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LPP-1 Infection of the Blue-Green Alga *Plectonema boryanum*

I. Electron Microscopy

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One-step growth and intracellular growth experiments were performed at high multiplicities of the virus LPP-1 during the infection of the blue-green alga *Plectonema boryanum*. The eclipse period lasts until 4 hr after infection, the latent period terminates at 6 hr, and the rise period continues until 14 to 16 hr after infection. The burst size was independent of multiplicity of infection over the ranges from 1 to 50. The burst size was 3,000 to 5,000 plaque-forming units (PFU) per infectious center or about 200 PFU per cell. Samples for electron microscopy were taken at characteristic times during the lytic cycle. The first sign of viral infection was the invagination of the photosynthetic lamellae at 3 hr after infection. Mature virions were visible at 4 hr. By 6 to 7 hr, many mature intracellular viral particles could be seen, with lysis beginning at 7 hr. By 10 hr after infection, all infected cells contained mature virions. No evidence for mass migration of preformed viral precursors was obtained. The invagination of the lamellae could be prevented by the early addition of chloramphenicol, which implies that this process requires protein synthesis.

A number of one-step growth experiments have been performed after infection with the blue-green algae virus LPP-1. A latent period of 7 hr and a burst size of 100 plaque-forming units (PFU)/cell was found by Goldstein et al. (6) by using LPP-1 at a multiplicity of infection (MOI) of 0.02 per cell. Ginzburg et al. (5) obtained identical results with LPP-1G at an MOI of about 0.07. The work on LPP-1G was recently extended by Padan et al. (12), and at very low multiplicity (0.1 PFU/trichome) the latent period was the same, but the burst size was 300 to 400 PFU/cell. The higher burst size was thought to be due to the lower MOI and to the incomplete removal of unadsorbed phage in the earlier work.

Before the lytic cycle for LPP-1 had been well characterized, the morphology of LPP-1 infection had been studied by Smith and co-workers (20–22). They originally proposed a model in which the whole virus enters the cell (20); this model was later retracted (22). They then proposed a theory of LPP-1 attachment (21) based on the T-even model (8). After injection of the deoxyribonucleic acid (DNA), the photosynthetic lamellae were thought to be pushed to one side, leaving a region called the "virogenic stroma" where the viral particles would later be formed. The phage DNA replicates in the host nucleoplasm and then migrates into spaces between the lamellae where they form into large (18 nm in diameter) helices. These helices then migrate into the virogenic stroma leaving "holes" between the lamellae: protein coats are then assembled on the helices in the virogenic stroma, which later develop into mature phage particles. This model requires a great deal of movement; viral DNA molecules must traverse several layers of membranes twice merely to end up where they began. The major evidence in favor of the model is the appearance of presumptive viral precursors between lamellae and the later appearance of "holes" between the lamellae (21). Therefore, we repeated the electron microscope observations on samples taken from a high multiplicity one-step growth experiment, having previously established the characteristic times for the eclipse, latent, and rise period. We found that the first visible sign of infection is the invagination of the photosynthetic lamellae and that the area thereby provided, between the lamellae and the plasma membrane, is the area in which virus particles later appear, in agreement with the observations of Smith (21). However, there does not

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appear to be a migration of macromolecules from the nucleoplasm or the photosynthetic lamellae to the virogenic stroma, but it seems that all viral synthesis occurs in the stroma. The intralamellar particles thought to be viral precursors are seen in uninfected cells as well, whereas the holes believed to be due to emigration of these precursors are probably an artifact of specimen preparation.

MATERIALS AND METHODS

Algae and virus. The alga used throughout this study was *Plectonema boryanum* 594, obtained from the Indiana University culture collection. LPP-1 is the isolate originally provided by R. S. Safferman.

The cells were grown in modified Chu no. 10 medium, as described previously (10), in one of two ways. A 5-ml amount of a concentrated culture was diluted into 100 ml of medium and grown in stationary culture at 25 C with a light intensity of 60 to 80 ft-c. Growth was logarithmic for 18 days. Ten- to fourteen-day old cultures (2×10^7 cells/ml to 10^8 cells/ml) were used for growth experiments; cultures as old as 18 days were used for plating.

For large amounts of virus, cells were grown in either 2-liter flasks or 20-liter carboys for 4 days at 25 C under 150 to 500 ft-c with aeration.

Illumination and plaque assay. Illumination for the cells was provided by banks of GE fluorescent lights (FR96 T10-CW reflector). These were supplemented with incandescent bulbs at a ratio of 90% fluorescent–10% incandescent. For growth and biochemical experiments, the intensity was kept between 600 and 800 ft-c. For growth on plates, an intensity of 250 to 350 ft-c was used. The plaque assay was identical to that described previously (10).

Virus infection and isolation. For preparation of virus stocks, cells were grown as above and infected at an MOI of 0.01. Complete lysis usually took 4 days, which resulted in titers between 2×10^{10} and 6×10^{10} PFU/ml. At this point, $CHCl_3$ was added to lyse any remaining intact cells, and the lysate was made 10^{-2} M in Mg^{2+} by the addition of $MgCl_2$. The smaller volumes were spun at 5,000 rev/min in a Sorvall GSA rotor for 10 min to pellet debris and then centrifuged in a Spinco 21 rotor at 19,000 rev/min for 90 min. The pellets were allowed to resuspend overnight at 4 C in 0.01 M $MgCl_2$, 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.5 (TM buffer). Alternatively, large amounts of virus were isolated by means of the dextran-sulfate 500-Carbowax 6,000 two-phase extraction technique described by Luftig (11). Both of these techniques yield stocks with titers of 10^{11} to 10^{13} PFU/ml. To purify the virus further, a sample of 0.2 ml to 1.6 ml of virus was layered onto a discontinuous CsCl gradient. This is formed by making a saturated solution of CsCl (G. Uhe and Co.) in 0.1 M $MgCl_2$, 0.01 M Tris (*p*H 7.5) and diluting the CsCl with this buffer to 70, 60, 50, 40, 30, and 20%. Gradients were made by gently pipetting 0.6 ml to 0.8 ml of each density into a 5-ml nitrocellulose tube and then layering the virus on top. This step separates the virus from a pink pigment that cannot be removed by repeated differential centrifugation. Centrifugation was performed at 30,000 rev/min for 45 min in an SW65 rotor.

A 5 to 20% sucrose gradient may be used either before or after the CsCl gradient, but this does not result in better purification. The purest virus stocks, as measured by the ratio of absorbancy at 260 nm/PFU, were obtained by running two CsCl gradients. This gave 1 $A_{260} = 4 \times 10^{11}$ PFU/ml.

One-step growth experiment. Cells were grown to a density of 10^8 cells/ml, centrifuged at 5,000 rev/min for 10 min in a Sorvall SS-34 rotor, and resuspended in sterile Chu medium about 12 hr before infection. Thirty minutes prior to infection, the cells were wrapped in aluminum foil and placed on a shaker rotating at 100 rev/min. Infection was carried out in light provided by a 15 w safelight with a Kodak no. 1 filter. The cells were then kept in the dark for 2 hr to allow adsorption. A 0.1-ml sample was diluted into 9.9 ml of 0.002 M ethylenediaminetetraacetic acid (EDTA), *p*H 8.0, to kill unadsorbed phage. The infected EDTA-treated cells were then diluted into Chu medium to a final concentration of 10^3 to 10^4 cells/ml (50 to 500 infectious centers/ml). In some experiments, the Chu was supplemented with 100 μg of gelatin per ml and 10^{-2} M $MgCl_2$.

