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Adsorption of Bacteriophages to Adhesions Between Wall and Membrane of *Escherichia coli*

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In plasmolyzed *Escherichia coli*, wall and membrane adhered to one another at 200 to 400 localized areas. This number of specialized wall areas per cell was of the same order of magnitude as the total number of bacteriophage receptors. When bacteriophages T1 to T7 were adsorbed to the bacteria, they were seen to attach almost exclusively to these areas. Comparisons of the number of adsorbed phage particles observed in ultrathin sections and the expected number of phages per cell were in agreement. These results suggest a sharing of receptive areas by the various phages. Adsorption to the wall-membrane associations would permit the virus to release its nucleic acid at an area closest to the cell's protoplasmic contents.

After the electron microscopic description of bacteriophage particles by Ruska (23), Luria and Anderson (17), and Luria, Delbrück, and Anderson (19), the mode of virus adsorption to surfaces of susceptible cells could be studied successfully: Anderson (2) clearly demonstrated that bacteriophages T1 and T5 adsorbed to their host cells with the tips of their tails. T-even bacteriophage adsorption involves the specific attachment of tail fibers to the bacterial receptor (13, 26, 32, 33), followed by changes in their contractile tail sheaths and baseplates (3, 14, 26, 27). However, the mode of attachment of other phage types, like T3 and T7, is still unknown (18).

Extracts from bacterial envelopes contain receptor activity and function as competitive inhibitors for virus attachment to bacterial host cells (6, 16, 31). Frank, Zarnitz, and Weidel (8) showed that the deoxyribonucleic acid (DNA) of bacteriophage T5 is ejected through the tip of the tail when the particles react with receptor preparations of bacterial wall extracts. After the virus is adsorbed to the receptors on the cell wall, its DNA passes into the cell (11). The binding capacities of whole bacterial cells, as well as changes in this capacity after chemical treatment, were studied by Tolmach and Puck (29). However, except for male-specific phages which adsorb to certain types of pilus (5) and a phage which adsorbs only to bacterial flagella (20), the morphology of the potential receptor sites on the wall of the bacterium has not been determined.

In plasmolyzed *Escherichia coli*, the protoplasmic membrane stays attached to the cell wall at numerous locations, whereas the major portion of the protoplast shrinks and retracts from the wall (*unpublished data*). When bacteriophages T1 to T7 are added to such plasmolyzed cells, the virus particles adsorb only to those areas at which wall and protoplasmic membrane adhere to each other. In this report, the morphological appearance of the adsorbed phages is described, and their number are quantitatively evaluated and compared to the number of phages expected.

MATERIALS AND METHODS

E. coli B was grown logarithmically in 10 ml of nutrient broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose; adjusted to *p*H 7 with 1 N NaOH) at 37 C under aeration (about 200 cc of air/min), with a generation time of 25 to 30 min, until cell counts between 5×10^7/ml and 2×10^8/ml were reached. The cells were then plasmolyzed by addition of equal volumes of nutrient medium (*p*H 6.8 to 7.0) containing 20 or 40% (w/v) sucrose and kept for about 2 min at 37 C with aeration. The cells plasmolyze almost immediately in the hypertonic environment (4).

For turbidimetric measurements a Klett-Summerson photometer was used. Besides colony counts, total cell counts were obtained by using a Neubauer chamber and a Zeiss phase-contrast microscope.

Bacteriophages T1, T2, T3, T4, T5, T6, and T7 had been maintained for many years in our laboratories. Lysates were obtained in nutrient broth in which the phage titer remained stable for at least 6 months. Storage of the virus at 4 C in F medium (25) or in 3 X D (9) yielded considerable differences in stability of the various phages: whereas the plaque-forming capacity of T2 remained unaffected when the

virus was stored in a synthetic medium, the titers of T3 and T7 dropped from 3×10^{10} to 1.5×10^{9} in 2 months. All adsorption studies were therefore performed with fresh lysates.

Phage adsorption to bacterial cells was carried out in nutrient medium with or without sucrose. For adsorption in sucrose (10 or 20%), the phages were, in general, kept in the sucrose medium for 10 to 60 min at 37 C; however, to investigate the influence of sucrose on the phage particle, in a number of experiments the exposure time of the phages to sucrose before adsorption was reduced to 1 min. The multiplicity of infection (MOI) was varied with the experimental requirements: when the plating efficiency in sucrose was to be tested, the MOI was kept at 1 or 0.1; for electron optical preparations, the MOI was kept between 25 and 100; in some of the tests with T5, the MOI was as high as 10^3.

The plating efficiency was tested on plates containing the corresponding amount of sucrose (i.e., 10 or 20%); 0.2 ml of the adsorption mixture plus 0.2 ml of logarithmically growing *E. coli* B in sucrose were mixed with 2.5 ml of soft agar containing the proper amount of sucrose and poured on L-agar plates of corresponding sucrose content. The plaques were counted after 12, 24, and, sometimes, after 48 hr of incubation at 37 C.

