10. Electron microscopy

Negative staining of infection mixtures was carried out using a modification of the technique described by DePamphilis and Adler (20). A copper grid with a Formvar membrane and carbon coating on one side was dipped into petroleum ether for 5 sec, and the excess fluid drawn off with filterpaper. This step removes the hydrocarbons which were adsorbed to the grid in the vacuum evaporator during carbon coating (20). The grid was then floated onto a drop of the infection mixture for 2 to 5 min. The grid was then transferred, without blotting, to a drop of 20% formaldehyde for 30 sec, and then onto deionized water for 15 sec. Excess water was removed by blotting from the side of the grid with filter paper. The grid was immediately placed onto a drop of 0.5 or 1% uranyl acetate, pH 4.5, for 1 to 2 sec and blotted so as to leave a thin film of stain on the grid surface. Grids were allowed to dry in the air. Once the sample had been applied to the grid, the surface of the grid was not allowed to

dry until the end of staining, otherwise poor staining and artifacts resulted.

Specimens were examined with a Philips EM300 standard transmission electron microscope operated at an accelerating voltage of 60 kV. A 20 µm objective aperture was used. A liquid nitrogen decontamination device was routinely used.

RESULTS

Final adsorption site of PBS 1

Pritikin and Reiter (49) reported that when PBS 1 particles which had been purified by CsCl density gradient centrifugation but never pelleted were used, 70 to 80% of the phage particles adsorbed to the host cell wall. On the other hand, when pelleted phages were used, only 4% of the particles adsorbed to the cell wall. Adsorption time was 10 min and the MOI was 2.8. These authors did not mention the possibility of the site of adsorption being the flagellar base. In their electron micrographs, it is not clear whether the phages are indeed attached to the flagellar bases. However, this possibility is suggested by the proximity of the phage particles to the flagella coming from the cell wall.

In order to determine whether the technique of phage purification affected the final adsorption site, lysates prepared either by CsCl density gradient centrifugation or by Millipore filtration were used to infect cells of strain SB19E. Adsorption time was

10 min and the MOI ranged from 0.1 to 10. In all cases, the final adsorption site appeared to be the flagellar base and not the cell wall. PBS 1 particles can be seen attached to the base of the flagellum with both uncontracted (Fig. 2) and contracted (Fig. 3) tail sheaths. The appearance of a ghosted phage in Fig. 4 indicates that DNA injection has occurred and suggests that this is the final adsorption and DNA injection site.

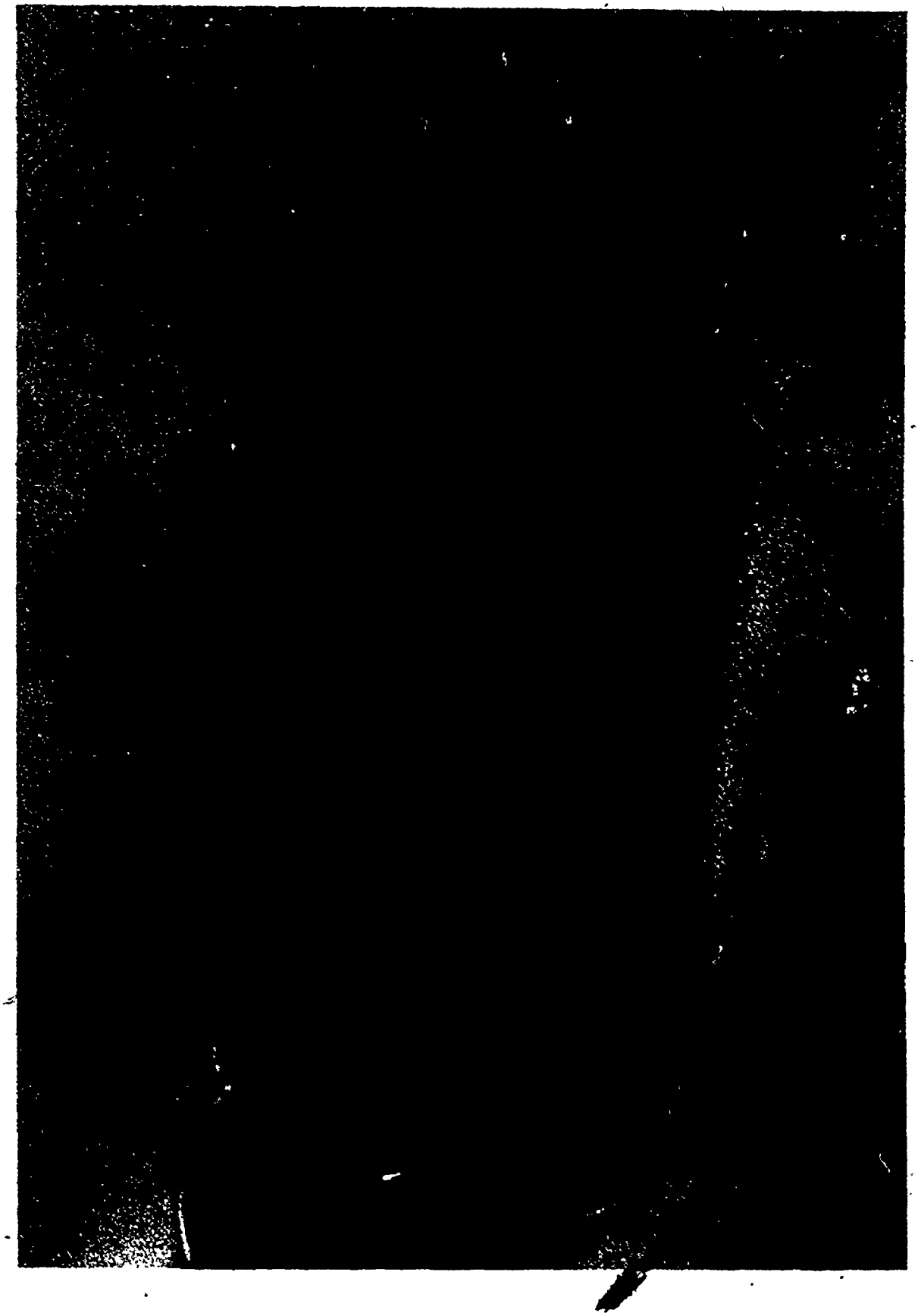
As Pritikin and Reiter (49) used a different growth medium (ETY) for the growth of host cells, it was thought that perhaps the composition of growth media might have some effect on adsorption. Cells of strain SB19E were grown in PA, TY, and ETY medium to a cell density of approximately 5 x 10^8 CFU/ml. Cells were infected at an MOI of 10 with lysates of PBS 1 that had been purified by either CsCl density gradient centrifugation or Millipore filtration. Adsorption was allowed for 10 min in a 37 C shaker bath. In all cases, phage particles in the contracted and ghosted (Stage IV) conformation were observed only at the flagellar base.