Electron microscopy. Infected cells were fixed by a modification of the procedure of Smith et al. (22). The infected cells were washed twice in 0.1 M cacodylate buffer and fixed for 1 hr at 4 C in a solution of 3% acrolein (Eastman), 3% glutaraldehyde (Ladd or Polysciences) in 0.1 M cacodylate (*p*H 7.2). After washing, they were postfixed in 1% OsO_4 for 0.5 to 1 hr at 20 C; this was followed by staining overnight in 0.5% uranyl acetate at 4 C. The cells were then embedded in 2% agar, spun into a pellet, cut into very small pieces with a sharp razor blade, and dehydrated through alcohol. These small pieces were embedded in Epon (9), usually with a concentration of 5A:5B. Sections were cut with a Dupont diamond knife on a Sorvall MT-II ultramicrotome. Grey to silver-grey sections were poststained in 3% uranyl acetate and with lead citrate (14). For the micrograph of protoplasts, the latter were prepared by the method of Biggins (2). The fixation was identical to that described except that all solutions contained 0.75 M mannitol, until the 70% alcohol step. Micrographs were taken on a Siemens Elmiskop 1 at 80 kv with a 50-μm objective aperture.

RESULTS

One-step growth curve of LPP-1. The filamentous nature of *P. boryanum* is the main difficulty in performing an accurate one-step growth experiment. Under our growth conditions, an average filament (trichome) contained about 20 cells. Therefore, to be perfectly sure that each filament was infected only once, we used an MOI of 0.01/cell (one virus/five filaments). Experiments performed at low MOI value gave essentially identical results to those obtained by Padan et al.

(12). We found that the latent period lasts until 7 hr after infection, the rise period continues until 16 hr, and the burst size is 200 to 300 PFU/cell.

We next attempted to do one-step growth experiments at high multiplicity, since the biochemical experiments anticipated require that every cell be infected. We encountered a difficulty, namely the loss of infectious centers, approximately at the time expected for the end of the latent period. This difficulty was overcome by supplementing the medium in the growth tubes with 0.01 M $MgCl_2$ and 100 μg of gelatin per ml. We then were able to determine the effect of MOI on the one-step growth curve (Fig. 1). The latent period and burst size are seen to be independent of multiplicity over the range from 1 to 50, although there may be a slight delay in lysis at the highest multiplicity. Since there are about 20 cells per trichome, the burst size of 3,000 to 5,000 is in good agreement with the result obtained at very low multiplicity.

Intracellular growth of LPP-1. The previous one-step growth experiment does not distinguish infected cells from free virus particles. To discover when mature phage first appear, the infected cell must be prematurely lysed. Chloroform is widely used for this purpose in bacteriophage infection (16).

The results of chloroform treatment during LPP-1 infection can be seen in Fig. 2. Mature virions are completely absent for about the first 4 hr (eclipse period); a rapid increase in the number of intracellular infective particles follows.

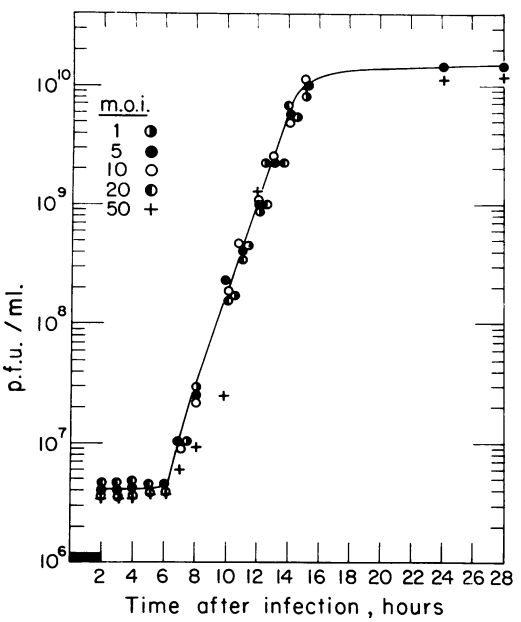


FIG. 1. *One-step growth curve for LPP-1 at various MOI.*

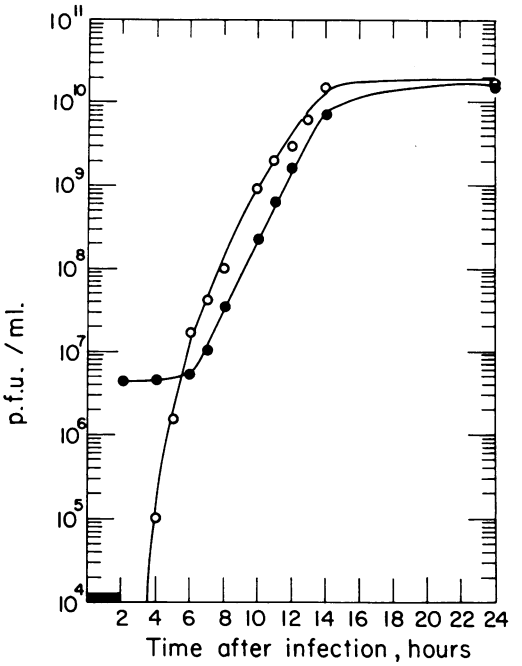
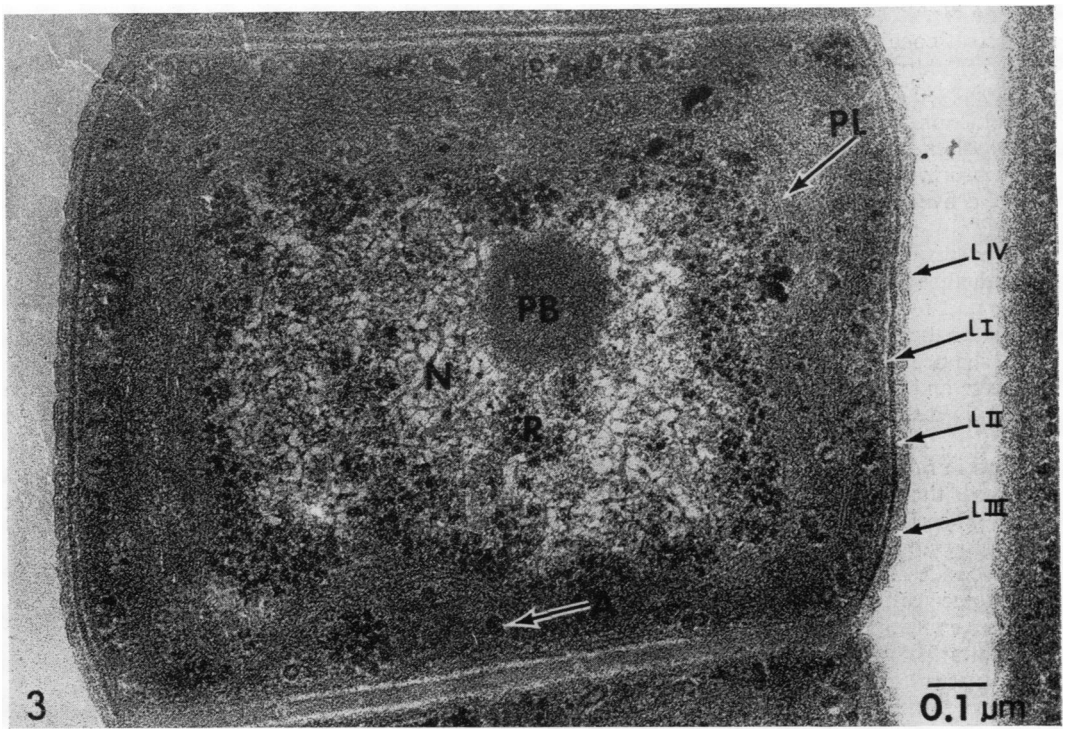


