JOURNAL OF VIROLOGY, Apr. 1967, p. 344-361
Vol. 1, No. 2

Morphology of a Virus of Blue-green Algae and Properties of Its Deoxyribonucleic Acid

RONALD LUFTIG AND ROBERT HASELKORN
Department of Biophysics, University of Chicago, Chicago, Illinois 60637

Received for publication 15 December 1966

The morphology of Safferman's virus of blue-green algae (phycovirus LPP-1) has been studied by electron microscopy and physicochemical methods. The virion has a short (100 to 200 A long, 150 A in diameter) forked tail, with an outer sheath, an inner core, and a capital attached to one of the vertices of a polyhedral head. The head capsid edge-to-edge distance is 600 A, based upon internal calibration of the magnification in electron micrographs by use of the line-line spacing of catalase crystals. Measurements of absorbancy and infectivity, and electron microscopy across the band of virus after zone centrifugation on a sucrose gradient, indicated that infectivity was correlated with the short-tailed particles described. The viral deoxyribonucleic acid (DNA) is linear, with a contour length of 13.2 ± 0.5 μ, measured by the Kleinschmidt method. Its sedimentation coefficient, $S^0_{20,w}$, is 33.4 ± 0.7 *S*. These values are consistent with a molecular weight of 27×10^6 for the viral DNA. Based upon buoyant density in CsCl and thermal denaturation, the guanine-cytosine content of the DNA is 53%. The viral DNA was used as template for in vitro ribonucleic acid (RNA) synthesis by *Escherichia coli* RNA polymerase. This RNA annealed to 18% of the sequences in the viral DNA, 0.5% of the sequences in bacteriophage T7 DNA, and 0.25% of the sequences in *Plectonema boryanum* DNA, at saturating levels of RNA in the Hall-Nygaard hybridization assay. The lack of homology with T7 DNA is of interest because the two viruses are very similar morphologically. The lack of homology with host DNA suggests that this algal virus is a poor candidate for transduction.

Although many attempts to isolate a virus of algae had been made previously, the first fruitful search was that of Safferman and Morris (22), who applied the Adams method for isolating viruses of microorganisms (1) to water from a waste disposal pond. They found a factor capable of lysing several species of *Cyanophyceae* (*Lyngbya*, *Plectonema*, and *Phormidia*), and proved conclusively that the factor was a virus. Subsequently, Safferman and co-workers studied the growth characteristics of the virus (23) and determined that it had a tail and contained deoxyribonucleic acid (DNA; 24).

Because of the potential importance of viruses of photosynthetic organisms, we have studied the morphology of the virus and its DNA in some detail. Since the virus host range is restricted to a small number of *Cyanophyceae*, in particular those which have lysozyme-sensitive cell walls (6), we expected that in most fundamental ways the virus of blue-green algae would be found to resemble bacteriophages. So far, this expectation has been borne out: the virus of blue-green algae is morphologically very similar to bacteriophage T7. By analogy with the nomenclature of many bacterial viruses, we proposed the name cyanophage for the virus of blue-green algae. However, Safferman prefers the term phycovirus, with the host range designated LPP-1. Throughout this paper, we shall refer to the virus of blue-green algae as cyanophage LPP-1.

Recently, a virus infecting the same species of algae has been isolated in Israel. It appears to be similar, if not identical, to cyanophage LPP-1 (20).

MATERIALS AND METHODS

Virus growth and purification. A 400-ml inoculum of a 2-week-old culture of *Plectonema boryanum* (IU 594) was added to 12 liters of modified Chu No. 10 medium [$Ca(NO_3)_2 \cdot 4H_2O$, 0.232 g; K_2HPO_4, 0.01 g; $MgSO_4 \cdot 7H_2O$, 0.025 g; Na_2CO_3, 0.02 g; ferric citrate, 0.0035 g; citric acid, 0.035 g; Hoaglands micronutrients (16), 1 ml, per liter]. Growth was at room temperature for 6 days (log phase; F. Kieras, *unpublished data*), with vigorous aeration, and with the use of two ring-type fluorescent lamps around a 14-liter carboy. A rough estimate of the number of algal cells was made with a Petroff-Hausser counter, with 2 μ taken

as the average length of a single cell. The culture was infected at a multiplicity of about 0.01, and 2 days later chloroform and 1 M tris(hydroxymethyl)aminomethane (Tris)-Mg acetate, *p*H 7.5 (10 ml per liter of lysate), were added to it. The lysate had a titer of 5×10^9 to 10^{10} plaque-forming units (PFU)/ml and was stored at 4 C.

Two methods were used for purification of the lysate. (i) Crude lysate (1 liter) was dialyzed against Carbowax 20 M for 24 hr and then redialyzed against 0.01 M Mg^{++}, 0.01 M Tris, *p*H 7.5 (TM buffer). Debris was removed by centrifugation for 10 min at 10,000 rev/min in a Sorvall centrifuge. This is the material used for DNA prepared by the Freifelder technique (10). (ii) Crude lysate was centrifuged at 17,000 rev/min in the 19 rotor of the Spinco L-2 ultracentrifuge for 1 hr. The pellet was resuspended overnight in TM buffer. This suspension was clarified by a low-speed spin, and the supernatant fluid was put on a 20 to 70% saturated CsCl step gradient, which was run at 28,000 rev/min for 40 min in the SW-39 rotor of the Spinco L-2. The resulting orange band was collected and run on a 5 to 20% sucrose gradient either in 0.01 M Mg^{++}, 0.005 M Tris, 0.005 M KCl (*p*H 7.5) or in TM buffer at 22,000 rev/min for 30 to 40 min in an SW 25 rotor. The large blue band was collected and concentrated by centrifugation at 19,000 rev/min for 90 min in the 40 rotor. The pellet was resuspended in TM buffer and again put on a CsCl step gradient as above. The final virus suspension had an absorbancy at 260 mμ of 14.8 and a titer of 1.85×10^{12} PFU/ml. This virus was used for electron microscopic examination.

Several crude lysates were partially purified in a more gentle manner. The virus was pelleted at 13,500 rev/min for 75 min in the 19 rotor of a Spinco L-2, allowed to resuspend for 24 hr in TM buffer, and then centrifuged at 3,000 rev/min for 10 min in a Sorvall centrifuge to remove insoluble debris.

DNA isolated for physical studies. A modification of the Freifelder technique (10) was used for preparation of viral DNA. A 1-ml amount of 30% sucrose, followed by 30 ml of viral lysate (5×10^{10} PFU/ml), was very slowly dripped down the side of an SW 25 centrifuge tube on top of 1 ml of 5 M $NaClO_4$. The tubes were centrifuged at 22,000 rev/min for 90 min at room temperature in a Spinco model L centrifuge. The bottom 1 ml was carefully removed, and the DNA was dialyzed overnight against SSC (0.15 M NaCl, 0.015 M Na_3 citrate, *p*H 7). DNA concentration was determined spectrophotometrically by use of an extinction coefficient at 260 mμ of 20 ml/mg-cm.

Plaque assay. Bottom (1.5%) and top (1.2%) agars were made with modified Chu No. 10 medium. Dilutions were made with TM buffer. For plating, 2.5 ml of top agar at 60 C was mixed with 2 to 2.4 ml of a 2-week culture of *Plectonema boryanum* and 0.1 to 0.5 ml of virus. Plates were incubated at 25 C for 3 to 4 days, illuminated with 100 to 200 ft-c of fluorescent light.

Electron microscope specimens. Specimens for electron microscopy were prepared by four different methods.

Plaque technique. Plaques were cut from soft agar with a sterile platinum loop (4), placed on a glass slide with a drop of 0.05 M Tris-0.015 M Mg^{++} (*p*H 7.1), and mashed with a blunt stirrer. The suspension was sucked back and forth 10 times in a pasteur pipette and then was examined by the droplet technique.

Droplet technique. This technique is similar to one developed by Fernandez-Moran and van Bruggen (9). Carbon-coated Formvar (0.2%) fenestrated films on 200-mesh copper grids, with or without carbon films placed over them, were used as the specimen substrate. A droplet of appropriately diluted viral suspension in TM buffer was placed on the film for 2 min; the grid was turned over onto negative stain for 2 to 4 min; then the edge of it was touched to filter paper and allowed to air-dry.

Fixed specimens were first inverted onto 10% Formalin or 1.5% glutaraldehyde for 30 min before negative staining, and then were transferred to the stain with a platinum loop.

For ethylenediaminetetraacetate (EDTA) specimens, the droplet of virus was floated on 0.35 M EDTA for 10 to 60 sec, or on 0.01 M EDTA for 2 min; then the grid was transferred to TM buffer for 20 sec before floating on the stain.

All specimens were made at 0 to 4 C. Negative stains were made with double-distilled deionized water, and were filtered before each preparation. The stains used were 1% phosphotungstic acid, brought to *p*H 7.0 to 7.4 with KOH (PTA), and 1 or 2% uranyl acetate, *p*H 4.3 (UA).