Deflagellation-reflagellation

Primary adsorption (Stage I) of PBS 1 to the host cell occurs on the flagellum of *B. subtilis* (51).

Fig. 2. PBS 1 particles at Stage II of adsorption to *B. subtilis* SB19E.

Negatively stained with 1% uranyl acetate. X 57,000.



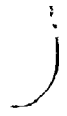


Fig. 3. PBS 1 particle at Stage III of adsorption to *B. subtilis* SB19E.

The helical tail fibres (arrow) are wrapped around the flagellum near the base. Negatively stained with 1% uranyl acetate. X 176,000.

Fig. 4. PBS 1 particle at Stage IV of adsorption to *B. subtilis* SB19E.

The phage appears ghosted indicating that its DNA has been ejected. Negatively stained with 1% uranyl acetate. X 176,000.

In order to determine the relationship of the length of the flagellum to the efficiency of adsorption, a deflagellation-reflagellation experiment similar to that of Raimondo *et al.* (51) was performed. Cells of strain SB19E were grown in PA (30 ml) for 4 h in a shaker bath. Cells (25 ml) were deflagellated for 1.5 min in the cold. The remaining 5 ml of culture was used to determine the rate of adsorption in untreated cells and viable counts. Deflagellated cells (5 ml) were centrifuged at 6,000 x g for 10 min at 4 C and resuspended in 5 ml cold PA. Viable counts were determined at this time. The deflagellated cells were divided among four 50 ml flasks (1 ml each) and incubated in a shaker bath for 0,8,17 and 27 min in order to permit reflagellation of the cells. After reflagellation, PBS 1 in 1 ml of AD was added to each flask to obtain an MOI of 0.02 and adsorption was allowed for 3 min in the shaker bath. Infection mixtures were fixed with 5% formaldehyde after adsorption. Total possible reflagellation times were therefore 3,11,20 and 30 min. Samples (1 ml) were immediately centrifuged at 8,000 x g for 5 min at 4 C and the supernatant fluids were assayed for unadsorbed phages. The results are presented in Table 3.

Table 3. Effect of relative flagella length on efficiency of adsorption[a]

Reflagellation time (min)	Unadsorbed phage (PFU/ml)	Efficiency of adsorption (%)
control	1.3×10^5	99.2
0[b]	1.4×10^7	17.6
8	4.6×10^6	72.9
17	3.9×10^5	97.7
27	4.4×10^4	99.7

[a] 1.7×10^7 PFU/ml was added to strain SB19E at a cell density of 9.3×10^8 CFU/ml.

[b] The sample receiving 0 min reflagellation represents a deflagellated control.

The results are in agreement with those of Raimondo *et al.* (51), indicating that the efficiency of adsorption is increased with longer flagella. The deflagellated control would have had 3 min reflagellation if the regeneration of flagella is not inhibited by the early stages of adsorption. Adsorption efficiency to the deflagellated control was 17.6%. This value may indicate that deflagellation was not complete or that non-specific adsorption occurred.

Adsorption to nonmotile strains of *B. subtilis*

To confirm the requirement of flagella for adsorption and to provide a control for subsequent experiments designed to investigate the specificity of adsorption, the efficiency of adsorption to two nonmotile, nonflagellated strains of *B. subtilis* was determined.

Joys (33) used the nonmotile strains of SB108 and MS14 and found no adsorption when cells were infected at an MOI of 0.02 for 30 min. In the experiments presented here, SB108 and MS14 were grown in PA to a cell density of 4×10^8 CFU/ml. Cells (1 ml) were infected in 50 ml flasks with PBS 1 suspended in AD (1 ml) to obtain an MOI between 0.02 and 0.04. Adsorption was allowed for 10 min in a shaker bath.

The infection mixture (1 ml) was centrifuged at 6,000 x g for 10 min at 4 C to remove cells and the supernatant fluid was assayed for unadsorbed phage. Strain SB19E was included as a control for adsorption to motile cells. The results are presented in Table 4.

The efficiency of adsorption to nonmotile cells ranged from 0 to 17% (Table 4). The range of values reported here reflect the sensitivity of this biological assay with respect to the procedural variation. Pritikin and Reiter (49) found 9% adsorption of PBS 1 to the cells of the nonflagellated strain SB171 using an MOI of 0.5 and 10 min adsorption. They considered this to represent a non-adsorbing control. According to the present data an adsorption efficiency of less than 18% may indicate the absence of adsorption. Electron microscopic observations revealed that no phage particles had adsorbed to the cells of strains MS14 or SB108 when infected with an MOI of 10. No flagellated cells were seen.

Cessation of motility

Meynell (42) using χ phage and Frankel and Joys (24), using PBS 1, observed the cessation of motility of the host bacteria following infection at a high MOI.

Table 4. Adsorption of PBS 1 to nonmotile strains of *B. subtilis*[a]

Strain	Initial phage titre (PFU/ml)	Unadsorbed phage titre (PFU/ml)	Efficiency of adsorption (%)
SB108	6.0×10^6	5.7×10^6	5
	1.3×10^7	1.1×10^7	14
	1.8×10^7	1.5×10^7	17
	1.0×10^7	9.3×10^6	9
MS14	6.0×10^6	5.5×10^6	8
	1.3×10^7	1.2×10^7	10
	1.1×10^7	1.1×10^7	0
	1.0×10^7	9.4×10^6	8
	1.0×10^7	8.8×10^6	14
SB19E	1.8×10^7	1.0×10^5	99

[a] all strains were grown to cell densities of 3.5 to 4.0×10^8 CFU/ml.