FIG. 2. *One-step growth and intracellular growth curves of LPP-1 at MOI = 10. Symbols: ○, intracellular growth, premature lysis with $CHCl_3$; ●, infectious centers. The burst size is 4,500 PFU/infectious center.*

Ultrastructure of P. boryanum. The ultrastructure of the blue-green algae has recently been elucidated by utilizing improved techniques for viewing thin sections in the electron microscope. Ris and Singh (15) studied a number of different species of blue-green algae and confirmed the fact that blue-greens are more closely related to bacteria than to other plants or algae since they do not contain any intracellular organelles such as nuclei and mitochondria. A later study on *Symploca muscorum* by Pankratz and Bowen (13) extended these findings and described a large number of structures that could be seen in the electron microscope, but could not be related to a specific composition or function. Recently, cell division and the structure of the cell wall have been studied in the unicellular blue-green *Anacystis nidulans* by Allen (1). The terminology introduced by these authors will be used in this paper.

Uninfected *P. boryanum* cells are shown in Fig. 3 and 4. The center region of the cell is the nucleoplasm which contains DNA, ribosomes, and structures called polyhedral bodies whose



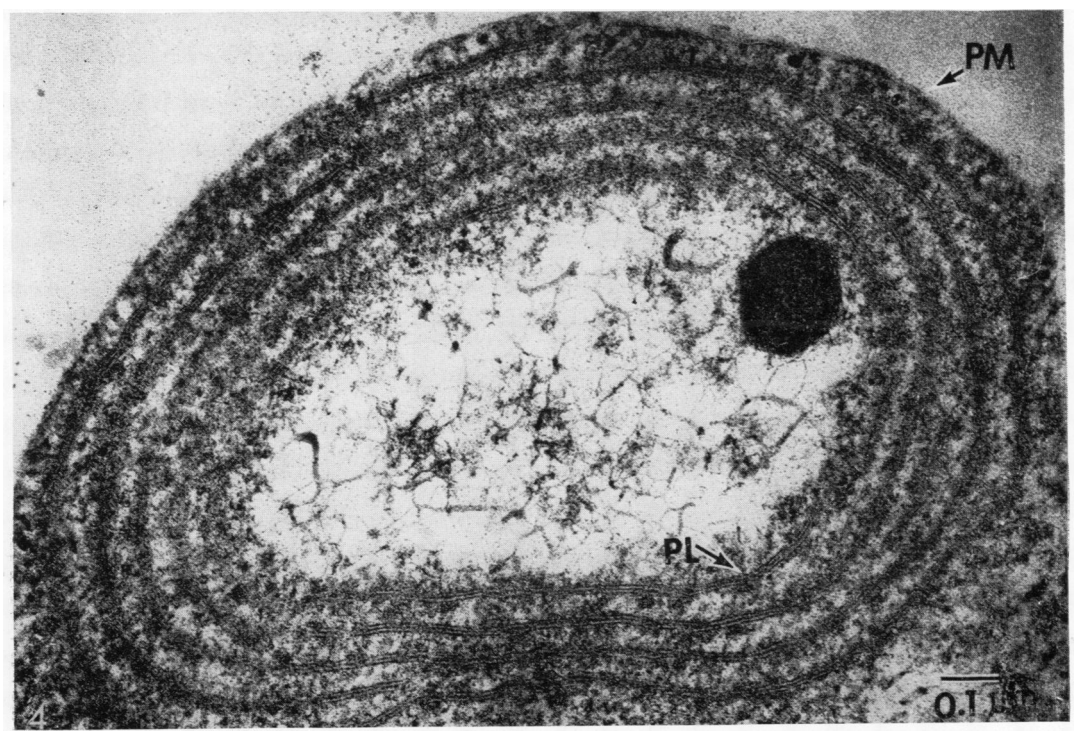


FIG. 3. *Uninfected Plectonema boryanum cell. The procedure for electron microscope preparation is given in Materials and Methods. Symbols: A, α-granules; N, nucleoplasm; PB, polyhedral body; PL, photosynthetic lamellae; R, ribosomes; LI, LII, LIII, LIV, cell wall (see text for details).* × *86,000.*

FIG. 4. *Uninfected P. boryanum protoplast. PM, plasma membrane.* × *87,000.*

function is unknown. Surrounding this area are a number of concentric lamellae which contain all of the cell chlorophyll and are the sites of photosynthesis (4, 17, 23). These photosynthetic lamellae sometimes appear as two unit membranes surrounding an electron transparent area (Fig. 11) measuring a total of 26 nm across. In other places, a five-layered structure, 16 nm across, is seen (Fig. 4) with three electron dense lines separated by two electron transparent spaces. However, whether the center dense line is due to fusion of two unit membranes cannot be determined.

Between the photosynthetic lamellae are a number of characteristic particles called α-granules by Pankratz and Bowen (13). These granules are 180 nm in diameter and from 30 to 50 nm long. They are found regularly spaced between the lamellae in most cells (Fig. 3, 11). At high magnification they give the appearance of being helical. Their function is unknown although it has been suggested that they are highly branched polyglucoside storage particles (3).

The blue-green algal cell wall is a complicated structure composed of four parts (1, 7). These are as follows from the inside of the cell outward: LI, an electron-transparent area of varying thickness next to the cell plasma membrane (Fig. 3); LII, an electron-opaque area 11 nm thick; LIII, another transparent region about 20 nm thick; LIV, a typical unit membrane. It is likely that LI is an artifact since it varies widely in size and completely disappears at times (Fig. 10, 11). This could be due to the separation of LII from the membrane during fixation or to a tangential sectioning artifact which makes the membrane appear to be separated from LII. In places where LII and the plasma membrane are closely appressed, the distance from the membrane to the outside edge of LIV is about 45 nm.

Large, electron-dense, amorphous structures can be seen in the nucleoplasm (Fig. 8). These structures resemble the polyphosphate granules found in yeast cells (H. Swift, *personal communication*). At times, vacuoles which contain a small amount of this granular substance are found, and these are presumably of similar origin.