Catalase technique. It has been determined by calibration with copper phthalocyanine crystals and a Fullam replica grating that the line-line spacing of CTR beef liver catalase crystals (Worthington Biochemical Corp., Freehold, N.J.) is 91 $\pm$ 3 A by UA or PTA staining (R. Luftig, *unpublished data*; R. Horne, *personal communication*). To use this spacing as an internal calibration for electron microscope magnification, the following technique was developed. A droplet of virus suspension was placed on a Formvar support as in the droplet technique. The grid was then inverted onto 2% UA for 90 sec. The grid was then removed with a platinum loop to a beaker containing 0.08 ml of catalase crystal suspension in 5 ml of 2% UA and stained for 2 min. The edge of the grid was touched to filter paper and allowed to air-dry. This technique gave much better stain penetration of the virus than did mixing catalase and virus together prior to staining. Measurements were made from prints with a 7× Bausch & Lomb magnifier calibrated in 0.1-mm divisions.

Kleinschmidt technique (18, 21). Viral DNA was diluted to a final concentration of 2 μg/ml in 1 M ammonium acetate and 0.01% cytochrome *c*. The DNA was spread on water according to the modification of Ris and Chandler (21). Plates were magnified 10 times on a Nikon model 6B comparator. After tracing the DNA strands, contour lengths were measured with a map measurer with better than 0.05% reproducibility. The error due to magnification by the comparator was less than 0.01%. The calibration of this magnification system was checked by measuring *Bacillus subtilis* phage ϕ-29 DNA prepared by K.

Bott. The contour length for 23 molecules was 5.72 ± 0.24 μ, in agreement with Anderson et al. (2), who found values ranging from 5.7 to 5.9 μ.

Electron microscopy. All work was done on a Siemens Elmiskop I electron microscope equipped with a Solatron voltage regulator and operated at 80 kv. Astigmatism was constantly checked and kept within 0.22 μ. A 200-μ condenser and 50-μ objective apertures were used at all times. Virus specimens were examined at a magnification of 40,000 to 45,000 diameters.

DNA prepared by the Kleinschmidt technique was examined with Pole Piece III, Intermediate lens at 0, a setting of minimal distortion, which gave a magnification of about 12,500 diameters. The latter was checked by taking two plates of a Fullam calibration grid (54,864 lines per inch) with each set of Ilford N-40 plates.

Sedimentation velocity. DNA prepared by Freifelder's method (10) was run at salt concentrations of 1 SSC (0.15 M NaCl, 0.015 M Na_3 citrate, *p*H 7) and 4.8 SSC. The factor

$$\left(\frac{1 - \bar{v}\rho_{20,w}}{1 - \bar{v}\rho}\right)\left(\frac{\eta}{\eta_w}\right)_{20C}$$

determined from the International Critical Tables was 1.032 and 1.188 for SSC and 4.8 SSC, respectively. A partial specific volume of 0.556 was assumed for the Na^+ salt of DNA (15). DNA solutions were placed into cells with no. 18 or no. 22 needles by dripping through an enlarged hole of the centerpiece or by slow injection with a plunger. No significant difference was found between results with no. 18 and no. 22 needles, in agreement with Davison and Freifelder's findings for T7 DNA (8). For DNA concentrations below 27 μg/ml a 30-mm Kel-F centerpiece was used; otherwise, a 12-mm one was used. The Spinco Model E Analytical Ultracentrifuge was run at 44,770 rev/min with temperatures between 15 and 25 C. Ultraviolet (UV) optics were used, and the film was traced with a Joyce-Loebl recording microdensitometer.

Guanine-cytosine (G-C) content of cyanophage LPP-1 DNA. For CsCl equilibrium centrifugation, a solution of 1.7 μg/ml of viral DNA with a density reference of 2 μg/ml of *B. subtilis* phage SPO1 DNA, provided by E. P. Geiduschek, was made up in SSC with CsCl (Harshaw Chemical Co., Hastings-on-Hudson, N.Y.) to a density of 1.7 g/ml. It was run in a Spinco Model E Analytical Ultracentrifuge for 27 hr at 44,770 rev/min with the temperature at 20 C. The density of SPO1 DNA was determined relative to *Escherichia coli* DNA (ρ = 1.710) by use of the relationship of Vinograd and Hearst (30), and was found to be 1.744 (D. Swinton, *personal communication*) at 25 C.

Thermal denaturation was determined by use of viral DNA prepared by Freifelder's method (10) and diluted to about 18 μg/ml in 0.1 SSC. The DNA solution was heated in a stoppered cuvette, and the absorbancy at 260 mμ was determined as a function of temperature, as described by Geiduschek (12).

Preparation of DNA for hybridization experiments. Viral DNA prepared by Freifelder's method (10) was gently shaken three times with distilled phenol saturated with 0.1 M phosphate buffer (*p*H 7). At each stage, the solution was twice centrifuged at 1,500 rev/min in an International centrifuge; first the phenol layer was removed, and then the aqueous phase was taken off. The DNA was dialyzed against 0.01 M Tris chloride-1.0 M NaCl (*p*H 7.5) until all traces of phenol were gone, and then it was dialyzed against 0.01 M Tris chloride (*p*H 7.5). All work was done at 0 to 4 C.

Preparation of labeled ribonucleic acid (RNA). Tritium-labeled RNA was synthesized on DNA templates in vitro by use of *E. coli* RNA polymerase as follows: Tris chloride (*p*H 7.5), 100 μmoles; $MgCl_2$, 10 μmoles; DNA, 100 μg; RNA polymerase, 300 Chamberlin-Berg units; adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), each 1 μmole; uridine triphosphate (UTP), 0.5 μmole; ^{3}H-UTP in 50% ethyl alcohol, 50 μc; spermidine, 1 μmole, all per ml. The RNA polymerase, generously provided by E. Brody, had a specific activity of 2,000 Chamberlin-Berg units per mg. The system was incubated for 30 min at 30 C; 5 μg of deoxyribonuclease (Worthington Biochemical Corp.) free from ribonuclease was then added, and the system was incubated for 5 min at 30 C. Next added were 0.4 ml of 1 M sodium acetate (*p*H 5.2) and 5 ml of water-saturated phenol. The system was shaken with phenol twice at 60 C, and the aqueous phase was then dialyzed as above for DNA.

Hybridization. A modified Hall-Nygaard technique (19) was used in which DNA was denatured before each experiment by heating for 10 to 15 minutes at 100 C and then quenched. This DNA was annealed with ^{3}H-RNA in 2 SSC by use of a 0.5 ml system and incubation at 60 C for 6 hr.

A 5-μg amount of pancreatic ribonuclease (Worthington Biochemical Corp.), which had been boiled to free it of deoxyribonuclease was added to each tube and incubated at 37 C for 15 min. The contents were filtered onto filters (coarse, type A; Schleicher & Schuell Co., Keene, N.H.) presoaked with 0.5 M KCl-0.01 M Tris(*p*H 7.5). After being washed four times with the latter solution and once with 80% ethyl alcohol, the filters were dried, placed in glass vials containing toluene-2,5-diphenyloxazole (PPO)-1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), and counted in a Packard liquid scintillation counter.

RESULTS

UA staining. The structural details of cyanophage LPP-1 are best seen with this stain. The virus has a hexagonal capsid and a tail of a length about one-fourth the head diameter, attached to one of the vertices (Fig. 1). Most of the head appears positively stained, whereas the light outer ring which is especially apparent on "ghosts" (Fig. 2a–f) appears in negative contrast. The ring measures 80 to 100 A in thickness. This agrees with the thickness of the halo surrounding those particles which frequently appear upon brief staining (20 sec) with 1% UA (Fig. 2g–i).