Raimondo *et al.* (51), using PBS 1 particles purified by CsCl density gradient centrifugation, found that no cells were motile 30 sec after infection at an MOI of 5. However, 50% of the cells were still motile when infected at an MOI of 1.

This phenomenon was examined with strains SB19E, 168, and five other motile, but PBS 1-resistant *Bacilli*. Cells were grown in PA to a density of approximately 5×10^8 CFU/ml. One drop of cells was placed on a glass slide and infected with one drop of PBS 1 at an MOI of 5. A coverslip was placed over the infection mixture which was then observed under the phase-contrast microscope, until cell motility ceased. Uninfected cells were also included as control, since motility ceases after a time possibly as a result of reduced oxygen tension under the coverslip. Under this condition, uninfected cells near the edge of the coverslip were still motile after 10 min, but cells in the centre area became immobile after 5 min. Therefore, all observations were made near the edge of the coverslip. Results are shown in Table 5.

In no case did the motility of an infected PBS 1-resistant *Bacillus* cease before the uninfected control. Both PBS 1-sensitive strains SB19E and 168 became completely immobile shortly after infection.

Table 5. Cessation of motility in infected cultures

Bacillus	Motility
B. subtilis SB19E	no motility after 90 sec
B. subtilis 168	no motility after 60 sec
B. megaterium	motile after 10 min
B. thuringiensis var. *alesti*	motile after 10 min
B. thuringiensis var. *thuringiensis*	motile after 10 min
B. amyloliquifaciens H (RUB 500)	motile after 10 min
B. cereus 819	motile after 10 min

Adsorption stages

Adsorption of PBS 1 can be divided into four stages according to the adsorption site and morphological conformation of the phage particle. Stage I involves the initial attachment of PBS 1 to any point along the length of the flagellum by means of the helical tail fibres. This is the only non-specific stage of adsorption (to be shown later) and is apparently reversible (51). Abortive infection occurs at this stage if the flagella are paralyzed (e.g.by KCN(51)) after the initial attachment of phage so that the phage particles cannot reach the flagellar base. An example of Stage I adsorption is shown in Fig. 5.

Stage II begins as the phage particle reaches the base of the flagellum and assumes the correct orientation for irreversible attachment to the specific receptor sites at the flagellar base. The tail sheaths in this stage are not yet contracted (Fig. 2).

At Stage III the tail sheath contracts as the tail core penetrates the cell wall at the flagellar base. Presumably at this stage cell motility ceases as these events occur at the same time after infection (30 to 60 sec). An example of Stage III is shown in Fig. 3.

During Stage IV the injection of phage DNA takes place and phage particles appear ghosted. The empty heads of ghosted phage appear dark as the stain penetrates the vacant space. An example of Stage IV is shown in Fig. 4.

In Stages II, III, and IV it appeared that the helical tail fibres coiled closely around the base of the flagellum to anchor the phage for penetration of the cell wall by the tail core after contraction of the tail sheath (Stage III, Fig. 3).

In addition to the four stages of adsorption described above, some contracted and ghosted phage particles were found on the flagella (Fig. 6). These may be phage particles which have contracted prematurely or they may be an artifact caused by the fixation and staining of the infection mixtures.

Determination of adsorption time sequence

Cells of strain SB19E were grown in PA to a cell density of approximately 5 x 10^8 CFU/ml (160 Klett units). To 5 ml of culture were added 5 ml of PBS 1 lysate (Millipore filtered) diluted with AD to obtain an MOI of 1.3, 3.3, and 20. Adsorption was carried out at 37 C in a shaker bath with moderate aeration. Samples (0.5 ml) were taken at various times and fixed with 5% formaldehyde (0.1 ml). The fixed samples were centrifuged at 3,000 x g for 2 min and resuspended gently in 1/2 volume of 0.1 M ammonium acetate. This washing step resulted in good staining of bacterial

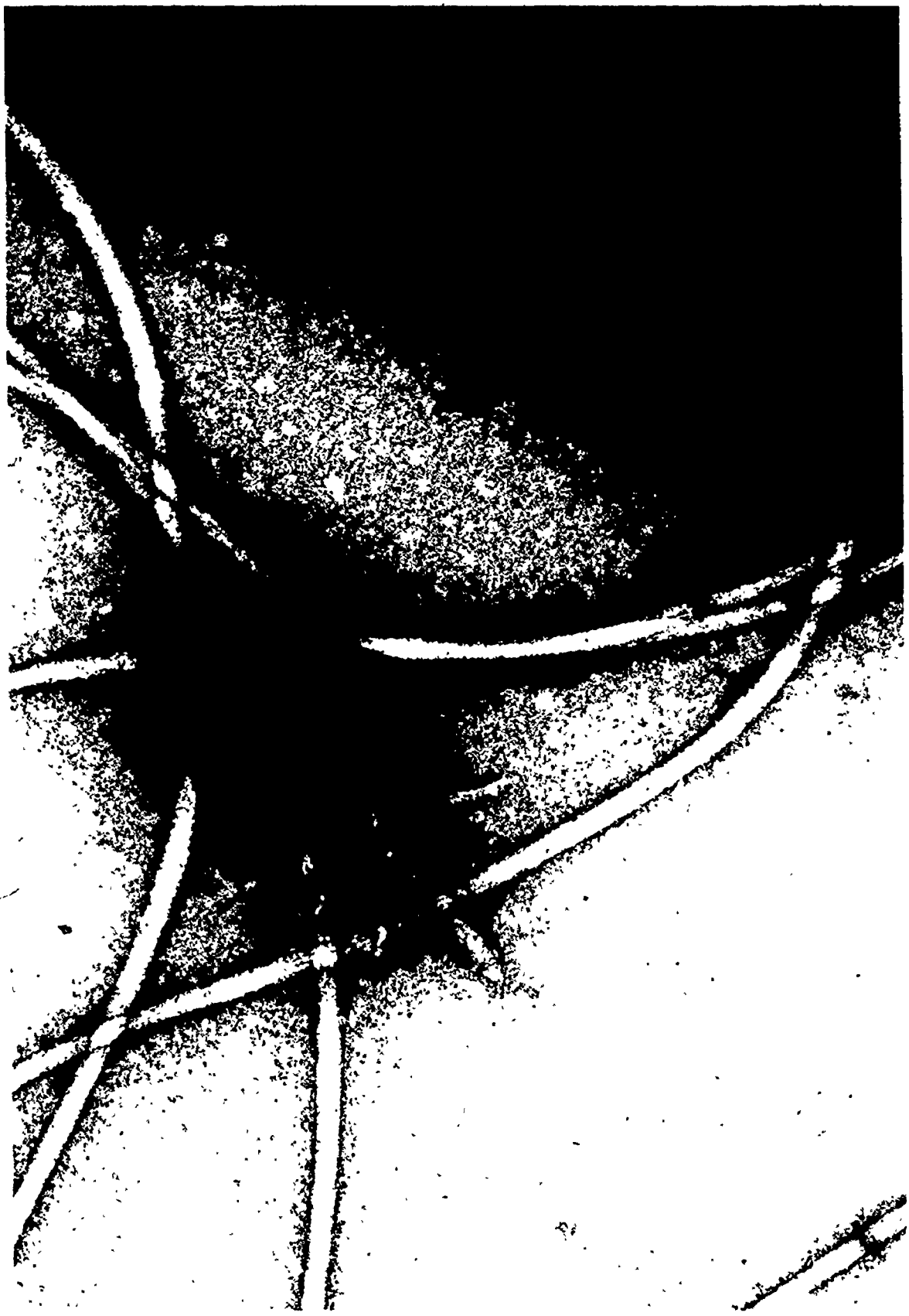
Fig. 5. PBS 1 particles at Stage I of adsorption to *B. subtilis* SB19E.