For electron microscopic studies of the cell-virus complex, the adsorption was carried out at 37 C for 2 to 4 min in aerated cultures containing 10 or 20% sucrose before the fixer was added. The adsorption period for T1 was prolonged to 8 min because of an apparent detachment of adsorbed phage particles during fixation and embedding. In some experiments involving phages T2, T4, and T5, adsorption of the phages was carried out in an osmotically normal nutrient medium to which the sucrose was added 1 to 2 min later in order to plasmolyze the cells. This procedure allowed one to observe a sufficient number of T4 adsorbed to plasmolyzed cells. The cell-virus suspensions were fixed by mixing with an equal volume of the following fixative: one part of 32% formaldehyde in water (made from paraformaldehyde) plus one part of nutrient medium containing 20 or 40% sucrose; shortly before fixation, the solution was adjusted to *p*H 7 by addition of 1 N NaOH. Thus, the final fixing solutions contained 10 or 20% sucrose, respectively. Fixation was carried out at room temperature in centrifuge tubes. After 2 hr, the bacteria were centrifuged at $7{,}000 \times g$ for 15 min in the angle head of a Sorvall centrifuge. The pellet was gently resuspended in 1% osmium tetroxide solution to which sucrose had been added shortly before fixation, to a final concentration of 10 or 20%. The *p*H was brought to 7 with 1 N NaOH or with Michaelis buffer. The OsO_4 fixation lasted for 1 hr at room temperature. The cell suspensions were then pelleted ($4{,}000 \times g$ for 10 min) and resuspended in an aqueous solution containing 0.5% OsO_4, 0.5% freshly prepared uranyl acetate, and 10 or 20% sucrose. The preparations were left in this fixative overnight at room temperature. After dehydration in acetone, the material was collected in pellets and embedded in Vestopal W (Jaeger, Zürich, Switzerland) and gray to silver-gray sections were cut with a diamond knife in a Porter Blum ultramicrotome equipped with an additional thermal advance or in an LKB ultramicrotome. The sections were stained in saturated uranyl acetate solutions for 1 to 2 min and stained further with lead hydroxide stain for 1 to 2 min in a CO_2-free atmosphere. The sections were studied and micrographs were taken on Ilford Special Lantern Contrasty plates, usually at a magnification of 40,000, in a Siemens Elmiskop I electron microscope equipped with double condenser and pointed filament. The instrument's magnification was calibrated with a cross grating of 2,160 lines/mm.

RESULTS

The plating efficiency of phages after adsorption and plating in media containing sucrose showed a dependency upon the sucrose concentration. In 10% sucrose, the plating efficiency for all phages was equal to that of the controls, with one exception: only 5 to 10% of T4 particles produced plaques in 10% sucrose. In 20% sucrose, T1 gave 100% plating efficiency, whereas all the other phages gave only 90 to 70% plating efficiency after 12 hr of incubation. After an additional incubation time of 12 hr, a few more plaques (about 10% more of the total number) usually developed, but in most cases the number of plaques did not reach that of the controls. T4 produced no plaques in 20% sucrose even when 10^4 infective units (measured without sucrose) were plated. Details will be published elsewhere (Bayer and Anderson, *in preparation*).

After the cells (*E. coli* B) and viruses (T2 and T5) were exposed to sucrose for various time intervals prior to adsorption, the adsorption rate seemed to be unchanged. When, after 3 or 4 min of adsorption, the cell-virus suspensions were quickly chilled and the bacteria pelleted, the yield of plaque-forming units (PFU) in the pellets or in the supernatant fluids remained, in general, unaltered, whether the cells had been plasmolyzed only 1 to 2 min prior to adsorption or had been grown in sucrose for four to six generations before adsorption. A reduction of 10 to 20% of the plating efficiency of plasmolyzed cells was sometimes observed (Bayer and Anderson, *in preparation*). Also, the number of PFU sedimenting with the bacteria appeared to be more or less the same whether the phages were exposed to sucrose (i) for 1 min or (ii) for 10 min to 1 hr before adsorption. However, similar to the behavior of freshly sucrose-exposed cells, the yield of PFU after 1-min exposure of the viruses to sucrose seemed to be slightly reduced (10 to 20%). When the adsorption rates for T2, T3, and T5 in L-broth and in L-broth containing 10% sucrose were compared, the following results were

obtained. In sucrose after 4 min of adsorption, only about 60% of phage T2 had adsorbed as compared to the controls. Only about 15 to 20% of T3 had adsorbed after 4 min in sucrose and about 20% after 8 min. Again, the values of the controls were considered as 100%. In contrast to the reduction of the adsorption rate for T2 and T3, T5 yielded the same adsorption rate in sucrose as in the controls.

In ultrathin sections, the morphological appearance of the cells after adsorption of bacteriophages T1, T3, T5, and T7 was found to be similar to that of the controls without phages adsorbed. The contour of the wall, the number and the dimensions of the protoplasmic ducts, and the protoplasmic contents remained unchanged. However, in the case of adsorption of bacteriophages T2, T4, and T6, a high degree of plasmolysis could be maintained only when multiplicities below 100 bacteriophages/cell were applied; even then, it was obvious that the extent of plasmolysis was much less pronounced than in uninfected controls. This phenomenon can be attributed to leakage through the protoplasmic membrane during the first few minutes of infection (22). On the other hand, our T5 strain did not seem to exert any influence on the maintenance of the osmotic pressure inside the cells, and the bacteria remained well plasmolyzed for the duration of adsorption, even after exposure to 1,000 phage/cell.