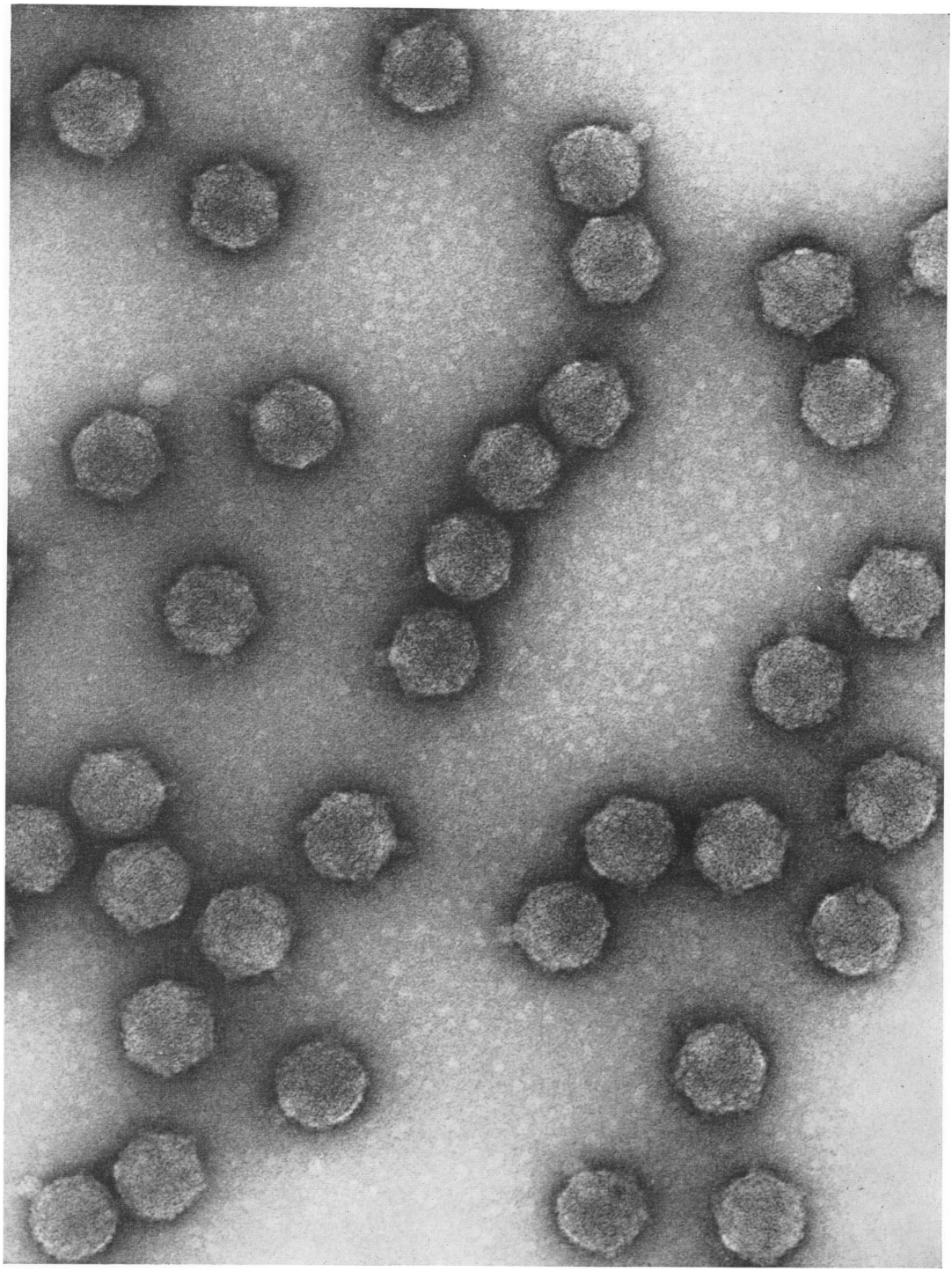

FIG. 1. *Particles of purified cyanophage LPP-1, stained with 2% UA. × 220,000.*

These particles are probably incompletely stained virions, since their total side-to-side diameter is 600 A, including the halo, and they are rarely seen upon longer exposure to stain. They constitute up to 50% of the field in briefly stained preparations.

It has been suggested that the purified short-tailed virus observed by negative staining (Fig.

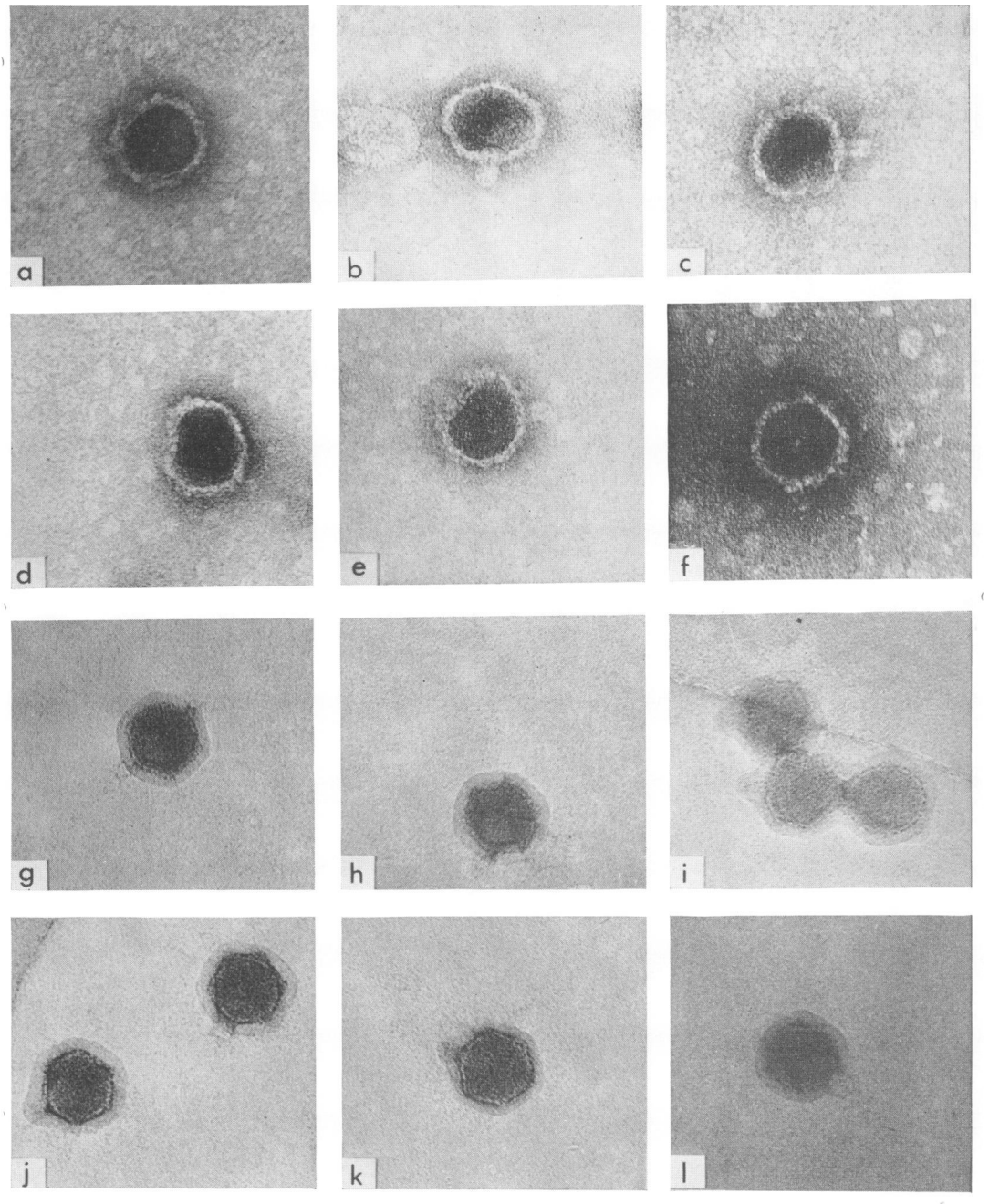

FIG. 2. *(a–f) "Ghosts" in purified cyanophage LPP-1 preparations stained with 2% UA. (g–l). Halo particles in purified cyanophage LPP-1 preparations briefly stained with UA.* × *220,000.*

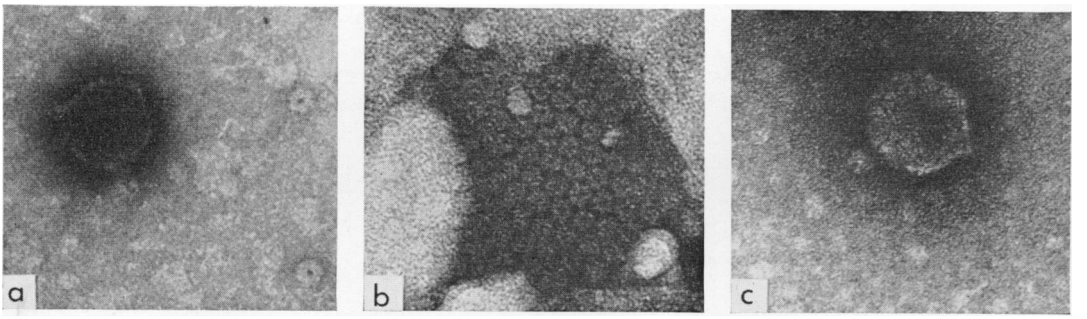

FIG. 3. (*a*) *Representative cyanophage LPP-1 particle from single plaque, stained with UA.* × *200,000.* (*b*) *Crystalline array of small particles (125 A in diameter), probably C-phycocyanin, in preparations of LPP-1 from plaques.* × *200,000.* (*c*) *Particle of cyanophage LPP-1 fixed with 10% Formalin prior to staining with UA.* × *220,000.*

1) may not represent the infectious virion but may be an artifact of virus purification or specimen preparation (26). We therefore performed the following experiments.