(A) Phages attached to the numerous flagella. X 33,000. (B) Detail of helical tail fibre attachment to the flagella. X 169,000. Negatively stained with 2% uranyl acetate.


A
B

Fig. 6. Contracted PBS 1 particle on a flagellum of *B. subtilis* SB19E.

The tip of the tail core does not appear to enter the flagellum. Flagellar base (F) is visible on the cell wall. Negatively stained with 1% uranyl acetate. X 205,000.



cell walls and consequently the phage at the flagellar bases could be observed with ease under the electron microscope. About 200 phage particles were examined for each sample. All phage particles attached in any manner to the bacteria were counted. Only bacteria possessing flagella were considered. Phage particles were scored for their morphological appearance (uncontracted, contracted, or ghosted) and position of attachment (flagellum or flagellar base) in each case. Results are presented in Table 6. Estimates were made on the time sequence for adsorption as well as for the various events occurring at each stage. These are summarized in Table 7.

Raimondo *et al.* (51) showed that infective centres were not formed if the infected cells were deflagellated during the first 4 min of infection. Pritikin and Reiter (49) demonstrated that PBS 1 infection is blocked by actinomycin D only within the first 4 min of infection. These observations suggest that it takes at least 4 min for PBS 1 to complete adsorption and to commence production cycle.

The percentages of phages counted at each stage of adsorption at various MOI remained relatively constant after 180 sec of infection (Table 6). This indicates that adsorption appears complete for most

Table 6. Effect of adsorption time and MOI on phage conformation and adsorption site[a]

Adsorption time (sec)	MOI	Ghosted phages at B (IV)	Ghosted phages at F	Contracted phages at B (III)	Contracted phages at F	Uncontracted phages at B (II)	Uncontracted phages at F (I)
10	1.3	0	1.5	0	0	68.1	30.2
60	1.3	0.5	0	37.1	0.5	55.9	5.9
90	1.3	30.4	0.4	54.9	1.3	9.4	3.6
180	1.3	44.5	1.4	43.2	5.9	3.6	1.4
300	1.3	43.7	12.1	34.0	8.8	1.4	0
600	1.3	45.7	15.4	32.2	5.8	1.0	0
10	3.3	0	0.5	1.0	0.5	53.1	44.9
30	3.3	0.9	0	14.5	0.9	67.5	16.2
60	3.3	4.3	0.4	58.0	1.8	24.6	10.9
90	3.3	8.7	0.5	73.6	1.0	11.5	4.8
180	3.3	47.2	1.9	42.1	4.2	2.8	1.9
300	3.3	40.9	2.8	39.9	5.5	0	0.9
600	3.3	33.0	25.9	26.9	14.2	0	0
30	20	1.4	0	32.7	0	14.7	51.2
60	20	4.6	1.4	41.3	1.4	10.1	41.3
180	20	16.0	1.0	39.3	1.0	3.9	38.8
360	20	12.9	3.3	37.8	2.4	3.3	40.2
600	20	16.1	2.2	33.6	4.9	3.6	39.5

[a] The number of phages examined for each sample varied from 202 to 276. The results were expressed as percent of the total phage particles examined.
Roman numerals refer to the stage of adsorption represented by phage conformation and adsorption site as described in the text.
B : flagellar base; F : flagella

Table 7. Time course of PBS 1 adsorption

Time after mixing (sec)	Adsorption stage	Description of the stages
0 - 5	I	phage particles attach at any point along the length of flagella by means of the helical tail fibres
5	II	phage particles appear at the flagellar bases
30	III	tail sheaths of phage contract, host motility ceases
90	IV	phage particles begin to appear ghosted as DNA injection is initiated
240	IV	phage DNA is totally injected and productive infection begins (49,51)

infected cells after 180 sec. This time required for completion of adsorption is consistent with the minimum time required for the initiation of productive phage development reported by the above authors (49,51).

The distribution of phage particles at Stages I, II, and IV at low MOI was markedly different from that observed at high MOI. The constant number of uncontracted PBS 1 particles attached to the flagella (Stage I) when the MOI was 20 indicates that these phages had become trapped on the flagella, possibly as a result of the loss of motility (24,51). At the lower MOI (1.3,3.3), very few, if any, phages at Stage I were found after 5 min adsorption. Presumably, the phages at the lower MOI were able to reach the flagellar bases before motility ceased. The appearance of PBS 1 particles in Stage III coincided with the loss of motility (30 to 60 sec after infection). It is conceivable that contraction of the tail sheath and penetration of the flagellar base by the tail core caused the loss of motility.