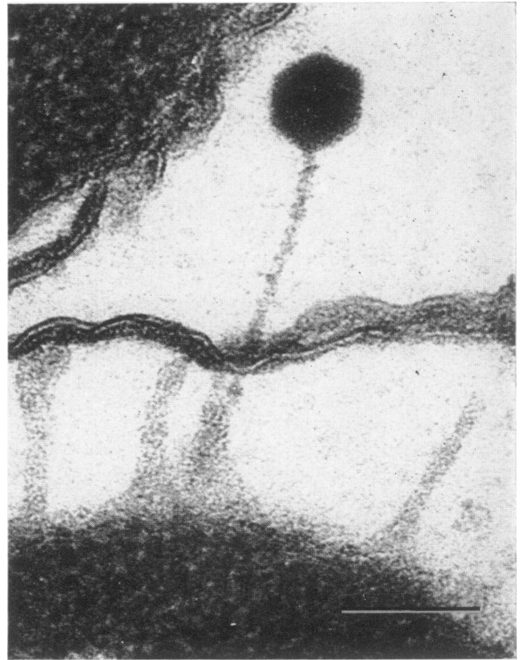

FIG. 2. *T5 adsorbed to plasmolyzed Escherichia coli B. The phage attached to an area where wall and membrane are associated via one of the protoplasmic "ducts." The adsorption time in this and all the following preparations was 3 min, except for T4.* × *180,000.*

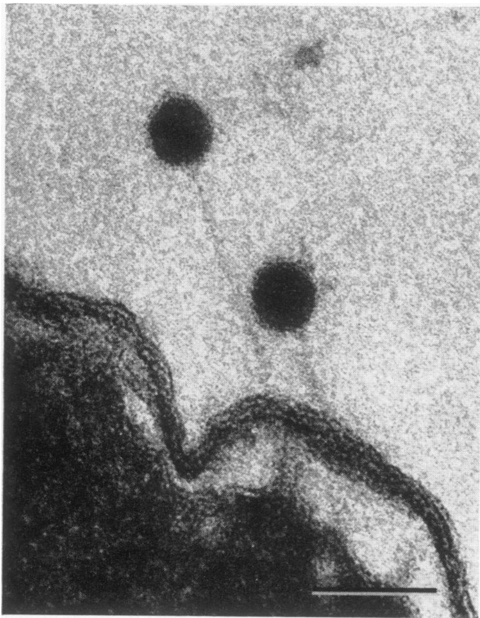

FIG. 1. *Bacteriophage T1 adsorbed to Escherichia coli B after plasmolysis in 20% sucrose. The virus-cell mixture was kept for 8 min at 37 C before fixation in formaldehyde, OsO_4-uranyl acetate, embedding in Vestopal, and staining of the section in uranyl acetate and lead hydroxide. This fixation and staining procedure applies for all of the micrographs.* × *160,000. The bar represents 1,000 A in all figures.*

The various bacteriophages studied in our experiments (T1 to T7) were usually seen to adsorb to those areas of the cell surface at which a protoplasmic duct terminated at the inner side of the wall (Fig. 1–10b). The morphology of these areas of the cell wall is described in a separate report. If one considers the restricted diameters of such contact areas (i.e., 200 to 250 A), one would expect instances in which the ultrathin section, although showing the adsorbed phage, would have missed most of the contact area. Such incidents occurred indeed (Fig. 4 and 5) but with a rather low frequency: of 100 phages (T5) seen adsorbed (i.e., with close wall contact), 21 tail tips did seem to have attached to an area other than a wall-membrane association or the attachment seemed only *near* such a site; 79 were clearly attached to the areas at which a protoplasmic duct was in contact with the wall. In ultrathin serial sections through cells with virus apparently adsorbed to an area without a wall-