Isolation of virus from plaques. Virus was isolated from plaques on soft agar by the method of Bradley (4) and was stained with UA. The gross morphology of this specimen is identical to that of purified virus (Fig. 3a). Also, the two plaque morphology variants, r and r^+ (23), appear structurally identical. Because of the crudeness of these preparations, there is high background noise, including some small (125 A diameter) hexagonal molecules, alone or in a crystalline array (Fig. 3b). These are of some interest, as they have the same size and morphology as C-phycocyanin (3). The crystalline arrays have been observed several times, and in each case are found superimposed on fragments of what appear to be membranes.

Fixation of virus prior to staining. Figure 3c shows that virus previously fixed in 10% Formalin before UA staining exhibits the same morphology as unfixed particles. We show individual virus particles in Fig. 3 because the particle concentration was low in both the plaque-isolated specimens and the fixed specimens, so that individual fields show few particles. However, in both cases, over 90% of the particles are indistinguishable from those shown in Fig. 1.

Analysis of the virus band on a sucrose gradient. If infective phage has a longer tail than those particles shown in Fig. 1, as recently suggested by Smith et al. (26), it would sediment more slowly than the short-tailed particles. Therefore, a crude lysate was partially purified, with great care taken to avoid steps that might shear long tails, and then was examined on a sucrose gradient. The results of absorbancy and infectivity measurements are given in Fig. 4. The infectivity is

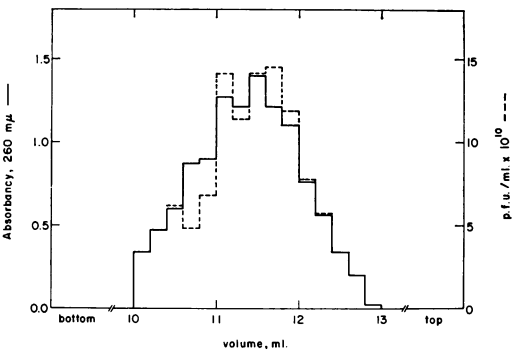

FIG. 4. *Zone centrifugation of gently prepared cyanophage LPP-1 on a sucrose gradient. Only the 3 ml containing appreciable absorbing or infectious material is shown, of the total of 30 ml in the gradient.*

directly correlated, within experimental error, to the absorbancy of the virus in each fraction, indicating that the physical particles and the infective particles have the same sedimentation coefficient. Electron microscopic examination of samples from fractions (2, 3) (5, 6) and (9, 10) showed only the short-tailed particles seen in Fig. 1.

Mg^{++} requirement. Infectivity of LPP-1 was irreversibly lost when Mg^{++} was omitted from the medium. This property of the infective virion can be directly observed with purified virus by electron microscopy of EDTA-treated virus (Fig. 10g–i). With the use of 0.35 M EDTA, all virions were disrupted within 10 sec, whereas, with 0.01 M EDTA, after 2 min less than 50% of the particles were broken. In most cases, fibers (presumably DNA) can be seen coming out of the head region, whereas the tail still remains attached to the particle.

Negative staining with PTA. The large majority

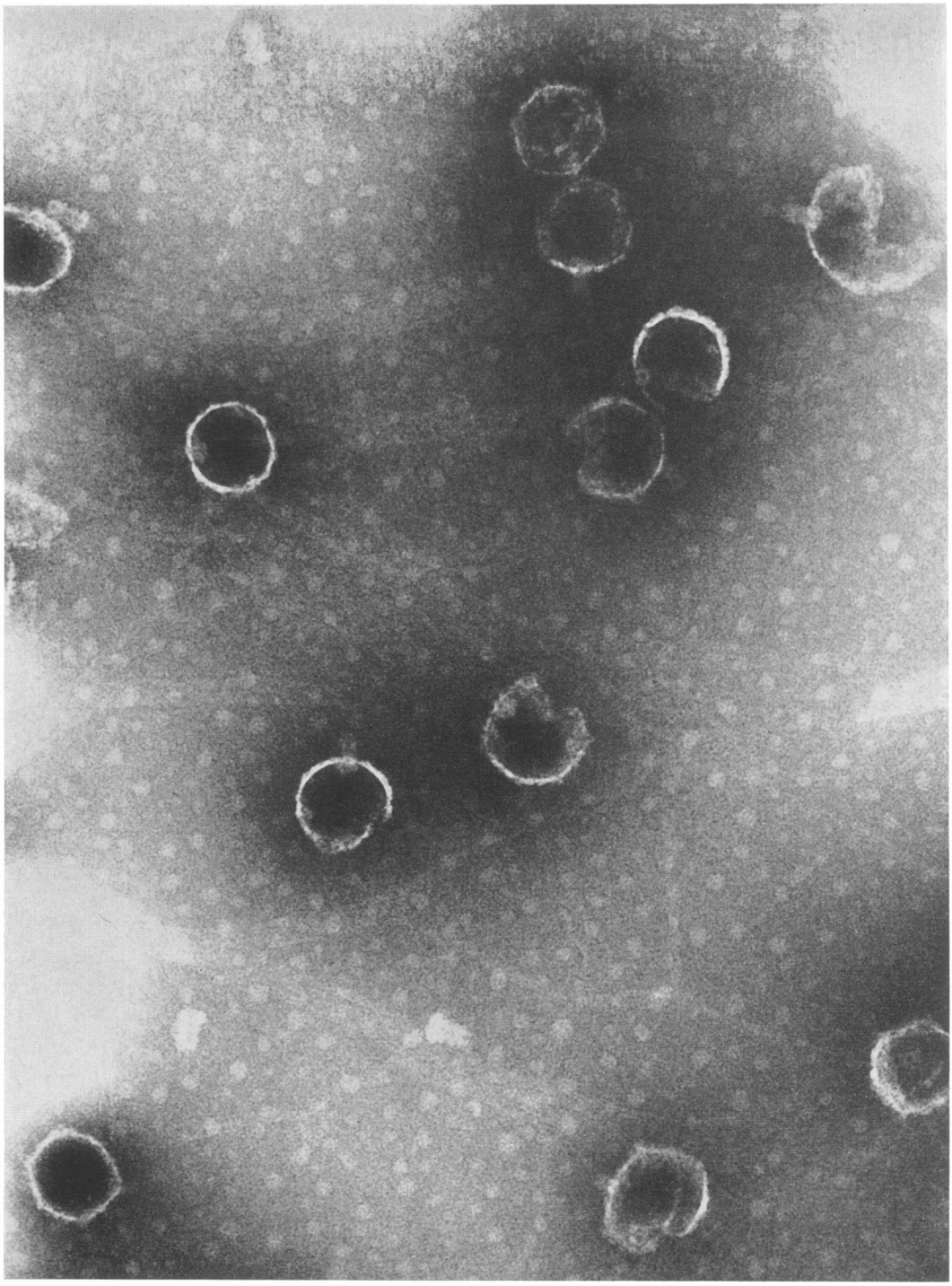

FIG. 5. *Particles of purified cyanophage LPP-1 stained with 1% PTA. Note swelling, disruption, DNA strands in the background, and two isolated tails toward the bottom of the field. At the top of the field is an isolated tail that may still be attached to a DNA filament.* × *220,000.*