Ultrastructure of LPP-1-infected P. boryanum. To view the morphological changes that occur after infection by LPP-1, samples for electron microscopy were taken at various times after logarithmically growing *P. boryanum* was infected by LPP-1 at MOI = 50. The growth curve for this experiment can be seen in Fig. 1. During various experiments, samples from 1, 3, 4, 6, 7, 9, 10, 12, 15, and 25 hr after infection were viewed in the electron microscope; representative results from the 4-, 7-, and 10-hr points were selected. These correspond to the end of the eclipse period, end of the latent period, and middle rise period, respectively.

The first sign of viral infection is the invagination of the photosynthetic lamellae (Fig. 6, 7). The lamellae are pushed inwards, pushing the nucleoplasm toward one corner of the cell. Viral particles will later appear in the space left by this invagination (the virogenic stroma). Invagination, which is characteristic of all infected *Plectonema* cells, is not seen 1 hr after infection but is seen at 3 hr after infection and is quite pronounced by 4 hr.

By 4 hr after infection, the virogenic stroma is seen to contain numerous particles. Some of these are probably ribosomes; others appear to be α-granules (Fig. 6, 7). Prior to infection, these particles were in the nucleoplasm and intralamellar space, respectively. Their appearance in the virogenic stroma suggests that some damage has been done to the lamellar system to permit these particles to penetrate into the stroma. Other small osmiophilic particles are seen throughout the cell (Fig. 8, 13). We believe these to be artifacts produced by electron bombardment of the large granular inclusions assumed to be polyphosphate.

Virus particles were also seen at 4 hr on one occasion (Fig. 7). Four hours corresponds to the end of the eclipse period, so that the probability of finding mature, intracellular virus at this time is extremely low. No large, partially formed viral precursors were seen at this time.

We will show elsewhere (18) that about 50% of the host DNA is broken down after infection. It is not possible to determine from the biochemical data whether half of the cells have all of their DNA degraded or whether all the cells have half of their DNA degraded. It is obvious from Fig. 7, 9, 10, 11, and 12 that there is still nucleic acid present in the nucleoplasm during viral development. Although the exact amount cannot be determined, we take this to mean that all of the cells have part of their DNA degraded.

After 7 hr, with viral DNA and protein synthesis continuing at a constant rate, viral assembly proceeds accordingly. By 10 hr, almost every cell appears to have begun viral assembly, and many lysed cells can be noted. One interesting phenomenon is shown in Fig. 10, 11, and 12. In these cells, the lamellae are invaginated in three places, and viral particles may be seen in all three stromata. In particular, Fig. 12 shows viral production at three different stages of development. It is possible that the lamellar invagination is asymmetric in three dimensions, and what appears to be three separate stromata in a single section is actually one continuous region. If the stroma is continuous throughout the cell, with the nucleoplasm pushed toward the middle or to one corner, then viral

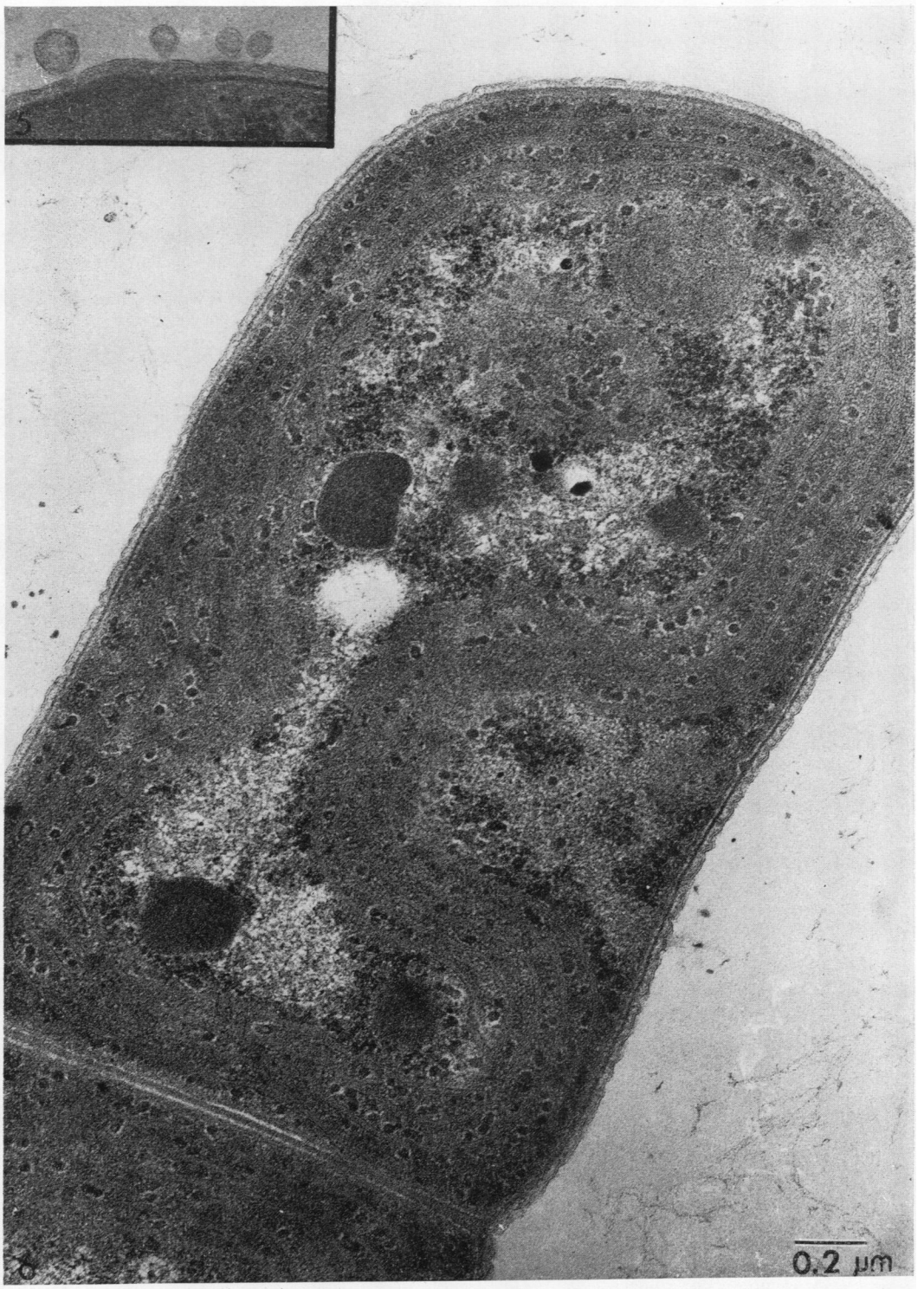
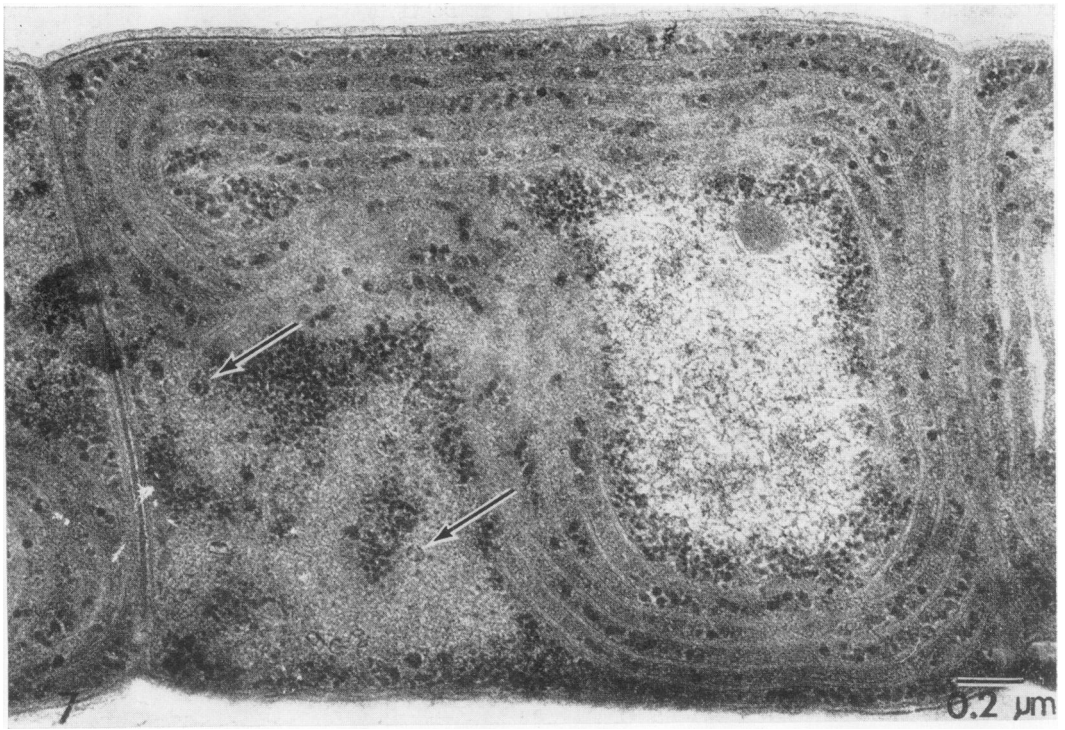


