FINE STRUCTURE OF THE BACTERIOPHAGE ATTACHMENT PROCESS

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ABSTRACT

COTA-ROBLES, E. H. (University of California, Riverside) AND M. D. COFFMAN. Fine structure of the bacteriophage attachment process. J. Bacteriol. **86**:266–273. 1963.—Electron microscopic examination of cells of *Escherichia coli* infected with coliphage T_2 suggested that the tail spikes of the virus attach to the cell wall. This is followed by a local disorganization of the cell wall and contraction of the virus tail resulting in the approximation of the virus to the cell. Ejection of the viral contents is followed by a retraction of the attached viral ghost from the infected cell. The bacterial cells reveal a fibrillar nuclear structure which in some cases surrounds material that appears to be of cytoplasmic origin.

The elaborate infective mechanism which bacterial viruses possess has been clarified by chemical analysis (Hershey and Chase, 1952; Barrington and Kozloff, 1956; Koch and Weidel, 1956) and by electron microscopic investigations (Anderson, 1952; Kellenberger and Arber, 1955; Brenner et al., 1959). However, as Kellenberger (1961) re-emphasized, the idea that bacteriophage acts as a hypodermic syringe has not been conclusively proven. There is no question that the phage tail sheath can contract. However, a question still remains concerning the precise association between the infecting phage and the cell wall.

Among the most revealing electron micrographs of thin sections of phage-infected bacteria that have yet been published are those of Mercer (1959). Unfortunately, the most striking example of a virus demonstrating a contracted tail did not show the virus attached to a whole cell. In this report, we present electron micrographs of thin sections of cells of *Escherichia coli* B infected with coliphage T_2. From our observations, we propose that little of the bacteriophage tail penetrates through the cell wall during infection.

MATERIALS AND METHODS

E. coli B was routinely cultured on Fraser and Jerrel's (1953) glycerol medium aerobically at 37 C. The cells to be infected with bacteriophage were harvested during the log phase of growth at a population density of 4×10^8 cells/ml. These cells were washed once with buffer (Cota-Robles, 1963) and suspended in buffer containing 0.35 M sucrose. The hypertonicity of the suspending medium effected plasmolysis of the bacterial cells which, although it does not pertain directly to this report, proved to be a useful tool. The usefulness of this tool was twofold: first, it permitted greater clarity in observation of viruses attached to the cell wall; and second, it presented a defined structure, the cell wall, that could be readily utilized as an aid in focusing at high magnifications.

Coliphage T_2 was added to the plasmolyzed bacteria at a multiplicity of 10 to 40 virus particles per cell. The viruses appear to attach to plasmolyzed cells almost as efficiently as they do to normal cells. Plasmolyzed cells infected with coliphage T_2 suffer the lethal effects of the virus. The high multiplicity was used to increase the likelihood of obtaining sections containing viruses attached to cells. The infection was permitted to proceed for from 4 to 8 min at 25 C in different experiments. During each experiment, infection was timed so that it preceded lysis from without by several minutes. Lysis from without was detected by phase-microscopic examination. The reaction was stopped by the addition of sufficient formaldehyde to yield a final concentration of the fixative of 3.7% (10% formalin). Fixation with formalin was permitted to proceed for 1 hr at 25 C. This mixture was then centrifuged and post-fixed with $OsSO_4$ according to the method of Kellenberger, Ryter, and Séchaud (1958). The infected cells were then subjected to the entire treatment utilized by these workers, including embedding in vestopal W. The only variation utilized was the handling of the cells

as pellets obtained by centrifugation, rather than as cells encased in agar blocks.

Sections were cut with glass knives on either a Porter-Blum microtome or a LKB Ultratome. The sections were collected on unsupported grids and post-stained with lead hydroxide according to the method of Watson (1958). Electron micrographs were taken with a Hitachi HU 11 electron microscope at accelerating voltages of either 50 or 75 kv.