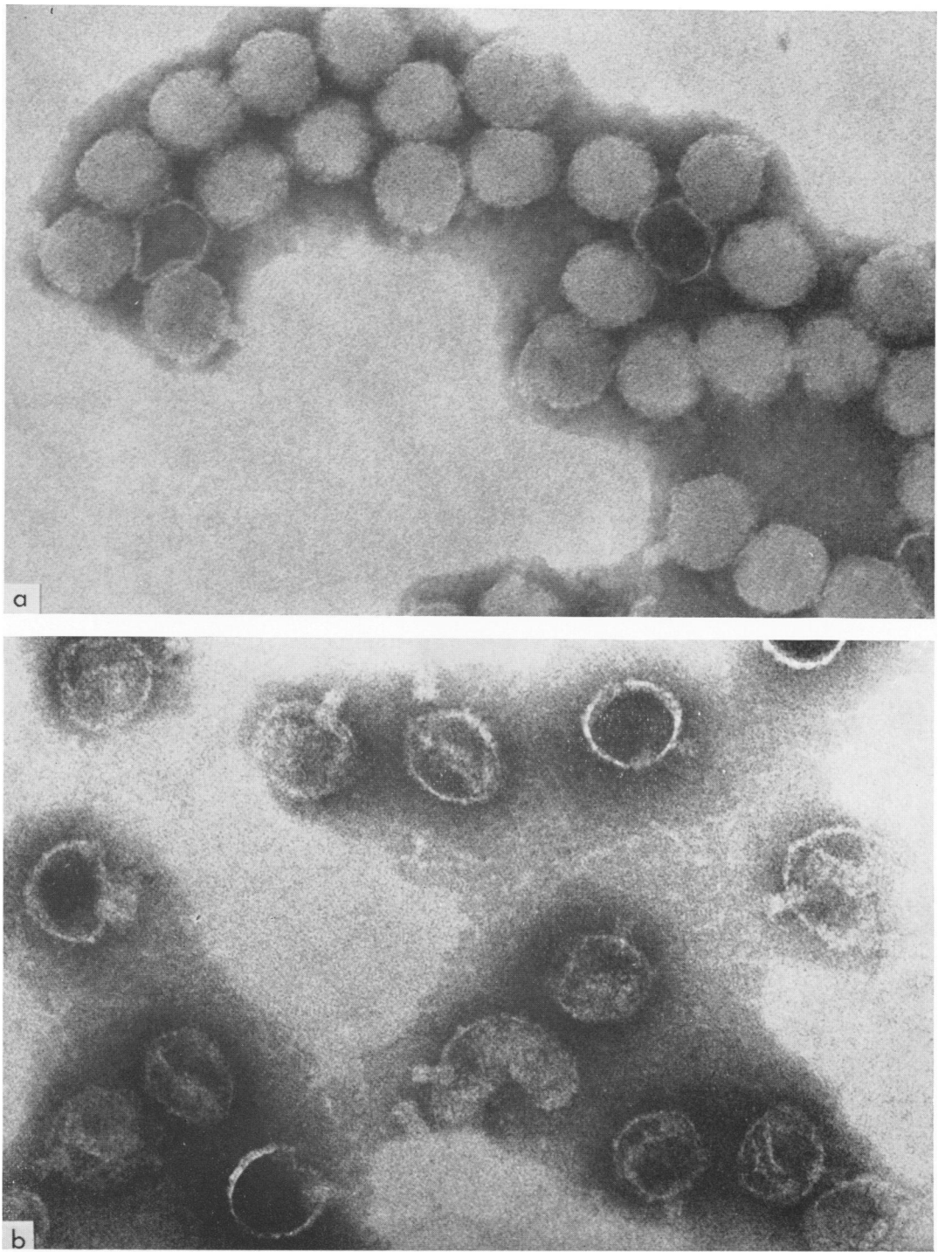

FIG. 6. *Effect of Mg^{++} upon disruption of cyanophage LPP-1 particles by PTA. Cyanophage LPP-1 dialyzed against 0.1 M Mg^{++} prior to staining (a) compared with LPP-1 dialyzed against 0.01 M Mg^{++} prior to staining (b). × 220,000.*

of virions stained with PTA for 2 min exhibited electron-dense heads (Fig. 5). Most of these heads appeared swollen or ruptured, similar to EDTA-treated virions stained with UA. In addition, the appearance of DNA-like strands in the background suggested that PTA might cause rupture of the virions by chelating Mg^{++}. To test this possibility, virus was dialyzed against 0.1 M Mg^{++}, before staining with PTA for less than 30 sec. In this case, 11 of 13 grids examined showed predominantly intact virions (Fig. 6a), whereas virus in 0.01 M Mg^{++} similarly stained with PTA yielded 13 of 19 grids with predominantly disrupted particles (Fig. 6b). Longer staining with PTA, in both cases, led to disrupted particles on 90% of the grids.

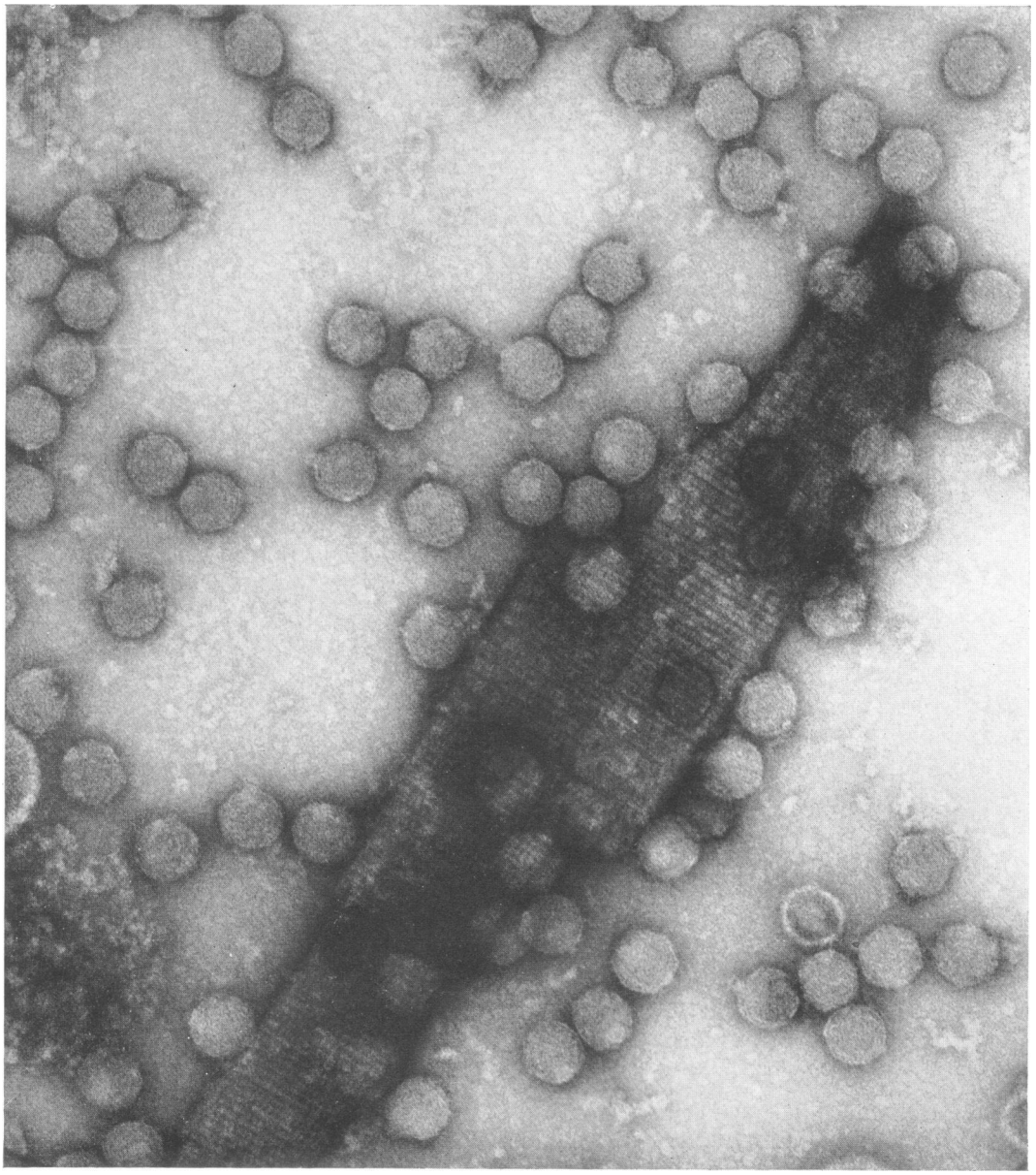

FIG. 7. *Particles of cyanophage LPP-1 stained with 2% UA and then mixed with stained catalase to provide an internal magnification standard. The catalase line spacing is 91 ± 3 A. × 146,000.*

Catalase crystal calibration for virus size determination. Safferman originally reported (22) that the average diameter of cyanophage LPP-1 was 660 A; in later work, this value was changed to 560 A (24). In view of this uncertainty, we measured the virus diameter by use of an internal magnification standard. In this technique, the magnification factor of a print is determined from the average periodicity of a catalase crystal measured over 10 to 15 lines on the print. At least three separate measurements are made per print. The need for an internal standard is evident from the fact that the catalase period varies over regions of the same grid. This is attributable to variations of thickness in the Formvar and carbon films as well as in the stain. For example, on two plates the catalase line spacings were 1.18 and 1.30 cm, and a typical virion in each measured 7.6 and 8.6 cm, respectively, indicating virus edge-to-edge distances of 586 and 595 A, respectively. Once the magnification was determined, virus particles near, but not under, the catalase crystal were measured in two directions of the hexagon, and only those particles for which this distance differed by less than 1% were selected as representative (Fig. 7). (The particles under the crystal seemed to be 20 A or more larger, perhaps owing to flattening.) The average edge-to-edge distance of 188 particles measured in two experiments was 597 ± 18 A. The histogram of one experiment (Fig. 8) was approximately Gaussian in character with a slight skew to the high side, which might be due to some flattening that occurs when the virus dries in the stain. The tail diameter was about 150 A with a length of 100 to 200 A.