FIG. 5. *Viruses adsorbed to the cell wall at 4 hr after infection.* × *95,000.*

FIG. 6. *P. boryanum cell 4 hr after infection with LPP-1; MOI = 50. At this stage of the infectious process, the only noticeable change in cellular morphology is the lamellar invagination.* × *72,000.*



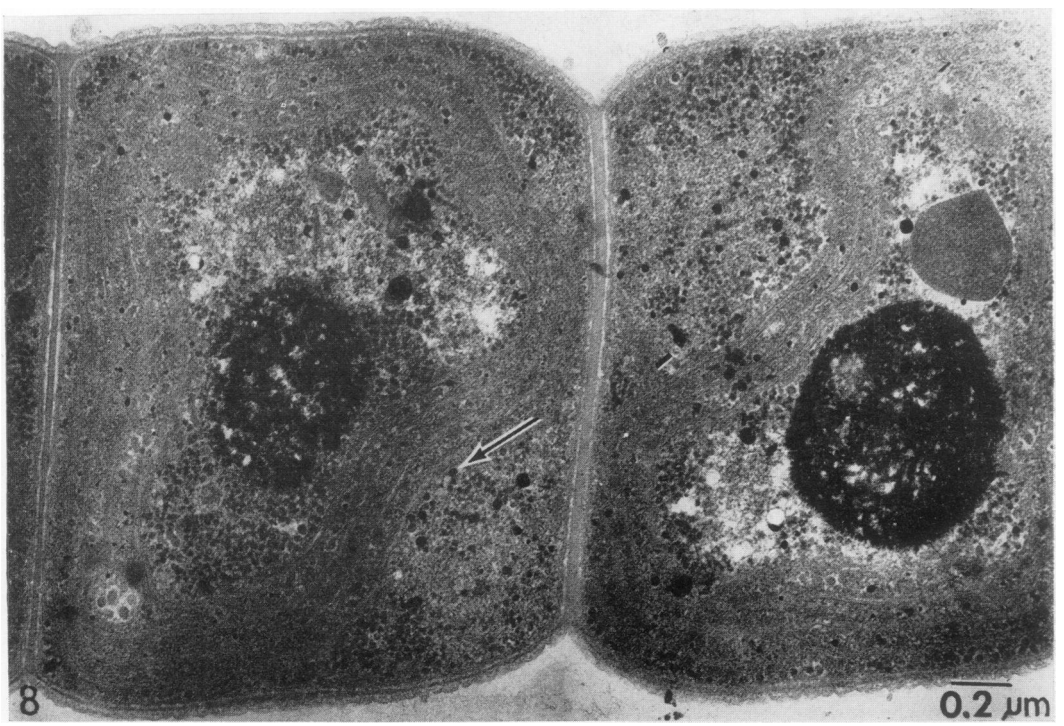


FIG. 7. *Four-hour infected P. boryanum. Lamellae are invaginated. In the virogenic stroma are particles that appear to be ribosomes and structures that are most likely viruses (arrows).* × *58,000.*

FIG. 8. *Four-hour infected P. boryanum. In the nucleoplasm are large, amorphous structures that resemble polyphosphate granules. The small, osmiophilic particles (arrow) seen throughout the nucleoplasm and the stroma are probably artifact, caused by partial destruction of the larger structures.* × *52,000.*