RESULTS

Figure 1 depicts a thin section through a plasmolyzed cell of *E. coli* B infected with T_2. The virus is firmly attached to the cell wall. An unusual pattern can be seen at the point of union between the virus and cell wall. There is a suggestion that the outermost layer of the cell wall has split and that this separated layer surrounds a small but discrete vesicle. This small vesicle, which measures approximately 70 A, is an unknown entity. The head of the attached virus is extremely electron-dense and the tail shows no evidence of contraction, indicating that the virus has not injected its deoxyribonucleic acid (DNA). The distance from the head of the virus to the cell wall proves to be 1,100 A, a value that corresponds quite closely with the recognized value for the total length of the tail of T_2 (Kellenberger, 1961). The dimensions of the phage head approximate 620 × 870 A. The cell presented in this photograph contains a well-defined nuclear region.

The dividing cell presented in Fig. 2 can be seen to be infected with at least two viruses (1, 2). Both of these viruses reveal a suggestion of a base plate with projections leading to the cell wall. One of the viruses (2) is empty, except for a slight amount of electron-dense material. There is, furthermore, a suggestion that the tail

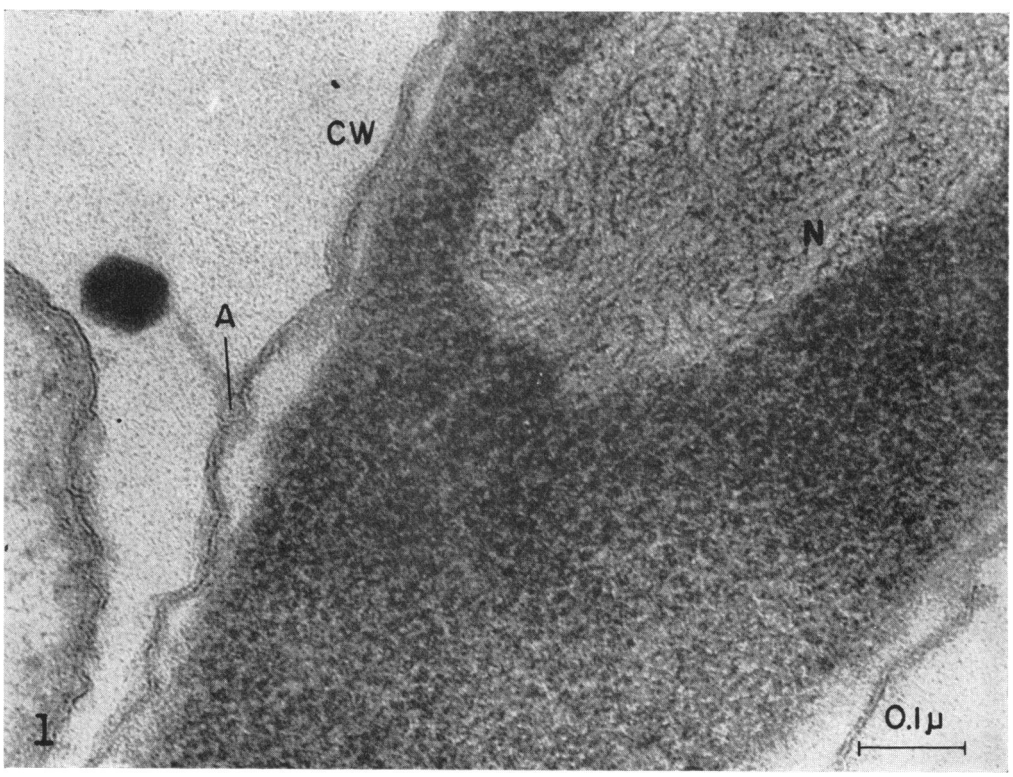

FIG. 1. *Thin section of a cell of Escherichia coli showing an intact coliphage T_2 attached to the cell wall. Note the unusual ultrastructure at the site of virus attachment (A). The nucleus (N) of the cell shows a complex arrangement of fibers. The cell wall (CW) reveals the triplex structure characteristic of gram-negative bacteria.*