Tail morphology. Four parts of the phage tail can be distinguished on virions stained positively with UA (Fig. 9) and negatively with PTA (Fig. 10a–f). First, there is a notched distal region (Fig. 9b–e, g–j, l, and 10g, h). Second, there is a hollow sheath which appears in two states, one extended (Fig. 9d, e, g, i, l, and 10b, c, e, g), and one shortened (Fig. 9a, b, c, f, h, j, and 10a, h). Third, there is a complex structure which we call a tail capital protruding into the head capsid (Fig. 5 and 10a, b, f, i). Finally, a tail core can be seen on those tails, still attached to head capsids, whose sheaths have shortened. In many cases, the tail sheath is compressed toward the *distal* region of the tail, appearing as two horizontal bands. The central cavity of the core is evident in both side views (Fig. 5, and 10a, b, c, e, i) and end-on views (Fig. 10d, f). We are fairly certain that the latter are really tails rather than contaminating phycocyanin, because they are seen in preparations of virus purified by banding twice in CsCl.

Molecular weight of cyanophage LPP-1 DNA. The molecular weight of the viral DNA was determined in two ways. One involved the sedimentation coefficient, and the other utilized the average contour length determined by the Kleinschmidt technique. The concentration dependence of the sedimentation coefficient of the viral DNA was determined at salt concentrations of 1 SSC and 4.8 SSC, with the results shown in Fig. 11. A microdensitometer tracing of a representative run is shown in Fig. 12. A least-squares analysis of the data in Fig. 11 is given in Table 1. Since the values at the two ionic strengths do not differ by more than the experimental error, we averaged them to give 33.4 ± 0.7 S for the purpose of calculating the molecular weight. When substituted into Studier's equation for native DNA (28), this value yields a molecular weight of 28.3 ± 1.7 × 10^6 daltons. We note from Fig. 11 that native DNA shows very little dependence of $S^0_{20,w}$ on ionic strength between 0.2 and 1, in agreement with Studier (28). We also observe a greater concentration dependence of $S_{20,w}$ at the lower ionic strength.

Native viral DNA molecules spread by Kleinschmidt's method (18, 21) are shown in Fig. 13. Measurements were made of such linear untangled molecules on various specimens, and the histogram (Fig. 14) represents contour lengths plotted in 0.1-μ divisions. Statistical evaluation of the data gives a length of 13.2 ± 0.5 μ, which corresponds to a molecular weight of 25.9 ± 1.0 × 10^6 daltons if the DNA is assumed to be in the crystallographic B form, with linear mass of 196 daltons per A (31).

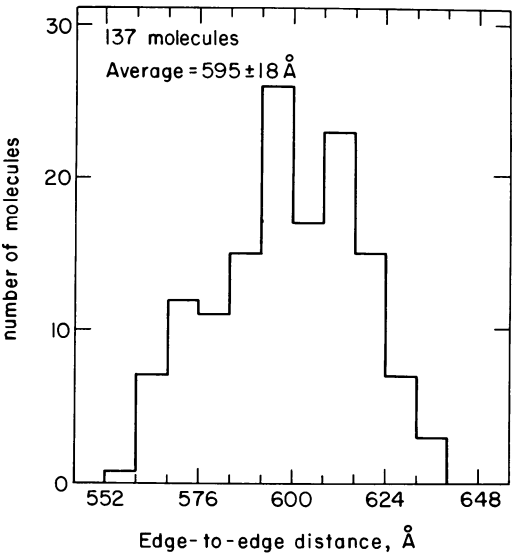

FIG. 8. *Histogram of cyanophage LPP-1 edge-to-edge distance measured on individual particles relative to the catalase line spacing (see text and Fig. 7).*

About 12% of the molecules are coiled, with a length approximately that of the linear forms. These appear in various stages of uncoiling (Fig. 15A, D, F), and are readily distinguishable from the rosette forms (Fig. 15B) seen in other systems (5, 11). Much more must be done, however, to determine what relation these coiled forms bear to the conformation of the DNA in situ.

G-C content of cyanophage LPP-1 DNA. The G-C content of the viral DNA was determined

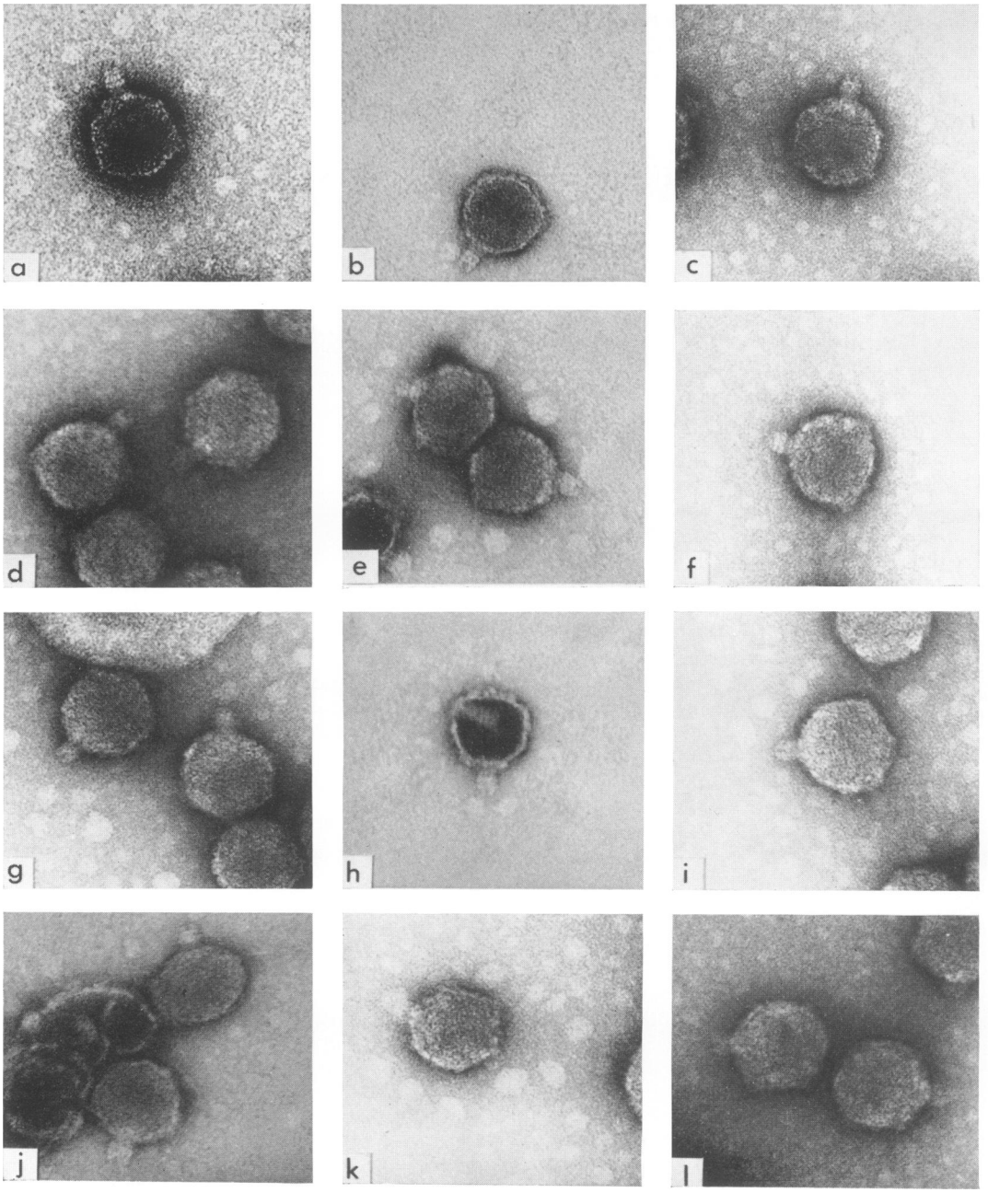

FIG. 9. *Fine structure of the cyanophage LPP-1 tail on particles stained with 2% UA.* × *220,000.*

from its buoyant density and from its thermal denaturation temperature. The density determined from CsCl equilibrium ultracentrifugation (Fig. 16) is 1.712 g/ml at 20 C, relative to SPO1 DNA of $\rho = 1.744$, by use of the relationship of Vinograd and Hearst (29). When corrected to 25 C by use of the formula of Vinograd and Greenwald (30), the value is 1.714 g/ml, corresponding to G-C = 53.7 ± 2.8%, by use of the equations of Vinograd and Hearst (29).

From the thermal denaturation profile of Fig. 17, the temperature for the midpoint of the transition is 75.5 ± 1 C in 0.1 SSC. For this solvent, using the equation: G-C = $(T_m - 53.9)(2.44)$ (M. Mandel, *personal communication*), this temperature corresponds to a G-C content of 52.7 ± 2%. This result is in sufficiently close agreement with the determination based on buoyant density to suggest that cyanophage LPP-1 DNA contains no odd bases as major constituents.