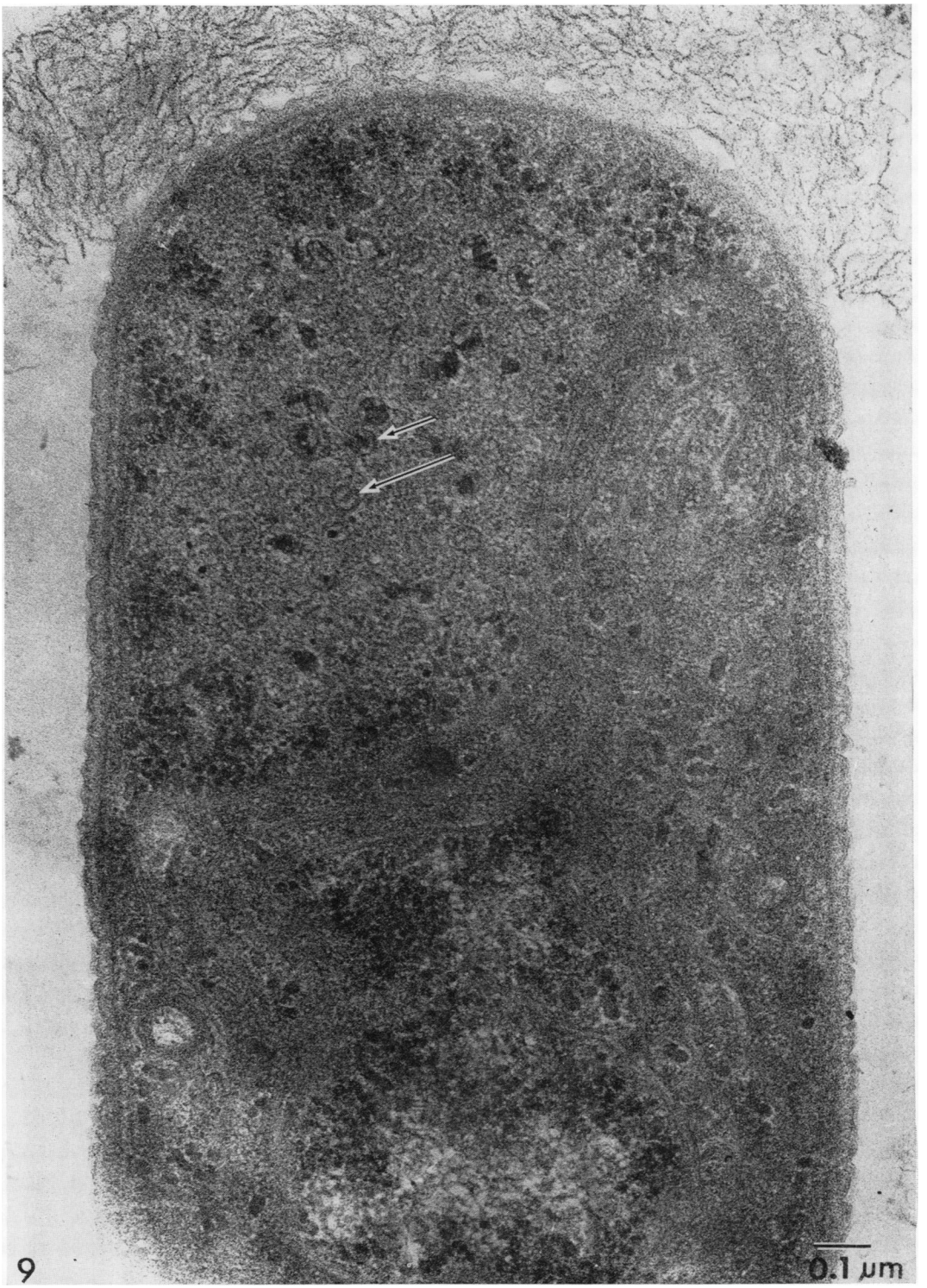


FIG. 9. *Seven-hour infected P. boryanum. The stroma of this cell contains many virus particles in different stages of assembly. Some viruses (small arrow) appear to contain DNA, whereas others (large arrow) do not. Whether this implies an order for viral assembly is discussed in the text. The unravelling of the cell wall was seen in many cells. However, it cannot be determined whether this is the normal lysis mechanism or merely due to preparation artifact.* × *105,000.*

FIG. 10. *Ten-hour infected P. boryanum. A variety of structures can be seen within the capsids. It is likely that this phenomenon is due to clumping of the DNA during fixation.* × *82,000.*

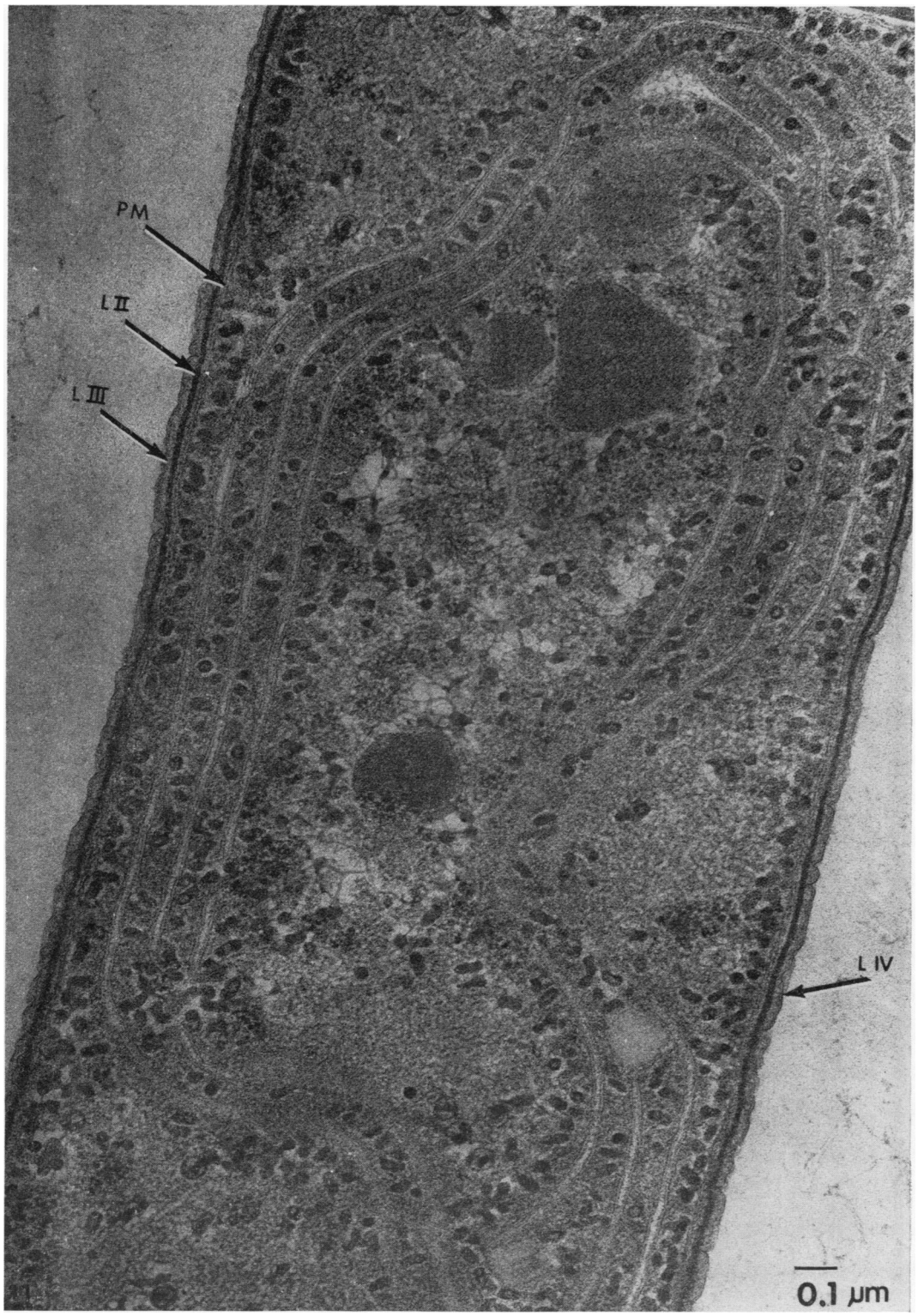


FIG. 11. *Ten-hour infected P. boryanum. Many α-granules may be seen throughout the cell. However, most of them are still in the intra-lamellar areas. Along most of the cellular periphery, the plasma membrane (PM) is closely appressed to LII of the cell wall, and LI is absent.* × *90,000.*