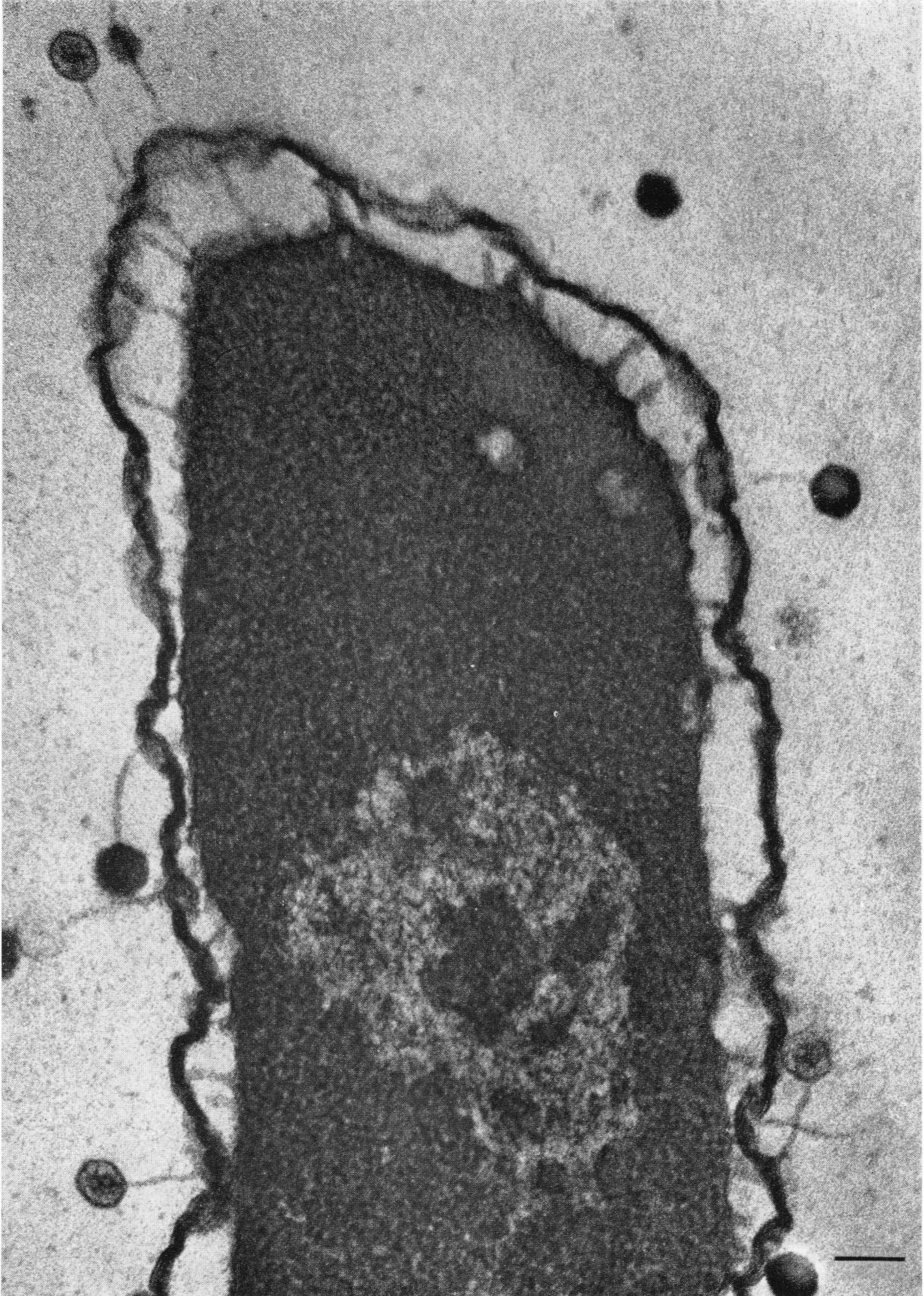

FIG. 3. *Larger area of a plasmolyzed cell to which T5 had been adsorbed for 3 min with a multiplicity of infection of 1,000.* × *103,000.*

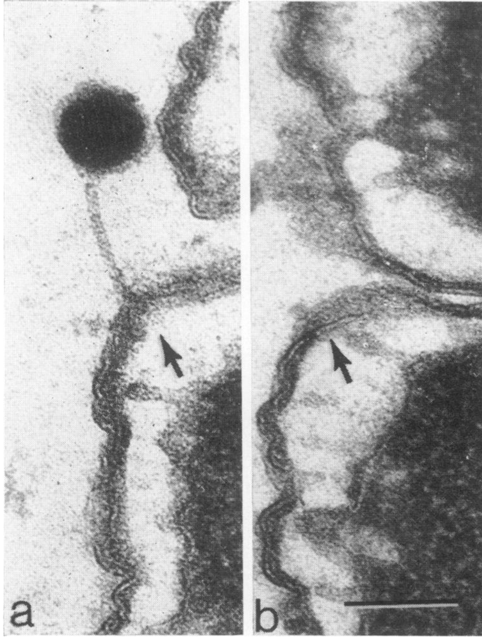

FIG. 4. *T5 adsorbed to a wall area at which the wall-membrane association becomes visible in the neighboring section (b). × 150,000.*

membrane association (Fig. 4), the contact zone of wall and membrane, not being visible in one section, became visible in one of the two neighboring sections; in these sections, the section plane showing the tail tip of the phage did not include the neighboring area of a wall-membrane association owing to an undulating cell surface. Therefore, the conclusion appears to be justified that probably all of the phage (T5) were adsorbed to areas of membrane contact. With regard to the various morphological "types" of wall-membrane associations, bacteriophages T3 and T5 did not seem to preferentially adsorb to one of the "types." Of a total of 60 adsorbed T5 particles, about 60% were found at sites at which the protoplasmic duct terminates at a funnel-like wall invagination, whereas about 20% were found at sites at which the wall does not curve in (Fig. 3 and 4a). Of 45 T3, 60% occupied sites of the former type (Fig. 10a) and 27% occupied sites of the latter type. About two-thirds of the wall-membrane associations showed a funnel-like invagination of the wall, whereas the remainder did not. These differences in the appearance of the wall-membrane complex did not seem to determine the specificity of the adsorption sites, at least with regard to T3 and T5.

When the bacteriophages (T2 and T5) were first adsorbed to the cells and the cells were then plasmolyzed, the phages were seen to have adsorbed in the same manner and to the same areas as just described for the reverse procedure. An adsorption before plasmolysis of the cells was the only way to show a higher number of T4 particles attached to the cell wall, since this virus did not adsorb in solutions of 20% sucrose and only to a small extent in 10% sucrose (Bayer and Anderson, *in preparation*).