A sample of viral DNA was sent to W. Szybalski and Z. Hradecna, who kindly supplied us with the following information. Thermally denatured LPP-1 DNA exhibits a single band in CsCl at a density 0.012 g/ml higher than the native DNA. Mixing the denatured DNA with poly IG results in a shift to higher denisty of almost the entire denatured band; a small fraction, presumably broken fragments, does not shift. This behavior is to be contrasted with T7 or λ DNA, for which poly IG selectively shifts

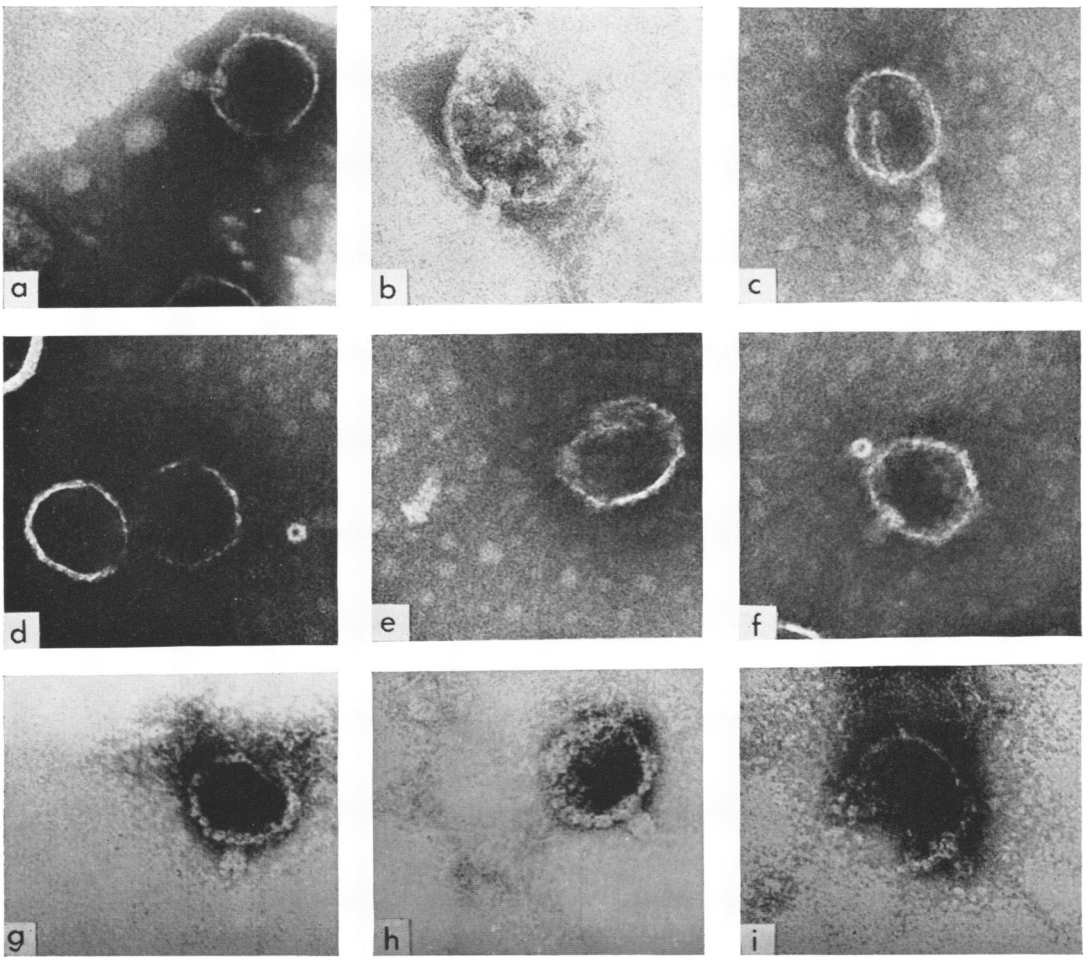

FIG. 10. *Fine structure of the cyanophage LPP-1 tail on purified, disrupted particles stained with 1% PTA (a–f). Figures d, e, and f come from the same field. Figures g, h, i are of EDTA-treated particles stained with 2% UA.* × *220,000.*

one of the strands to higher density (W. Szybalski and Z. Hradecna, *personal communication*).

Nucleic acid hybridization. It has been shown by Green (14), using DNA-RNA hybridization, that there is about 8% homology between bacteriophage λ and its host *E. coli*. As a first step in determining the relationship between cyanophage LPP-1 and its host, *P. boryanum*, we looked for homology between the two DNA preparations by DNA-RNA hybridization. RNA was synthesized in vitro by use of the viral DNA as template for the *E. coli* DNA-dependent RNA polymerase. This RNA was then annealed to DNA from cyanophage LPP-1, coliphage T7, and *P. boryanum*.

The *E. coli* RNA polymerase transcribes native coliphage DNA in a predominantly asymmetric manner (13). This was found to be true also for LPP-1 DNA as template: 86% of the RNA product was ribonuclease-sensitive after self-annealing at RNA concentrations which were saturating with respect to expression of self-complementarity.

To calculate the degree of homology, we annealed fixed amounts of each DNA with increments of cyanophage LPP-1 RNA as illustrated in Fig. 18, and extrapolated to the plateau of each curve. The per cent homology, expressed as the maximal weight fraction of RNA annealed, was 8.8, 0.25, and 0.12% for LPP-1, T7, and *P. boryanum* DNA, respectively. As the synthesis is predominantly asymmetric, this indicates about 18% homology between cyanophage LPP-1 DNA and RNA. The values of 0.25% for host DNA and 0.5% for T7 DNA indicate relative nonhomology with LPP-1 DNA. Since we do not know the absolute efficiency of annealing, it is not possible to state the extent to which the figure of 18% represents restriction of portions of the DNA to transcription by the *E. coli* polymerase in vitro. It is possible, of course, that *P. boryanum* DNA is homologous to regions of cyanophage LPP-1 DNA that were not transcribed in vitro.

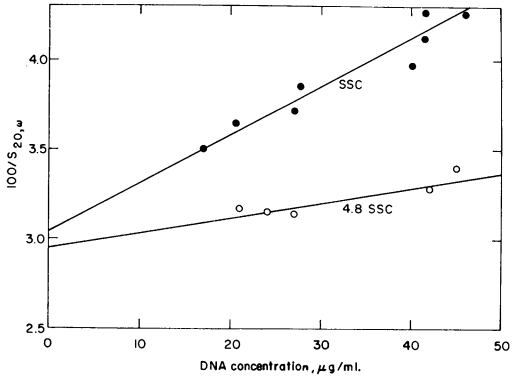

FIG. 11. *Concentration dependence of the sedimentation coefficient of cyanophage LPP-1 DNA at two salt concentrations.*

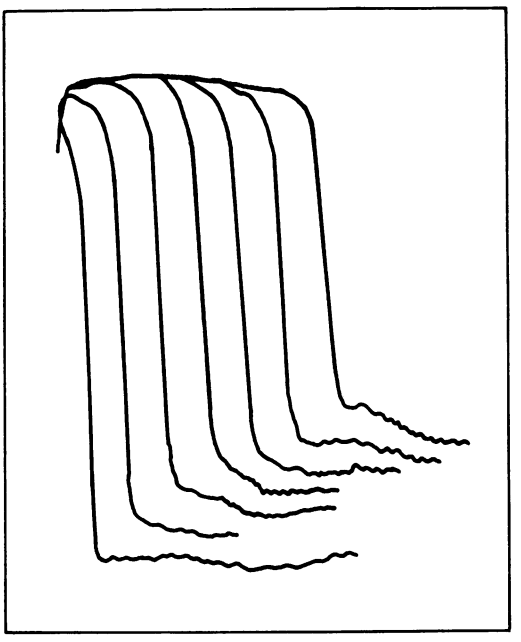

FIG. 12. *Microdensitometer tracing of ultraviolet absorption photographs of the boundary of cyanophage LPP-1 DNA at 40 μg/ml. Pictures were taken at 4-min intervals at 44, 770 rev/min.*

TABLE 1. *Least-squares analysis of the data in Fig. 11*

Determination	Salt	
	SSC	4.8 SSC
Slope	0.0270	0.0086
Intercept	3.04	2.95
$S^{\circ}_{20,w}$ (*S*)	32.9 ± .8	33.9 ± .5

DISCUSSION

On the basis of the electron microscope observations, we propose the structure shown in Fig. 19 for cyanophage LPP-1. The evidence for the details of this model has been presented in the Results, but a brief summary at this point is in order. We can clearly distinguish an outer coat for the head in Fig. 2 and the tail fork is seen on most particles of Fig. 9. The sheath and core regions are most evident on particles where the former appears shortened into two horizontal bands, e.g., Fig. 9a–c, whereas the capital and