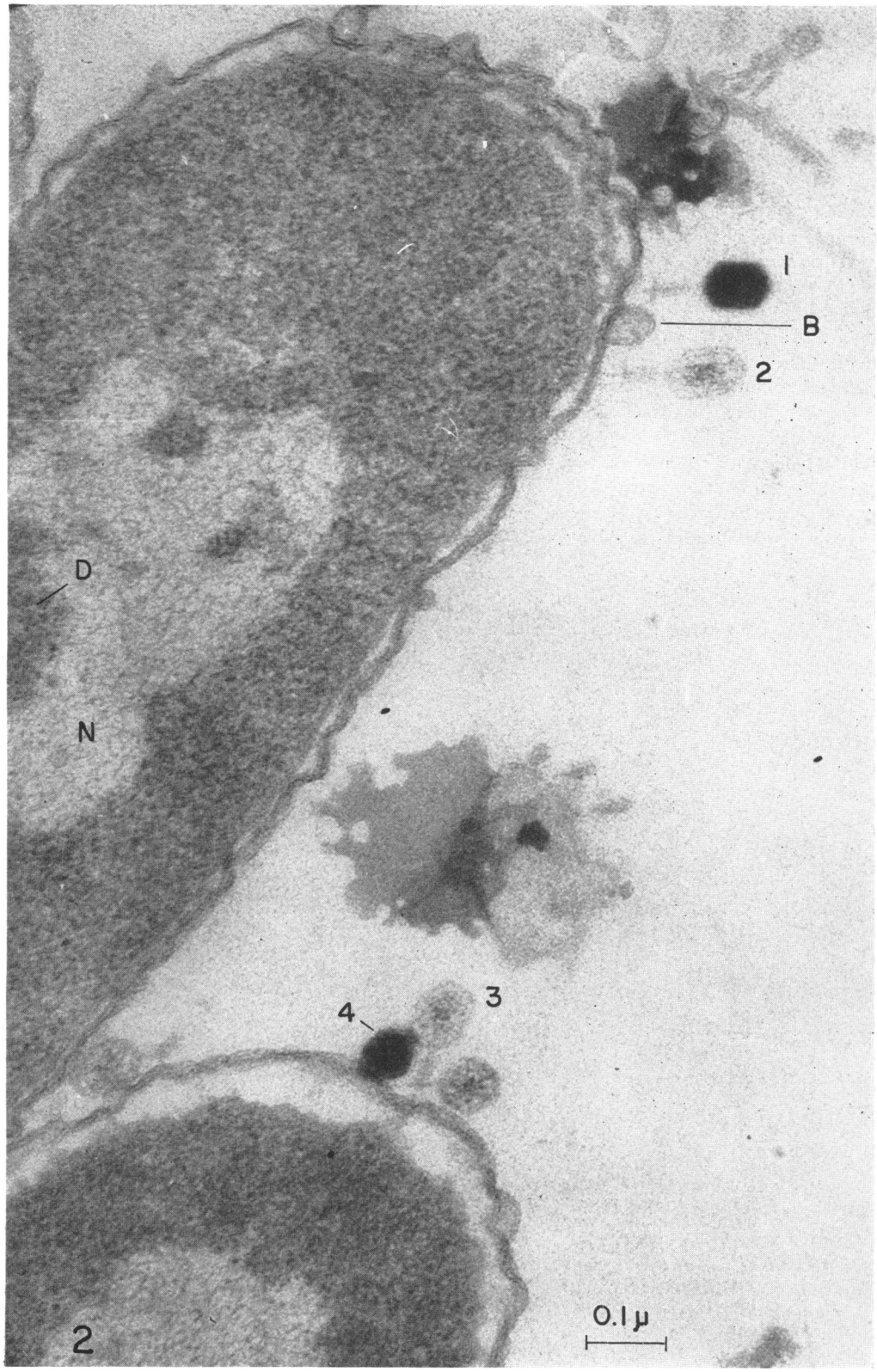

FIG. 2.