A saturating adsorption of phages to all accessible receptors on the bacterial surface cannot be expected in these experiments for technical

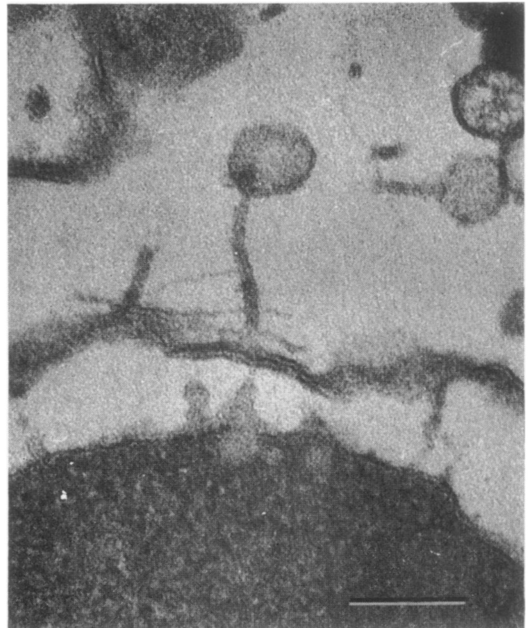

FIG. 5. *T5 adsorbed to a plasmolyzed cell; fibers are associated with the tails. × 150,000.*

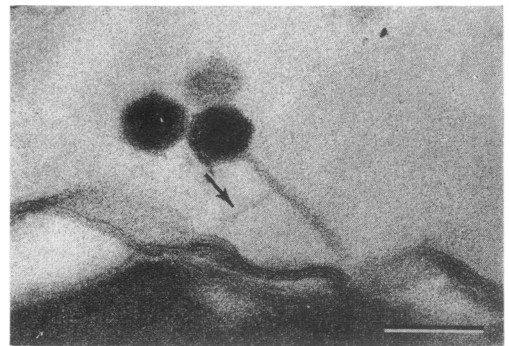

FIG. 6. *T5, showing thin fiber attached to the wall × 120,000.*

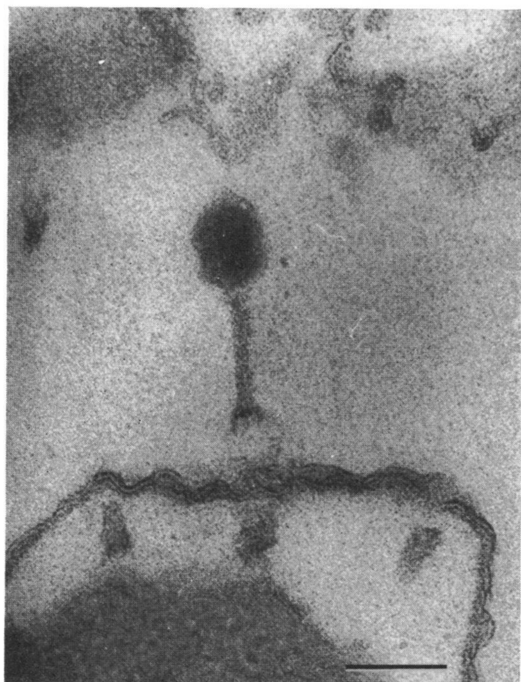

FIG. 7. *T6 in the proximity of a wall-membrane association.* × *160,000.*

reasons. Because of an active reversion of plasmolysis by the cells (4), an adsorption time of 2 or 4 min, during which the cells remain clearly plasmolyzed, limits the number of adsorbed phages: assuming an adsorption rate constant for T2 of 6.4×10^{-9} ml/min (1), and using virus concentrations of 1.5×10^{10}/ml and cell concentrations of 1.5×10^{8} to 2×10^{8}, one would expect 50 to 80 adsorbed phages per cell (*see* Discussion). A higher multiplicity of infection of T2, T4, or T6 would cause a breakdown of the osmotic barrier and would, eventually, lead to lysis from without. In the case of T5, multiplicities of 10^{3} have occasionally been used, but the adsorption rate of T5 appears to be slower than that of T2. We found a k value of 2.3×10^{-11} ml/min for T5. Since saturation cannot be expected in our adsorption system, many of the potential receptor sites are expected to remain unoccupied.

In spite of the dehydrating effects of sucrose in concentrations of 10 and 20%, the dimensions of the bacteriophages and of some of their organelles did not seem to differ from those of controls without sucrose, and both agreed with previous reports (28). However, detail in some of the structures was lost: cross "striations" of the contractile tail sheaths of the T-even phages were difficult to observe, and the tail fibers of these phages were often invisible.

Despite the many similarities in the mode of virus adsorption to the areas of wall-membrane complexes, the various types of bacteriophages tested exhibit during adsorption some special features which are a consequence of morphological and physiological differences among the phage strains.

T1. Although T1 showed a plating efficiency of 100% in 20% sucrose, it was difficult to keep it adsorbed during our fixation and embedding procedures and only 1 to 5% of the expected number of virus particles per section were found attached to the cells. These particles had their flexible tails attached to the areas of wall-membrane association. The tails often appeared longer than normal or otherwise distorted. Most of their heads were empty; only occasionally were full heads seen (Fig. 1). This can be explained by the prolonged adsorption time of 8 min, enough time for the injection of the DNA of most of the particles.