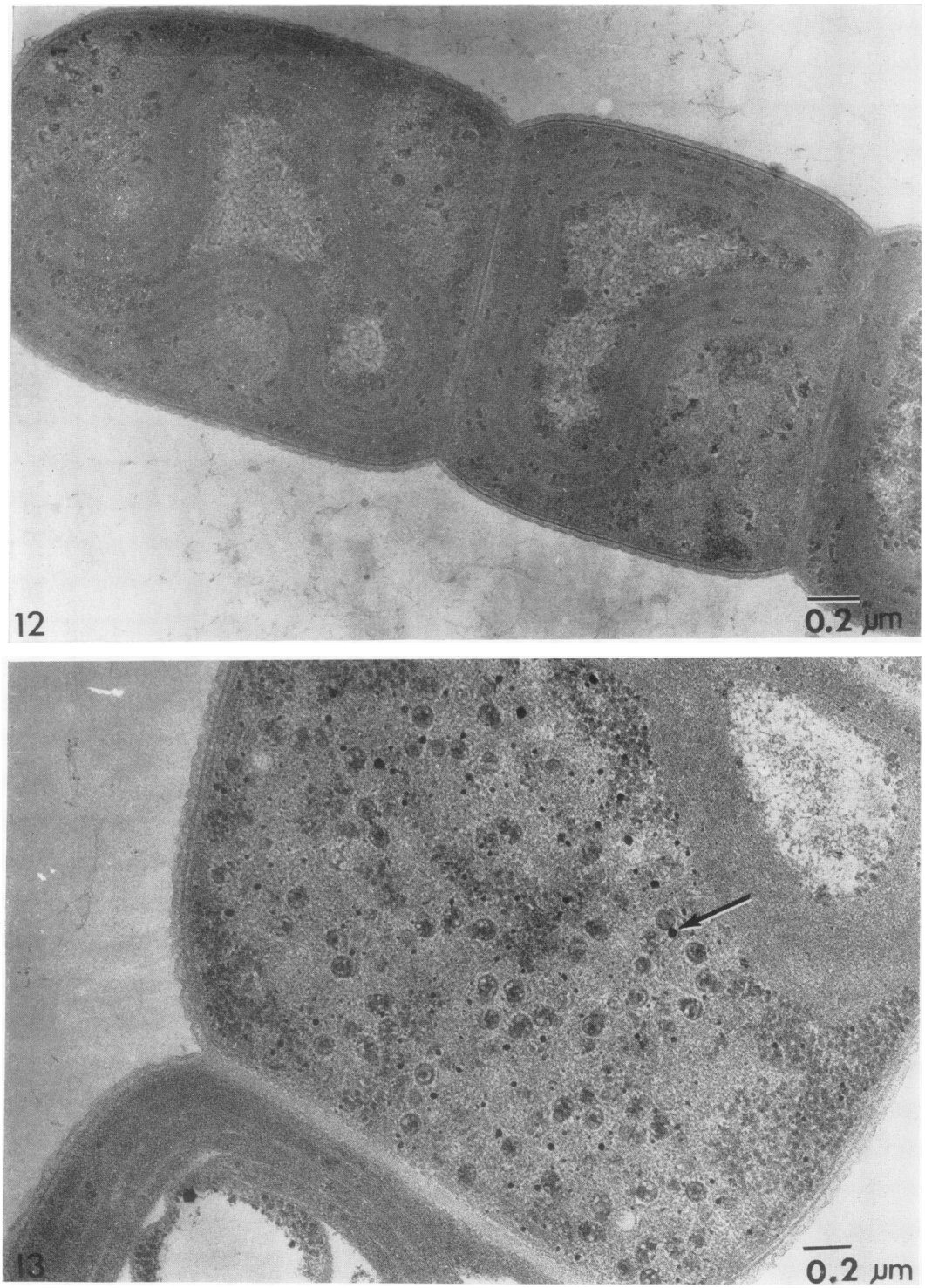


FIG. 12. *Ten-hour infected P. boryanum. Cells in a trichome showing various stages of viral assembly and multiple stroma in a cell.* × *30,000.*

FIG. 13. *Ten-hour infected P. boryanum. Dark, osmiophilic granules may be condensed DNA, but are most likely of some other origin.* × *42,000.*