of this virus has contracted. We assume that this virus has injected its DNA. However, there does exist the possibility that this virus was devoid of its DNA prior to attachment. We do not wish to belabor this point, but if this virus had injected its DNA into the cell, it would appear unlikely that the phage tail penetrates the cell wall to any appreciable degree. In addition, the dimensions of the head are larger than those of the neighboring intact virus (1), a fact that would appear contradictory to the reports of Cummings and Kozloff (1962) that the head of coliphage T_2 must be in its short form in order to permit ejection of DNA. Even though this photograph cannot resolve these problems, it does suggest that the virus head has a double-layered external covering and that the base plate may be resolved (albeit poorly) in thin sections. A curious bleb (B) suggestive of wall material can be seen between the tails of these two viruses. The nature of this bleb is not established, but its appearance is not unique in our micrographs. More recent observations suggest this bleb may be wall material.

The dividing cell depicted in Fig. 2 has two well-defined nuclear areas. However, these areas do not present a homogeneous appearance. In fact, areas of considerable electron density are readily observed within these nuclear regions. The density and organization of these isolated zones resemble quite closely the density and organization of the cytoplasm. This observation is not an unique one, since Chapman and Hillier (1953) reported similar inclusions in nuclear sites of *Bacillus cereus*.

Figure 2 presents a transverse section through a cell of *E. coli* which has several interesting viral associations. One of these is exemplified by the ghosted virus (3) which appears to be attached via its tail to the cell wall, but which in addition has a particle (4) of extreme electron density in close association with its head and with the cell wall. The marked electron density of this particle suggests that it could be the DNA of a virus. We cannot decide on the basis of a single micrograph what the nature of this particle is, but we currently feel that it is a second virus sectioned through an unusual angle. There does exist the possibility that this particle does originate from the ghosted virus (3), but, if the particle is DNA from the ghosted virus, it did not exit through the tail but rather through the head.

We feel that the virus presented in the first two micrographs suggests that an infecting virus does not come exceedingly close to the host cell. This does not mean that we have not seen an occasional virus which may be in close apposition to the cell. However, the numerous observations we have made conform generally to those depicted above. Figure 3 is but another example of what we interpret as the general pattern of infection of *E. coli* B by coliphage T_2. Here a ghosted virus can be seen to reside some distance from its host cell.

We have presented evidence that a ghosted virus may not be in very close association with the cell. However, we have not presented convincing evidence that a virus with a discernibly contracted tail does not project into the cell. We feel that this point is clearly decided in Fig. 4. Here the ghosted virus contains a tail that is markedly contracted. A neck region is discernible, as is a region of marked density, which we assume to be the base plate. Our calculations show this base plate to be 50 A from the cell wall proper. The dimensions of the head of the ghosted virus are essentially identical with those of an intact virus. However, a central ridge which is not usually resolved can be readily observed. The head of this virus closely resembles those described by Cummings and Kozloff (1962; Plate I, Fig. C).

The cell presented in Fig. 4 contains a well-defined nuclear region within which one can observe areas of marked electron density. In addition, this cell contains one well-defined, membrane-delimited "vesicle." The term vesicle is used advisedly here, since the structure in

FIG. 2. *Section through a dividing cell of Escherichia coli infected with at least two viral particles. The first virus (1) still retains its DNA and appears to be attached by its tail spikes to the cell wall (CW). Its tail is not noticeably contracted. One of its tail spikes is attached to a bleb (B) of wall material. The second virus (2) is virtually completely ghosted and its tail appears to be in a contracted state. The ghost wall appears to be composed of a double-layered structure. The cell nucleus (N) is fibrillar but contains areas of unusual electron density (D). Other attached virus can be seen in this photograph. One virus (3) is in close association with an electron-dense particle (4).*