T5. This virus was studied rather extensively during our investigation. Like T1, the virus adsorbs with the tip of its flexible tail (Fig. 2). In a few cases, two phages were seen to have attached to the same area of the wall. The heads of the virus particles were found to be in all stages between "filled" or "empty" (Fig. 3). Occasionally, the flexible tail of the phage showed fibers which seemed to be intimately fused with

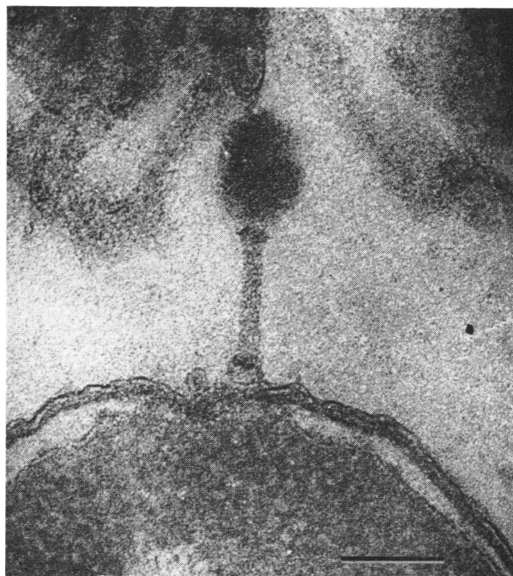

FIG. 8. *T6 adsorbed to cell wall with its short fiber elements or "pins."* × *160,000.*

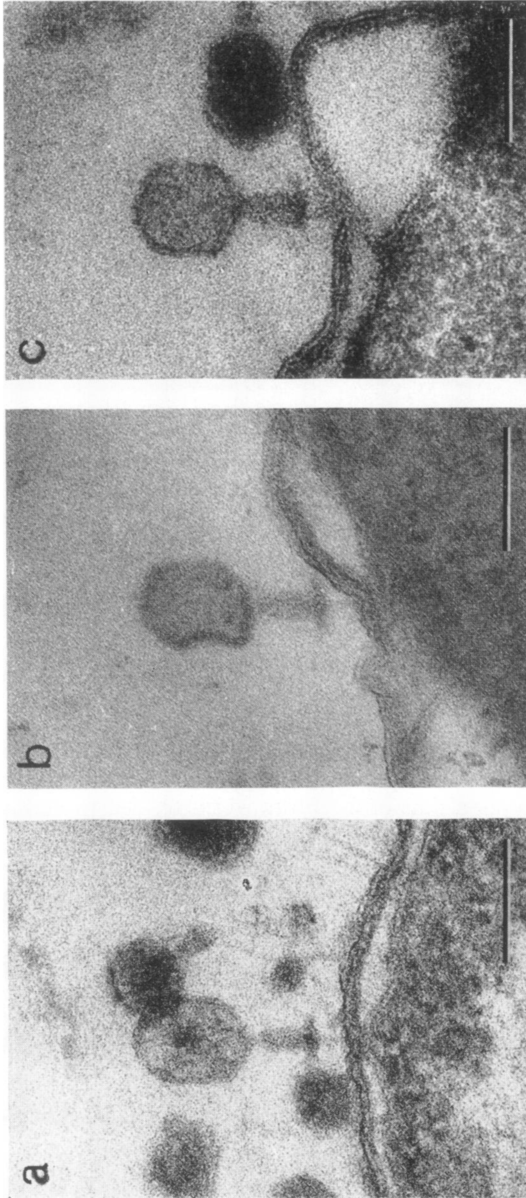

FIG. 9. (*a*) T2 after injection of its DNA; the phage is still adsorbed to the wall. The needle points to a wall-membrane association. × 160,000. (*b*) T4 adsorbed for 2 min before the cell had been plasmolyzed in sucrose and then fixed. × 160,000. (*c*) T6 after "injection."