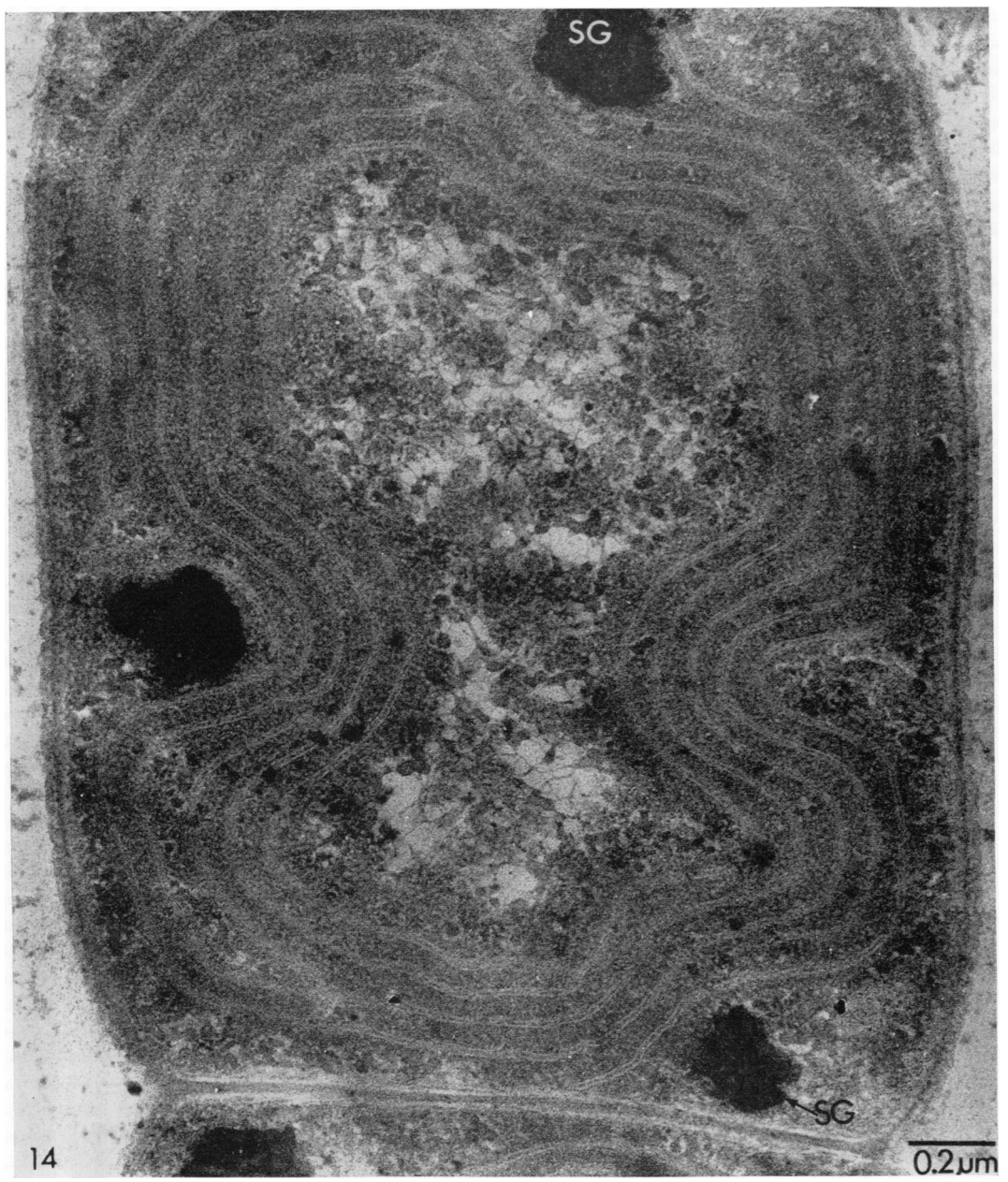


FIG. 14. *Ten-hour infected P. boryanum plus 100 μg of chloramphenicol per ml at 1 hr. Cells were infected, at MOI = 20, treated with 100 μg of chloramphenicol per ml at zero time and at 1 hr, and left in the light until 10 hr after infection. This cell, treated with chloramphenicol at 1 hr, is partially invaginated in many places. However, no cells were found that had lamellae that were completely invaginated. It is most likely that more than 1 hr of viral-directed protein synthesis is necessary for complete lamellar invagination. SG, structural granule.* × *76,000.*

development would begin preferentially at one point (possibly nearest to the place at which the DNA was injected).

Various conformations of the DNA within the protein coat can be seen in Fig. 10, 11, and 13. This is strikingly similar to the observations on T2-infected *Escherichia coli* reported by Zagury et al. (24). These authors consider this asymmetric appearance of the viral DNA in the capsid to be due to nonhomogeneous packaging of the DNA. Although this is possible for both T2 and LPP-1, the nonhomogeneity is most likely a fixation artifact. Glutaraldehyde, although cross-linking protein very effectively, does not preserve nucleic acid quite as well. The DNA has a tendency to clump, and, when later stained with uranyl acetate, appears as a very dense ball (E. Kellenberger, *personal communication*). This clumping may explain the finding of viral particles that appear to lack DNA (Fig. 9). If the DNA has clumped at one side of the virus, a thin, tangential section may appear empty.

The mode of attachment of LPP-1 to the *P. boryanum* cell wall can be seen in Fig. 5 and 10. If the tail is 20 nm long in isolated particles (10, 11) this would allow the tail to extend through LIII to LII. If a core protrudes at this stage, then it is possible that the DNA could be injected at or within the plasma membrane. The exact depth to which the tail penetrates can not be determined although it appears that only the viral capsid is outside the cell after attachment.

It has been mentioned that lamellar invagination is the first morphological sign of infection. To determine the number of cells that showed this characteristic at a given time, various grid areas were scanned at random. Only longitudinally oriented cells in these areas were counted for this purpose, since it is too easy for a transverse section near the edge of a cell to appear normal. The results of this survey are listed in Table 1. The number of cells with invaginated lamellae increases greatly between 3 and 4 hr and continues to a maximum at 7 hr. It is not thought that this maximum level (10 to 15% not invaginated) indicates the number of uninfected cells, which is known to be much less than 1%. More likely, sections through part of an infected cell appear normal, since the invagination is not necessarily uniform throughout the cell.

It was of interest to determine whether virus-directed protein synthesis is necessary for the invagination process. If invagination were due to a mechanical process stemming from virus attachment, it would be expected to occur in the absence of protein synthesis. However, if expression of a viral gene was necessary for invagination, it might be possible to stop invagination by inhibiting protein synthesis. To differentiate between these two possibilities, *P. boryanum* was infected with LPP-1 (MOI = 20) and chloramphenicol (19) was added either at the time of infection or 1 hr later. The infected cells were collected at 10 hr and prepared for electron microscopy as before. Those cells treated with chloramphenicol at the time of infection showed no sign of viral attack. At 10 hr after infection, there were no viral particles and no invagination. Fewer than 10% of the cells showed even the slightest change in lamellar conformation. In all other respects, these cells appeared normal. When chloramphenicol was added 1 hr after infection, there was some invagination at 10 hr (Fig. 14). However, there were no cells that had the lamellae pushed completely to one side. It can be seen in Fig. 14 that there is a slight invagination of the lamellae in a number of places. It appears that allowing only an hour of protein synthesis has enabled cells to invaginate partially. This rules out a mechanical process and implies that more than 1 hr of virus-directed protein synthesis is required for complete invagination.

TABLE 1. *Invagination of lamellae after LPP-1 infection*

Time after infection (hr)	No. of cells counted[a]	Cells with lamellae invaginated
		%
1	150	0
3	310	27
4	316	59
6	336	85
7	318	83
9	73	85
10	504	89
12	388	95

[a] Longitudinal sections of infected cells were counted from random grid areas.

DISCUSSION

P. boryanum doubles every 24 hr under the growth conditions used for the LPP-1 lytic cycle experiments. The complete lytic cycle for LPP-1 requires slightly more than half the doubling time of the host, about 14 hr. This relationship between the length of the viral lytic cycle and the growth rate of the host is approximately the same as that of the T-even bacteriophages. The burst size found for LPP-1 is also similar to that of the T series of bacteriophages. Approximately half of the particles in our purified virus preparations are infective. Assuming the same to be true in lysates, each cell produces 300 to 500 LPP-1 *particles*.

Based on the electron microscope observations, we propose the following scenario for LPP-1 infection. The virus adsorbs by its tail, which eventually penetrates to the inner edge of wall

layer LIII. Viral DNA is injected through, or transported across, the plasma membrane. In the accompanying papers, we show that viral protein synthesis and host DNA breakdown begin within the first hour after infection. The former is required for the first morphological change observed, the invagination of the photosynthetic lamellae, noticeable by 3 hr. Intracellular viruses are first seen at 4 hr, and cell lysis begins at 7 hr. By the 10th hr all unlysed cells contain intracellular virus, and by 15 hr very few cells remain unlysed.

We have not mentioned viral precursors in this scheme. We found no evidence for migration of viral DNA from stroma to nucleoplasm, for the formation of helical DNA condensates in the intralamellar spaces, or for the migration of such condensates back to the stroma. The observations that led Smith and co-workers (21) to postulate this complex scheme appear to have rather simple explanations. The helical structures in the intralamellar spaces are probably the α-granules of Pankratz and Bowen (13) which we saw in proliferation in both infected and uninfected cells. The holes which these helices are supposed to have left after their return to the stroma were indeed seen once by us. They were due to poor infiltration of Epon into the cells, and were found in both infected and uninfected cells. There is no compelling reason to assume that any viral syntheses occur outside of the virogenic stroma.

The process of lamellar invagination invites speculation. It occurs between 3 and 7 hr after infection and requires protein synthesis. We show elsewhere (18) that a very early viral product, requiring protein synthesis, is responsible for the solubilization of approximately half of the DNA of the host. The time courses of host DNA solubilization and lamellar invagination are very similar. Inhibition of protein synthesis 1 hr after infection permits partial host DNA breakdown and partial lamellar invagination. Therefore, we suggest that lamellar invagination is due to the breakdown of host DNA.

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