Influence of MOI on motility and adsorption

Results presented in Table 6 suggest that the final distribution of phage particles on the host cells may depend on the MOI. Raimondo *et al.* (51) reported a

dependence on MOI and the extent of cessation of motility of cells of strain SB19. These authors found that with an MOI of 1, 50% of the cells were motile, at an MOI of 2.5, 10 to 25% of the cells were motile, and at an MOI of 5, no motile cells were seen after 30 sec of infection.

In the present data (Table 6) 40% of the phages counted for the MOI of 20 were on the flagella (Stage I), whereas at an MOI of 1.3 no phages were found on the flagella after 5 min adsorption. Since motility appears to be blocked much sooner after infection when a high MOI is used, the loss of motility may account for the high proportion of phages in Stage I when cells are infected at a high MOI of 20. Conversely, after 10 min of adsorption, 45.7% of the phages counted were at Stage IV when the MOI was 1.3, while at an MOI of 20, only 16.1% were observed at this stage. This suggests that motility is required for the phage to reach the flagellar base and proceed to Stage IV. At the lower MOI, more phage particles are able to reach the flagellar base than in the case of high MOI. Early cessation of motility presumably traps phage particles on the flagella (Stage I).

It was also observed that not all flagella with "trapped" phage particles had the phage at their

base in immobilized cells. This observation suggests that adsorption of the phage to all flagellar bases is not required to stop cell motility. Raimondo *et al.* (51) reported that the adsorption of one phage particle to one flagellum of *B. subtilis* rendered the entire complement of flagella nonfunctional.

Minimum time for flagellar base attachment

To determine the minimum time required for the phage to reach the flagellar base (Stage II) and the effect of the growth medium on adsorption, the following experiment was carried out. Cells of strain SB19E were grown for 4 h in PA, TY and ETY and infected with PBS 1 in AD at an MOI of 1.7. Cells were infected in test tubes kept in a water bath at 37 C. Samples (1 ml) were fixed with 5% formaldehyde 5 sec and 10 sec after infection.

Electron microscopic examinations revealed that about 20% of the phages counted were at Stage II after 5 sec of adsorption. The type of growth medium employed had no effect. Apparently, PBS 1 particles can either move down the flagellum to the flagellar base very rapidly or a certain percentage of the phage particles attach initially on the flagella at or very close to

the flagellar bases. Adsorption times of less than 5 sec could not be accurately tested.

Adsorption of PBS 1 on other *Bacillus* species

The process of initial attachment of PBS 1 during infection, namely the three helical tail fibres wrapping around the host flagellum, appeared to us to afford little means of specificity apart from perhaps limitations imposed by flagellar diameter. To determine whether PBS 1-resistant bacteria with similar flagellar diameter did indeed adsorb the phage, adsorption efficiencies were determined and electron micrographs taken for a number of PBS 1-resistant *Bacilli* infected with PBS 1.

The efficiencies of adsorption of PBS 1 was determined with *B. subtilis* strains SB19E and 168 as positive adsorption controls and nonflagellated strains SB108 and MS14 (Table 4) as negative adsorption controls. Various PBS 1-resistant *Bacillus* species (Table 8) were tested for PBS 1 adsorption. Host culture (1 ml) was mixed with 1 ml of PBS 1 particle which were purified by CsCl density gradient centrifugation and resuspended in AD and incubated for 10 min in a shaker bath. The MOI ranged from 0.01 to 0.09. Low MOI values were employed so as to eliminate the possibility

of phage particles being trapped by the cells without actually adsorbing to them. Infection mixtures were centrifuged at 6,000 x g for 10 min and the supernatant fluids assayed for unadsorbed phages. Results are presented in Table 8.

Under the conditions employed, the PBS 1-sensitive controls allowed 94 to 100% adsorption whereas the nonflagellated controls allowed between 0 and 17% adsorption. As discussed earlier, an efficiency of adsorption less than 18% is considered to be doubtful or negative due to the experimental error associated with this assay. The results presented in Table 8 indicate that PBS 1 particles could adsorb to all the PBS 1-resistant *Bacilli* tested.

Electron microscopic observations of cells infected at a high MOI revealed that all of the PBS 1-resistant *Bacilli* tested were indeed capable of adsorbing PBS 1 particles. Adsorption of PBS 1 on *B. megaterium* and *B. thuringiensis* var. *alesti* is shown in Figs. 7 and 8, respectively. Similar observations were also made with other PBS 1-resistant *Bacilli*.

In order to determine at which stage of adsorption productive infection is blocked in the resistant *Bacilli*, cells were infected at an MOI of about 100 and adsorption allowed for 30 min. These

Table 8. Adsorption of PBS 1 to various *Bacillus* species

Bacillus	Efficiency of adsorption (%) at various MOI						
	0.01	0.02	0.03	0.04	0.05	0.07	0.09
B. subtilis SB19E		99 100	94				
168		96					
MS14 (nonmotile)		8 8 14	0	10			
SB108 (nonmotile)		5 9	14	17			
B. megaterium			0	13 13 0	17	5	39
B. cereus 819		22 0 21 0	25	0			
B. thuringiensis var. *thuringiensis*		22	15	33			
B. thuringiensis var. *alesti*		15 38	38 30		44		
B. amyloliquifaciens H (RUB 500)	23	20	7 14		44		

Fig. 7. PBS 1 particle attached to a flagellum of *B. megaterium*.

Negatively stained with 1% uranyl acetate. X 52,000.