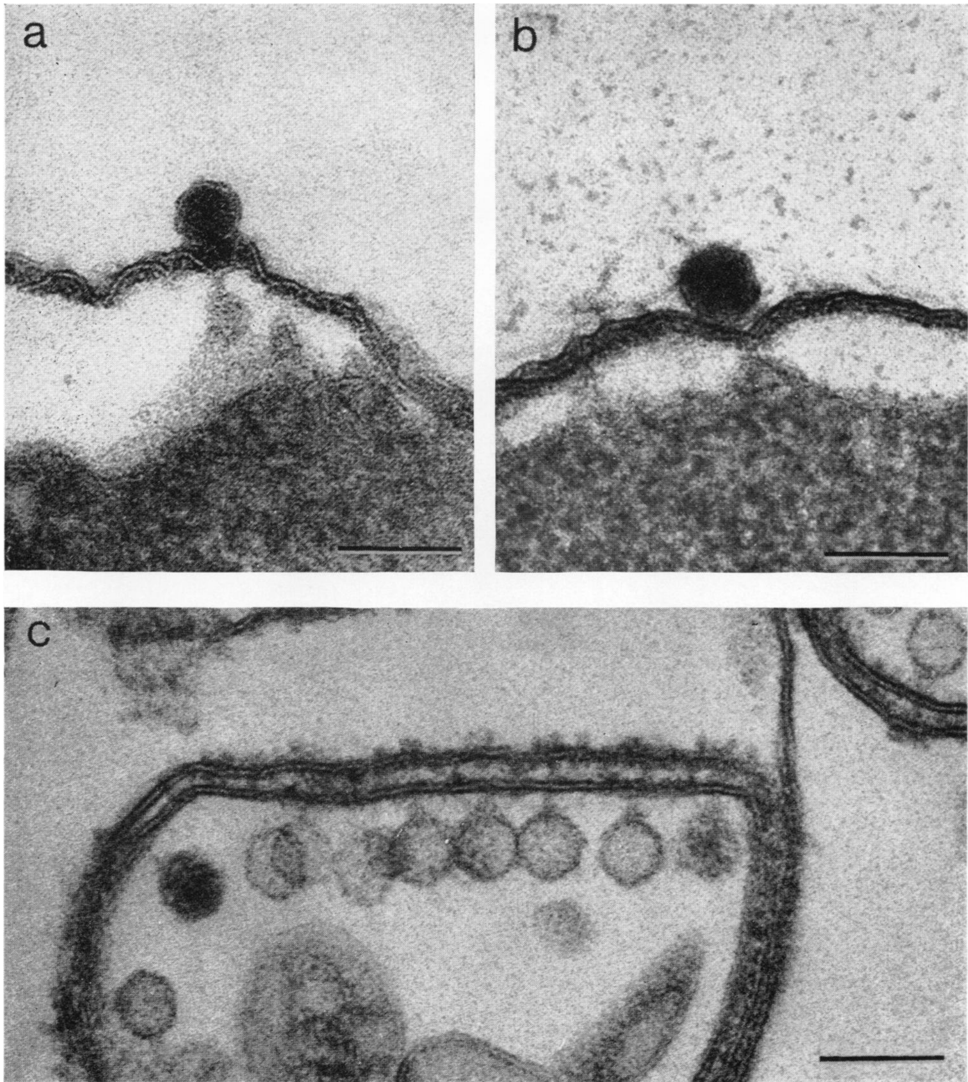

FIG. 10. (*a*) *T3 adsorbed to a wall-membrane association.* × *160,000.* (*b*) *T7 adsorbed to a wall-membrane association.* × *160,000.* (*c*) *A number of T3 virions adsorbed to the wall of a lysed cell.* × *160,000.*

the structure of the tail (Fig. 5); they were seen either attached to the wall or sometimes pointing in a direction more or less parallel to the cell surface (Fig. 5). The fibers seemed to be in contact with the phage tail at any location between tail tip and phage head (Fig. 5 and 6). The chemical nature (e.g., tail protein or free DNA) of the fibers had not been established. When the fibers were seen adsorbed to the cell, they were stretched obliquely from the tail and adsorbed at areas distant from the zone where the tail tip had attached (Fig. 6).

T2, T4, and T6. These phages are treated together because of their many morphological similarities. Having a contractile sheath, this group engages in a number of successive events during adsorption and infection which have been recently described by Simon and Anderson (26, 27). T6 could be seen oriented toward its prospective site of adsorption when the tip of its tail was about 600 A away from the bacterial surface (Fig. 7). A following step involves the approach of the end plate to about 130 A from the wall (Fig. 8). Short "pins" of 120 to 130 A length seem to have made contact with the wall surface. When the sheath has contracted, the end plate is found

about 260 to 300 A from the outer contour of the wall. The needle or core is seen to have aimed at the wall-membrane complex [Fig. 9a (T2), Fig. 9b (T4), Fig. 9c (T6)]. In this state of adsorption, the heads are "empty."

T3 and T7. Although smaller than the phages described above, T3 and T7 particles that still contain their DNA are rather easily found in sections because of their high contrast. When adsorbed, these phages form a close contact with the wall-membrane association (Fig. 10a, b). The depth of "penetration" is not measurable because of a lack of discernible structures at the contact area. However, the conical promontories on these phages are evidently the organelles by which they attach to fragments of the cell wall shown in Fig. 10c. This figure also shows a phenomenon which can regularly be observed in the crude lysates of phages T2, T3, T4, T6, and T7. The wall residues are often densely "coated" with phage particles, as though the viruses had been packed with sterically closest proximity.

Discussion

The fact that the various phages attached to the sites of wall-membrane association leads me to assume that the receptors are exposed at these areas. To evaluate such a model, one has to discuss the validity of the various data and estimate the errors associated with the methods. The number and appearance of the wall-membrane associations seem unchanged whether or not phages have been adsorbed. This fact excludes the possibility that adsorbed phages might cause the adhesion of membrane and wall. The question could be raised as to what extent phages might be desorbed during fixation and embedding, and as to whether such a desorption occurs selectively by leaving only those phages adsorbed which are attached to a wall-membrane association, whereas virus particles adsorbed to other areas of the wall are desorbed. Such a desorption during fixation and embedding was observed with T1 only, although the plating efficiency of T1 in sucrose was not reduced. Among those few T1 particles found in thin sections, very rarely did one observe "full" heads as seen in Fig. 1; this could be interpreted by assuming that especially the early stage of adsorption is easily reversible. The reason for a desorption could very well be the partial destruction of T1 receptor sites by formaldehyde as described by Tolmach and Puck (29). Thus, a quantitative estimation of the number of T1 phage remaining adsorbed during fixation, dehydration, and embedding has presently to be left open.

A selective desorption or any other mechanism by which bacteriophages could escape observation in the embedded state would be expressed in a reduction of the number of phages in the ultrathin sections. To evaluate this possibility, one has to compare the number of virus particles per cell which are seen in ultrathin sections in the adsorbed state and the number of phages theoretically expected to have adsorbed under these experimental conditions. The equations of Krueger (15) and Schlesinger (24) describe the adsorption kinetics for bacteriophage; their application to our systems reveals the following data: the number of phage T2 (1.5×10^{10}/ml) adsorbed per bacterial cell (1.5×10^{8}/ml) in 3 min would be 94 when one assumes a value of 6.4×10^{-9} ml/min (1) for the adsorption rate constant k. When a more or less cylindrical cell, 2 μ long, is sectioned along its main axis with a section thickness of 600 A, one obtains about 33 cross sections. The number of phage visible per cross section would then be $94/33 = 2.8$; I found 1.2 phages per cross-section. For T3, the expected value is 36 phages per cell after 3 min of adsorption [5×10^{9} phage/ml and 5×10^{7} cells/ml assuming a k value of 3×10^{-9} ml/min (21)], i.e., 1.1 phages per section. I counted 0.36. However, I found in preliminary studies that the k values in sucrose seemed to be lower than in L-broth. For T2, the value was about 60% of the controls; for T3, the value was about 15% of the controls. Reduction of the k value would reduce the expected number of adsorbed phage to a value closer to the microscopic observations.