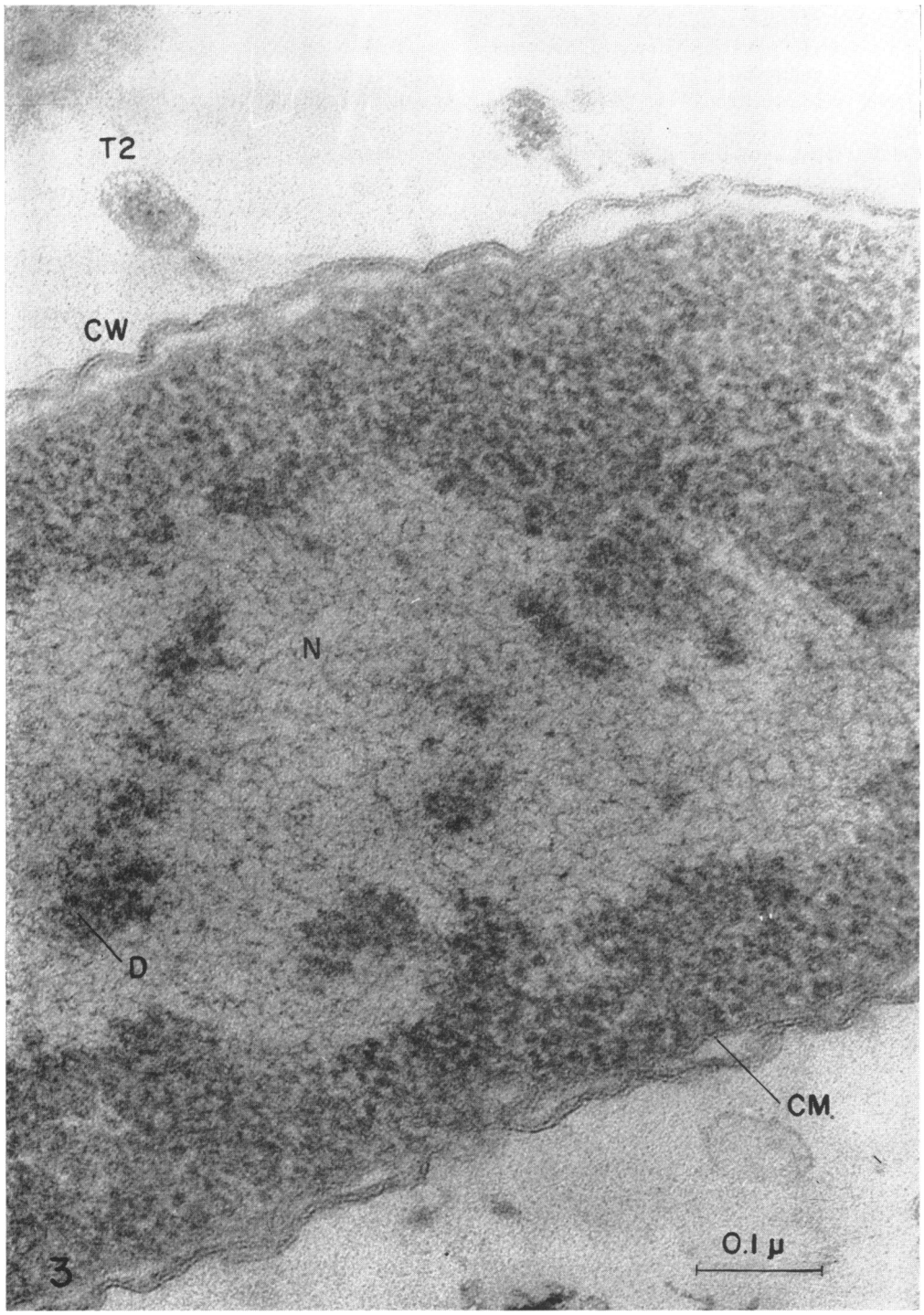

FIG. 3. *High-magnification electron micrograph of a cell of Escherichia coli infected with coliphage T_2 (T2). The attached virus appears to have a contracted tail, yet the virus head retains considerable electron density. The fibrillar nucleus (N) contains areas of an electron density (D) that closely resemble the electron density of the cytoplasm. The cell membrane (CM) can be resolved as a triplex structure.*