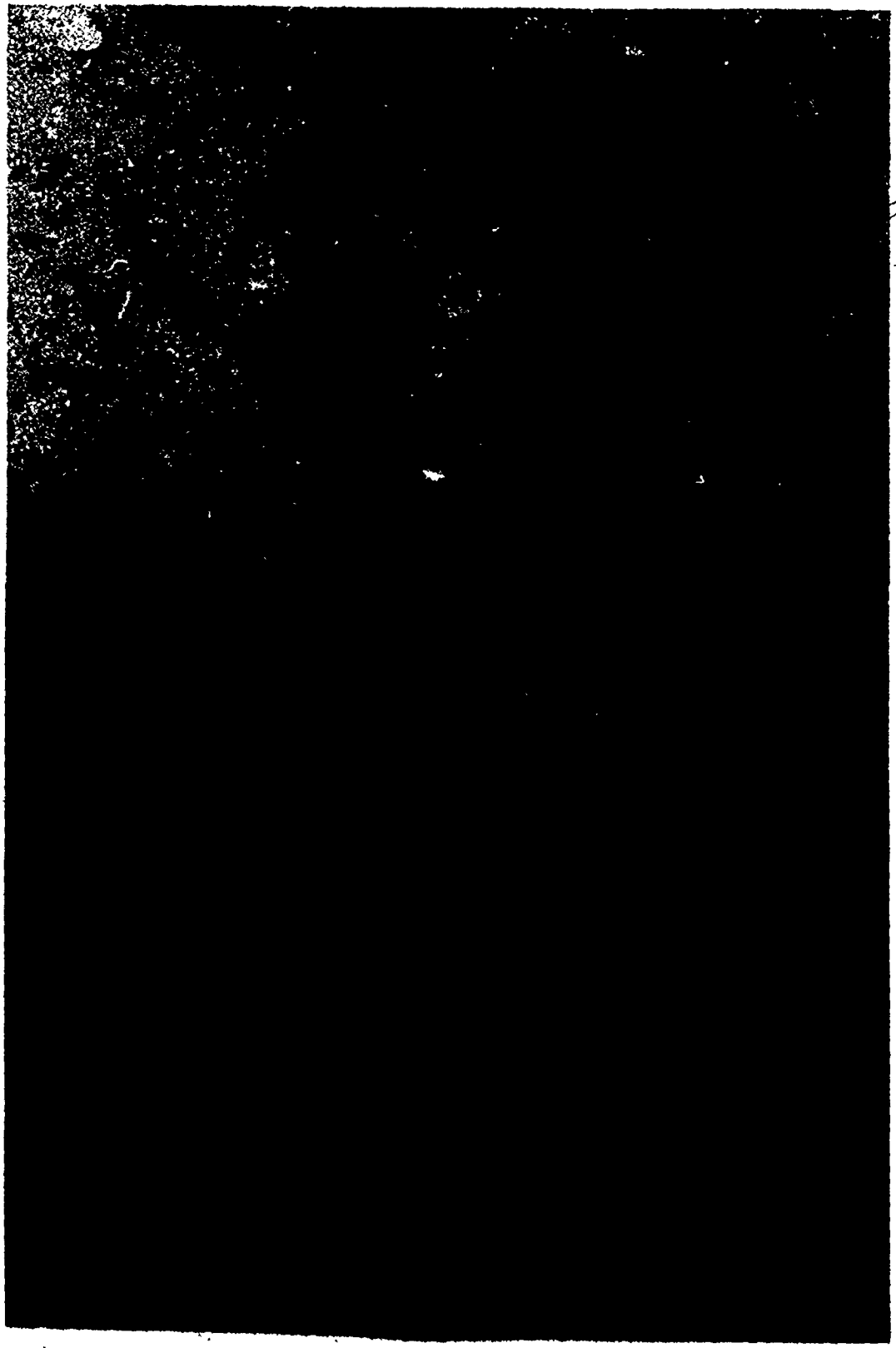
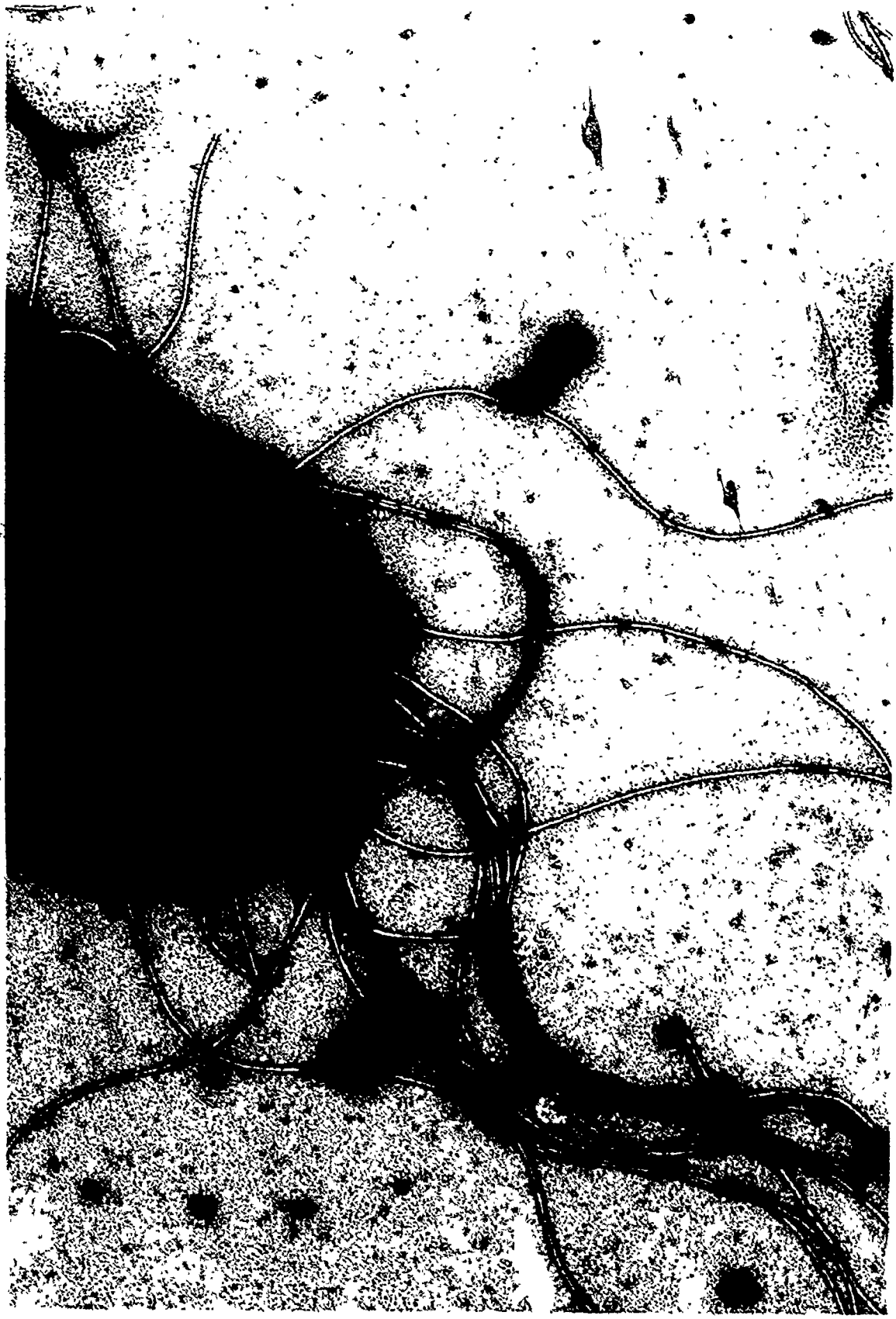