For computation of the total number of viruses per cell from the number of adsorbed viruses visible in the ultrathin sections, the section thickness has to be determined. The thickness is derived from the interference color of the section. However, at the present time, an error of about 30% in the section thickness might be expected, since the distinction between "gray-colored" and "silver-colored" sometimes proves to be difficult.

Since plasmolyzed cells are actively reverting the state of plasmolysis by increasing their osmotic pressure, there is not enough time available to saturate all of the receptor sites on the cell surface with viruses. Thus, the number of unoccupied receptor sites will always exceed the number of sites having viruses adsorbed. Considering the receptor capacity of a cell being determined by the number of wall-membrane associations, the following values can be compared. A logarithmically growing *E. coli* cell contains 200 to 400 wall-membrane associations. The saturation capacity of *E. coli* seems to match this number, as determined by other investigators. Watson (30) found a maximum value of 300 T2 adsorbed to heat-killed *E. coli* B; Delbrück (7) found a saturation value of 250 phage ("B2" and

"P2") per *E. coli*; Schlesinger (24, 25) counted 300 WLL phage (T-even) per *E. coli*. If one assumes that this number of receptors is also applicable to the other phages which adsorb to the wall of *E. coli* B, one also has to assume a sharing of receptor sites per individual wall-membrane association. A sharing of receptors can be demonstrated by first saturating the receptors with one phage and afterwards exposing these cells to another phage. An adsorption inhibition of the second phage should result. That this is in fact the case for T2 and T6 has been demonstrated by Watson (30), who found that cells when saturated with T2 did not adsorb T6; both phage strains had the same adsorption rate when tested alone. The result was the same when X ray-inactivated T2 was used for coating the T6 receptors. However, one has to take into consideration that 300 of the relatively large T-even phages will occupy probably more than half of the cell surface, thereby at least partially excluding those phages which have been added later. This investigation on the early stages of adsorption avoids this problem of "crowding" and leaves enough space for phages to approach the receptor sites more or less independently of sterical hindrance. There exists some additional support for the theory of sharing the receptive areas derived from observations on combinations of phage resistance of *E. coli* (L. E. Jackson et al., Bacteriol. Proc., p. 27, 1967). These authors propose that T2, T3, T4, T6, and T7 "may share a common or closely linked attachment locus on the bacterial surface."

Each of the areas of wall-membrane association, therefore, could tentatively be conceived as an array of different receptor sites. The arrangement of the receptors on such a wall area would have to meet the adsorption requirements of every phage strain which adsorbs on the wall. This leads to an interesting aspect: the receptors must be displayed in a spatially defined pattern or mosaic. When phages equipped with a large adsorption apparatus attach to a cell with their long tail fibers, the fibers adsorb to an area about 700 A from the center of the "needle" (26, 27), considerably farther outside of the area of subsequent injection than the receptor areas for smaller phages such as T3, T7, or P22. The end plate of T3, T7, and P22 by which these phages attach consists of rather short elements only. Without these elements, the phage is unable to adsorb at all (12). The lateral distance from the "pins" of the adsorption apparatus to the central axis of the T3 or T7 virus [its neck or conical element through which the phage DNA is probably injected—or ingested (10)], measures about 100 A. In conclusion, the zone on the wall of *E. coli* exhibiting various receptors could tentatively be described as a mosaic or a display of spatially separated receptor sites exposed around a central area at which the phages would inject their DNA. This central portion, the "bull's eye," would then be the entrance through which the nucleic acid is passed into the protoplasm. In a plasmolyzed cell, the passageway to the protoplasm would be longer and would consist of the hollow "duct" formed by the extension of the protoplasmic membrane at the site of a wall-membrane association.

If one considers the areas of wall-membrane contact as the only places where the receptors are normally exposed, and where synthesis of wall substance possibly occurs, the assumption can be made that in "older" areas of the wall the receptor sites are masked and remain inaccessible until they are uncovered by an enzymatic or chemical attack. This would explain the observation that wall residues in lysates frequently show wide areas more or less evenly coated with phage which have their DNA released (Fig. 10c). On an intact wall, however, a phage would adsorb to an area of wall-membrane association and would thus have the "advantage" of releasing its nucleic acid closest to the protoplasmic contents.

Rapid degeneration of the osmotic barrier of the plasma membrane occurs with MOI of around 100 T-even phages per cell, so that the state of plasmolysis is difficult if not impossible to maintain. Whether this indicates the direct puncture of the protoplasmic membrane by the "needle" of the phage cannot be decided, since the inner contour of the wall and the structure of the membrane seem to fuse at the locations of wall-membrane association, the areas to which the phage adsorb. A leakage through the membrane could be caused by an (enzymatic) alteration, degradation, or removal of a small portion of the membrane without a need for a mechanical device to penetrate the membrane. Thus, the mode of bacterial uptake of nucleic acid molecules from phages might possibly involve the aid of additional mechanisms of active transport (10).

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