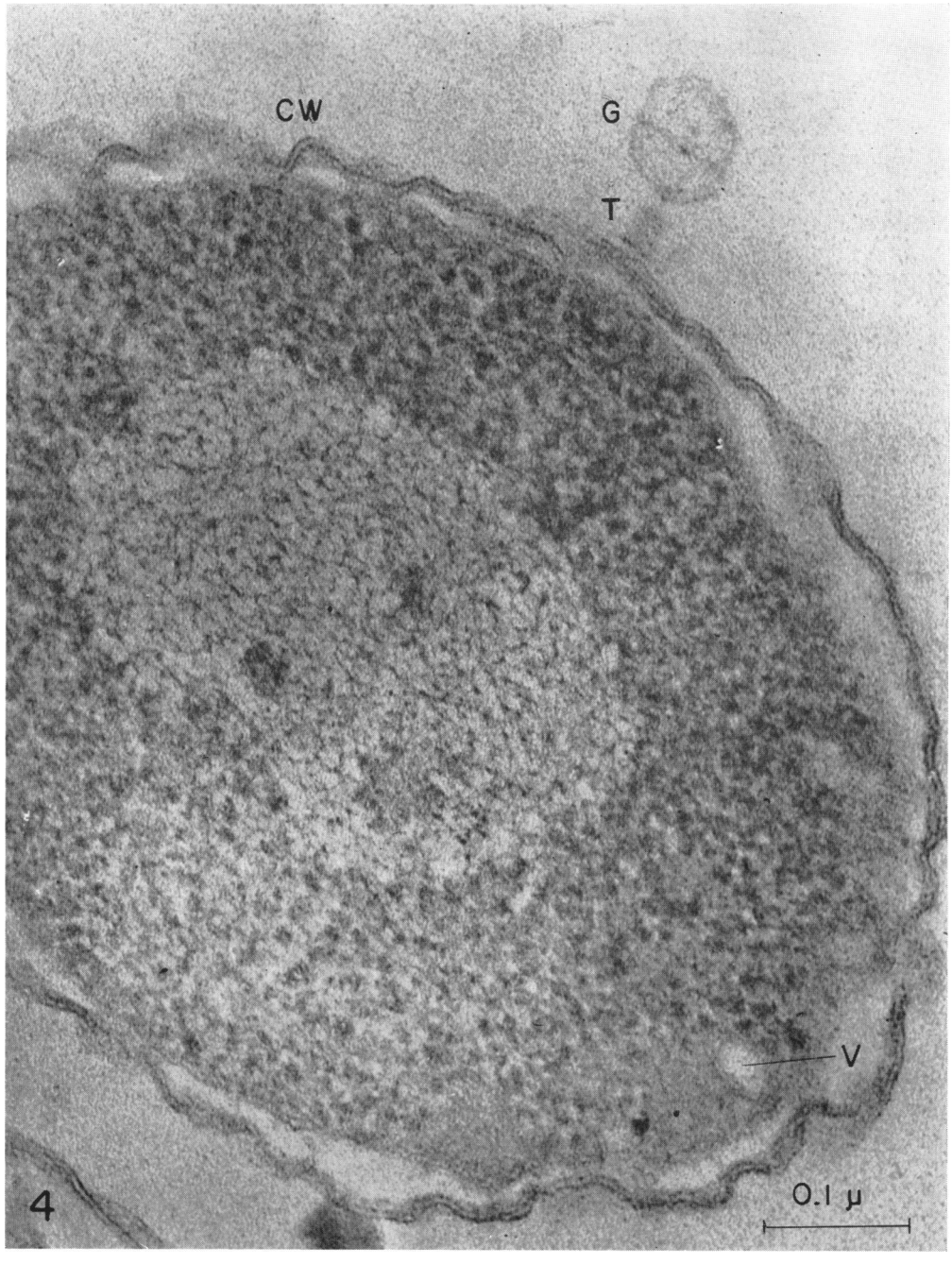

FIG. 4. *Thin section of a cell of Escherichia coli showing an attached ghost (G) of coliphage T2. The viral ghost shows a markedly contracted tail (T). The distal portion of this tail appears to be the base plate. The distance from this base plate to the cell wall is approximately 50 A. The nucleus again presents a complex fibrillar arrangement with areas of unusual electron density. A membrane-bounded vesicle (V) can be resolved. The area of cytoplasm that borders this vesicle appears to be agranular. This photograph was made from a negative of uneven density by the masking method of Gonzales (1962).*