Fig. 8. PBS 1 particles attached to flagella of *B. thuringiensis* var. *alesti*.

Negatively stained with 1% uranyl acetate. X 40,000.



infection mixtures were then centrifuged at 3,000 x g for 2 min and resuspended in 1/2 volume of 0.1 M ammonium acetate and examined under the electron microscope. The latest stage of adsorption found for each species is presented in Table 9. Formaldehyde fixed and unfixed cells of strain SB19E were infected and observed as controls.

Stage II adsorption was the furthest stage observed with any of the PBS 1-resistant *Bacilli*. This suggests that the specificity associated with the adsorption process may reside in the flagellar base. The appearance of phage particles on the flagella or formaldehyde-killed cells of strain SB19E supported the claim of Raimondo *et al.* (51) that phages can attach to nonmotile cells having inactive flagella. The actual efficiency of adsorption by formaldehyde-killed cells of strain SB19E was not determined. The absence of Stage II adsorption in these cells suggests that flagellar motion is required for the movement of phage particles down the length of the flagella to the flagellar bases. This requirement was mentioned by Schade *et al.* (56) for the adsorption of χ phage to *E. coli*.

None of the PBS 1-resistant *Bacilli* tested lost their motility after infection with PBS 1 (Table 5). This observation, together with the absence of Stage III adsorption in the PBS 1-resistant *Bacilli* suggests

Table 9. Extent of adsorption in PBS 1-resistant *Bacilli*

Bacillus	Furthest adsorption stage observed
B. subtilis SB19E (control)	IV
SB19E + HCHO	I
B. megaterium	II
B. cereus 819	II
B. thuringiensis var. *thuringiensis*	II
B. thuringiensis var. *alesti*	II
B. amyloliquifaciens H (RUB 500)	II

that some stage of adsorption leading to a productive infection appears to be required for the loss of motility in PBS 1-sensitive strains of *B. subtilis*.

DISCUSSION

From the electron micrographs presented in this thesis it appears that the site of final adsorption and DNA injection of PBS 1 is the flagellar base and not simply the cell wall.

The time course for the four adsorption stages was estimated from the data in Table 6 and is presented in Table 7. It appears that Stage I and II adsorption can occur almost immediately after infection. Stage III may require proper orientation of the phage particle at the flagellar base site before tail sheath contraction can occur. Tail sheath contraction can also occur spontaneously in isolated phage particles. These prematurely contracted phages are usually seen in small numbers in normal phage lysates. A minimum of 30 sec is required for Stage III to be seen. A large proportion of phage particles were seen at Stage IV as early as 90 sec after infection, indicating that DNA injection had already occurred at the base of flagella.

Infection by most bacteriophages seems to leave motility unaffected until the latent period is advanced (i.e. just prior to the bursting of the host cell), or even until lysis (42,43). The opposite is true of

the flagellotropic bacteriophages as motility of the host cell is generally lost soon after adsorption. Meynell (42) found that addition of χ phage to *Salmonella abortus-equi*, at a sufficiently high MOI, caused rapid cessation of motility with concomitant cell clumping. She found that phages which were damaged by ultraviolet radiation or prolonged centrifugation caused immobilization as efficiently as infective phage. This suggests that the loss of motility was due to the adsorption of χ phage and not productive infection.

Lovett (37) showed that PBP1, at an MOI of 20 immobilized *B. pumilus* after 4 min infection. Fukuda *et al.* (25) reported that motility of *Caulobacter vibrioides* is immediately lost upon ϕCp34 infection. *C. vibrioides* has a single polar flagellum (25). Recently, Lotz *et al.* (36) reported that addition of phage 7-7-1 to a culture of motile *Rhizobium lupini* H13-3 cells lead to immediate arrest of active cell movement and to a clumping of bacteria.

Frankel and Joys (24) found that PBS 1 at an MOI of 10 could immobilize *B. subtilis* SB108b (a flagellated revertant of SB108) completely within 2 to 3 min. Raimondo *et al.* (51) reported that *B. subtilis* SB19 lost motility after 30 sec infection by PBS 1 at an MOI of 5. Rapid loss of motility after infection by PBS 1 was also observed for *B. subtilis* SB19E and 168 in the present study (Table 5).

Some question still remains as to whether active flagellar motion is required for phage adsorption and productive infection by PBS 1. Most studies on flagellotropic bacteriophages report a requirement for active flagella for infection (3,24,25,32,36,42). Raimondo *et al.* (51) found PBS 1 adsorption at a reduced efficiency to the inactive flagella of *B. subtilis* protoplasts. They also found productive infection occurring in nonmotile mutants possessing flagella lacking the long period helix. These straight flagella might still retain their twisting or rotating motion at the flagellar base (29). This motion, while insufficient to cause cell motility, might be sufficient to allow Stage II adsorption, thereby leading to a productive infection. In the present study it was found that PBS 1 particles could adsorb to formaldehyde-killed cells, but only as far as Stage I (Table 9). Brownian motion of the cells may have been sufficient for Stage I adsorption, but independent movement of the flagella appears to be required for the phage particle to move along the flagella to the flagellar base and to reach Stage II. That phage particles can attach at points on the flagella, distal to the cell wall, and move down to the base was demonstrated by the deflagellation-reflagellation experiments of

Raimondo *et al.* (51) and corroborated in this study (Table 3). The longer the flagella, the greater the efficiency of adsorption and number of infectious centres that may result (51).