question appears to be an inward loop of the cell's cytoplasmic membrane and not an independent vesicle.

DISCUSSION

The electron micrographs presented in this article demonstrate quite clearly that the heads of the strain of coliphage T_2 that we have studied are uniform in size. The size is not altered appreciably after injection of DNA into the cell. Even though this be true, after injection a central ridge in the head of the virus may become evident. The origin of this ridge is uncertain. It seems unlikely that it is an artifact. The reason we have not seen it more often in our photographs may be strictly a technical one associated with sectioning or microscopy. On the other hand, it could be absent if the phage were ghosted by osmotic shock rather than by injection of DNA into the host cell, a situation that might not involve a specific alteration of the morphology of the phage head. A third possibility may be related to the extent of DNA ejection at the time of fixation. Several ghosts of T_2 that we present in this paper appear to contain some internal electron-dense material. This material could be masking the central ridge. We feel that any or all of these three conditions could explain why we have resolved this central ridge so rarely. Furthermore, our observations indicate that the strain of coliphage T_2 that we have studied does not possess heads of two vastly different lengths.

Our observations suggest that an opening occurs in the cell wall prior to contraction of the tail of the virus. Furthermore, our photographs suggest that the tail spikes may be the integral units of attachment. The virus tail does contract following attachment, but it does not appear to penetrate the wall to any appreciable degree. In fact, among our observations, the minimal distance between the major bulk of the phage tail and the cell wall was never less than 50 A.

Our reconstruction of the sequence of events during bacteriophage infection is based on observations similar to those described in Fig. 2, 1, 4, and 3. The virus attaches to the cell wall via tail projections (Fig. 2), causing a local disorganization of the wall (Fig. 1). The tail of the virus contracts. The viral DNA is injected (Fig. 4). The virus ghost retracts from the wall but still retains its attachment (Fig. 2, virus 2).

An ancillary aspect of these studies concerns itself with the nucleus of *E. coli*. Our photographs reveal that the nuclear region contains fibrous strands which are quite similar to those presented by Robinow (1962) in his recent review. As Robinow pointed out, little has been learned of the morphology of replication of bacterial nuclear material. Murray (1960) and Kellenberger (1959), as well as Giesbrecht (1958), do offer some suggestions about the organization of the bacterial nuclear material. Our photographs touch upon this point. Even though our observations are scanty and are not based upon examination of serial sections, it appears to us that the nucleus of *E. coli* is not a compact body. Rather, we visualize it as an intricately organized fibrous structure into which cytoplasmic elements project. Thus, a given section through a nucleus may reveal cytoplasmic components that appear to be embedded within nuclear material (Fig. 2, longitudinal section). Similarly, a section through a cell could reveal a single nuclear structure that contained no cytoplasmic component (Fig. 2, transverse section).

All available evidence indicates that the bacterial nucleus is devoid of a personal limiting membrane. Our observations suggest that the nucleus may be held in place by the surrounding cytoplasm. However, we do not feel that this indicates a nonspecific property of the bacterial cytoplasm, but rather that it underscores the complex organization of the bacterial cell. We have recently observed that treatment of *E. coli* cells with toluene not only results in the disorganization of the cytoplasmic membrane but of the nucleus as well (Cota-Robles and Coffman, *in preparation*). This action of toluene upon the cytoplasmic membrane is not unexpected. The action upon nuclear organization is not expected, and it may presage a close association between lipid components and integrity of the bacterial nucleus.

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