It was noted that the proportion of phages at Stage III and IV at an MOI of 1.3 and 20 remained relatively constant after about 3 min of adsorption (Table 6). Reductions in the number of phages seen at Stage III and IV for an MOI of 3.3 were found at 600 sec after infection, but these were offset by a rise in the number of contracted and ghosted phages found on the flagella. These changes occurred because some of the contracted and ghosted phage particles were dislodged from the flagellar bases.

If cell motility is required for Stage III adsorption and motility is being lost within the first few minutes of infection it is reasonable to suggest that the loss of motility is responsible for the constant proportions of Stage III and hence, Stage IV adsorption seen after 3 min infection. Similarly, the constant Stage I adsorption seen for phages after 30 sec infection at an MOI of 20 is a consequence of the host cell motility being lost within the first 30 sec and thereby trapping many phage particles attached to the flagella.

How might the adsorption of phage particles render the entire complement of flagella inactive? It has been reported that a single PBS 1 particle can immobilize a cell even if the viral genetic material has been lost following osmotic shock (51). The phages therefore block flagellar movement mechanically, rather than by productively infecting the cell.

DePamphilis and Adler (20) presented a detailed model of the flagellar basal end for both Gram-positive and Gram-negative bacteria. This model consists of a series of rings attached to the cell wall layers and cytoplasmic membrane through which passes a rod that acts as a rotor, being attached to the flagellar hook. In Gram-positive bacteria, only two rings are present due to the reduced complexity of the cell wall structure. The S ring is attached to the cell wall, and the M ring is attached to the cytoplasmic membrane. Berg (5), in his article on flagellar motion stated that:

"The evidence at hand suggests a model for the rotating motor (for flagellar motion) in which the torque is generated between two elements in the (flagellar) basal body, the M ring and the S ring. ...The torque could be generated by the active translocation of ions through the M ring to interact with the charged groups on the surface of the S ring. ...

The direction of the rotation (of the flagellar base) would be determined by the ion flow".

Other theories for flagellar motion are presently being considered (57). If ion flow is indeed important for flagellar motion, it is conceivable that any disruption of the cell membrane at or around the M ring would destroy any existing ionic gradient necessary for the flagellar rotation proposed by Berg (5). Perhaps such a disruption at one flagellar base could destroy the ionic gradients for the entire complement of flagella, rendering them all inactive. If so, this could account for the reported ability of one phage particle to inactivate a multitrichous bacterium (51). Penetration of the phage tail core through a site at or near to the M ring may cause the aforementioned disruption of ionic gradients that would immobilize all of the flagella on the infected cell. It was shown that damaged, non-infective PBS 1 particles can still immobilize host cells as efficiently as infective phage (51). It is possible that phage possessing no DNA (i.e. ghosted by osmotic shock (51)) may still be able to contract their tail sheaths, thereby causing the observed loss of motility. It is also noteworthy that loss of motility in *B. subtilis* appeared concurrently with the occurrence of Stage III adsorption. In the present study it was

shown that PBS 1-resistant *Bacilli* adsorbed phages as far as Stage II, but, even at a high MOI no loss of motility was observed. The failure of phage particles to contract on these cells suggests that perhaps Stage III adsorption is required to immobilize the host cells.

The fact that noncontractile phage (group B and C) can also cause the loss of motility (25,37,42) does not support the suggestion that penetration of the flagellar base by the phage tail core (Stage III) causes immobilization. Meynell (42), using damaged, noninfective χ phage, showed that productive infection (i.e. DNA injection) is not required for loss of motility.

That Stage II adsorption can cause the loss of motility cannot be ruled out, since less than 10% of the phages adsorbed to the PBS-1 resistant *Bacilli* reached Stage II and it was difficult to detect less than 10% loss of motility in an actively motile culture. A very high MOI was required to find significant Stage II adsorption in the PBS 1-resistant *Bacilli*. The low number of phages seen attached at Stage II in these cells compared to the PBS 1-sensitive SB19E cells might indicate that the phage particles must attach properly to the specific receptor site at the flagellar base in order to inhibit flagellar motion and proceed to Stage III adsorption. Inability to stop the flagellar motion soon after reaching the base could

allow the phage particles to be dislodged by the continuing flagellar movements. If proper attachment to the receptor site at the flagellar base is required for the phage particle to remain there, then this would explain the low proportion of Stage II adsorption seen in the PBS 1-resistant *Bacilli*, even after 30 min adsorption time. Greater than 50% of the phages attached to sensitive host cells reached Stage II after only 10 sec of adsorption (Table 6). It appears, therefore, that Stage I is the only nonspecific stage of adsorption, and is the only reversible stage (51). The Stage II adsorption seen in the PBS 1-resistant *Bacilli* may, in fact, be only "pseudo-stage II", not having attached properly to the receptor site at the flagellar base.

It seems certain that Stage I adsorption of PBS 1 is not sufficient to cause the observed cessation of motility as suggested by Berg (5). Further studies are required to distinguish the effects of Stage II and Stage III adsorption on motility. A solution to this question might provide some insight into the current studies on the mechanism of flagellar motion.

SUMMARY

The adsorption of PBS 1 begins with the attachment of phage particles to the flagella of *B. subtilis* (Stage I). The phage can attach to both active and inactive flagella. Flagellar attachment is nonspecific as PBS 1 was shown to attach to the flagella of *Bacillus* species other than the normal host *B. subtilis*. The phage particle then quickly moves down the length of the flagellum to its base, the final adsorption site. Flagellar motion appears to be required for flagellar base attachment (Stage II). After proper attachment at the flagellar base, the phage tail sheath contracts sending the tail core through the final adsorption site (Stage III). The phage DNA is then injected at this site (Stage IV).

Stage I adsorption does not cause loss of motility in PBS 1-resistant *Bacilli*. The loss of motility observed upon infection of sensitive cells by PBS 1 may be associated with either Stage II or Stage III of adsorption.

LITERATURE CITED

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