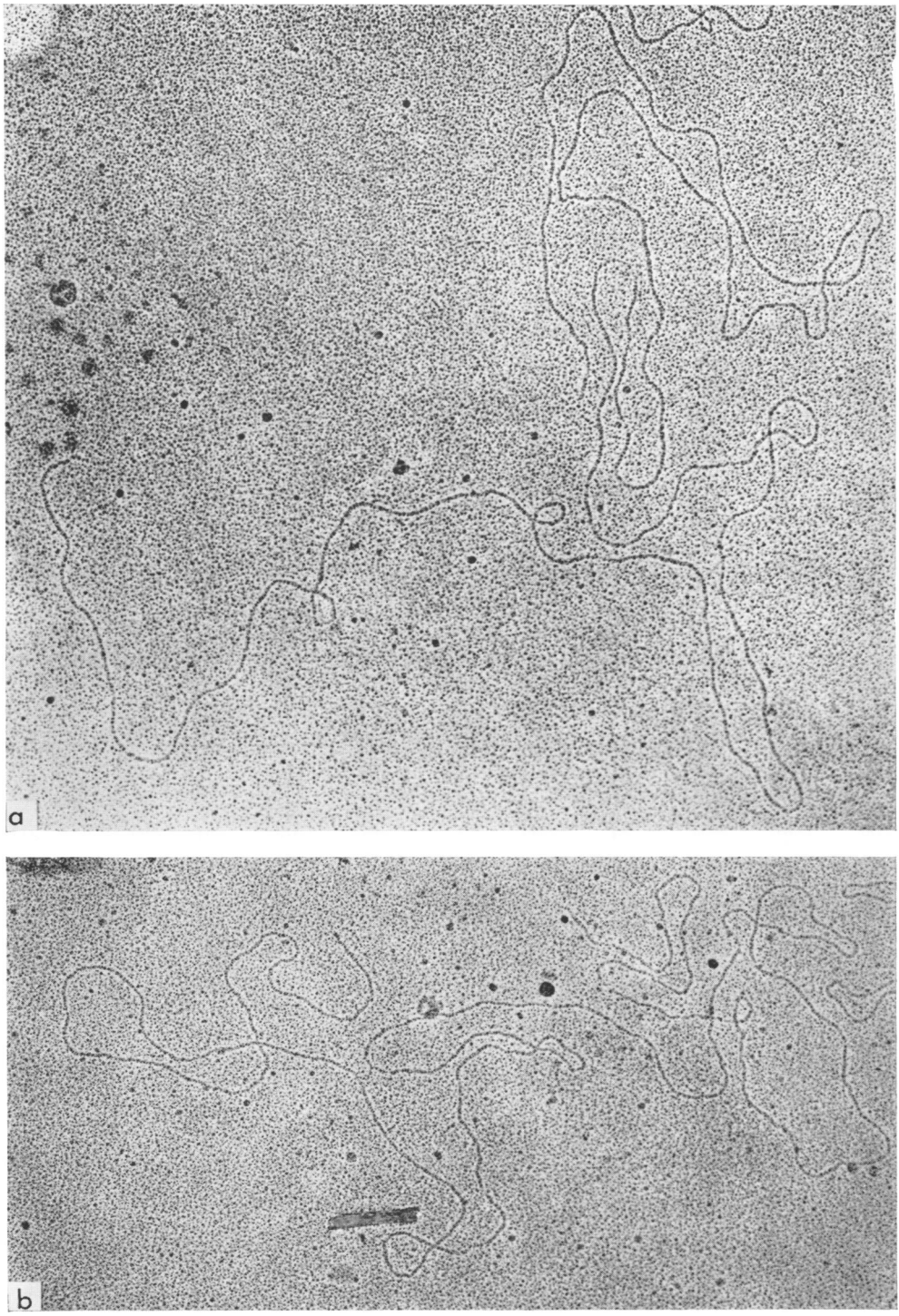

FIG. 13. *Two complete linear molecules of cyanophage LPP-1 DNA spread and shadowed by the Kleinschmidt method. That in (a) measures 13.4 μ, that in (b) 13.3 μ × 50,000 (a), × 42,000 (b).*

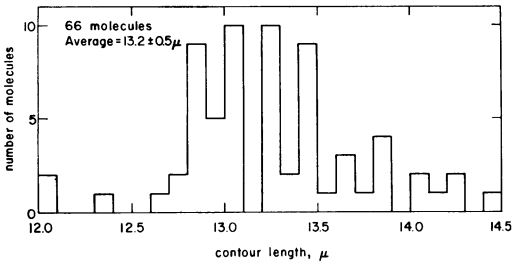

FIG. 14. *Histogram of cyanophage LPP-1 DNA molecular lengths.*

central cavity are best seen on disrupted particles stained by PTA (Fig. 5 and 10).

Head. The 400 to 440 A region inside the outer coat exhibits intense staining with UA, which indicates the presence of nucleic acid (17). There appears to be competition between this positive staining and negative staining of the protein subunits. The former effect is greatest on those particles whose heads contain a number of concentric circles (Fig. 9j–l and Fig. 1). This appearance is consistent with our observations of the coiled DNA molecules using the Kleinschmidt technique (Fig. 15). Since this DNA was prepared very gently, it is not inconceivable that the coiled molecules reflect the in situ packaging of DNA in the head of the virus. Several coils contain 10 or 11 concentric circles and, as the diameter of the DNA molecule is 20 A (27), tight packaging of the coil yields a diameter consistent with the inner region of the head. Finally, if the inner volume is approximated by a sphere 440 A in diameter, the total volume is about 4×10^7 A^3. This is precisely the total volume occupied by a cylinder of 20 A in diameter and 13 μ long.

Tail. We have demonstrated by several techniques that the free, infective virion has a short tail. Smith and co-workers have suggested that the short-tailed particles are artifacts, because their electron micrographs of crude lysates show several virus particles attached to membranes by what could be long tails (26). Padan and coworkers also showed many particles attached to membranes by tails whose total length is twice that we observed for the tail of the free virion (20). However, the pictures of Padan et al. show clearly that the extra length is contributed by the tail core, which protrudes from a sheath whose length agrees with our value for the tail. It therefore seems likely that the structure we propose describes the free virion, and that the tails on particles seen by Smith et al. and by Padan et al. owe their length to adsorption to membrane fragments.

Cyanophage LPP-1 DNA. The molecular weight calculated from the contour length is lower by about 10% than that calculated from the sedimentation coefficient. Since the histogram (Fig. 14) is skewed to the high side, there may be a preferential loss of long, linear molecules. An alternative explanation is that DNA examined by the Kleinschmidt technique may be trapped in a configuration intermediate between the B and A forms of DNA.

One of the main sources of interest in cyanophage LPP-1 is its possible use in transduction. Although appropriate genetic systems for exploring this possibility have yet to be developed, it seemed worthwhile to investigate host-virus nucleotide sequence homology by use of DNA-RNA hybridization. Comparison with Green's positive result for the λ-*E. coli* system (14) suggests that LPP-1 is an unlikely candidate for transduction of *P. boryanum*. Of course, it is still possible that *E. coli* RNA polymerase fails to transcribe the region of homology in LPP-1 DNA.

ACKNOWLEDGMENTS

One of us (R. L.) thanks H. Fernandez-Moran and E. F. J. van Bruggen for instruction in the techniques of electron microscopy. We are grateful to H. Fernandez-Moran for his initial research on this project and subsequent gifts of electron microscope materials, to L. Bogorad for the use of his electron microscope, to William Bloom for the use of his shadowing apparatus, to H. Ris for instruction in the Kleinschmidt technique, to R. Safferman for the original stocks of cyanophage LPP-1 and its hosts, and to G. Grofman for assistance in making prints.

This investigation was supported by Public Health Service research grant AI-06279 and training grants 5 T1 GM 780 and 1 T1 AI 238. R. H. holds a Research Career Development Award from the U.S. Public Health Service. R. L. is the recipient of the James Franck Dissertation Fellowship in Biophysics established by the Samuel Fels Fund.

LITERATURE CITED

1. ADAMS, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. ANDERSON, D. L., D. D. HICKMAN, AND B. E. REILLY. 1966. Structure of *Bacillus subtilis* bacteriophage φ29 and length of φ29 deoxyribonucleic acid. J. Bacteriol. **91**:2081–2089.
3. BERNS, D. S., AND M. R. EDWARDS. 1965. Electron micrographic investigations of C-Phycocyanin. Arch. Biochem. Biophys. **110**:511–516.
4. BRADLEY, D. E. 1965. The morphology and physiology of Bacteriophages as revealed by the electron microscope. J. Roy. Microscop. Soc. **84**:257–316.
5. CARO, L. G. 1965. The molecular weight of Lambda DNA. Virology **25**:226–236.
6. CRESPI, H. L., S. E. MANDEVILLE, AND J. J. KATZ.

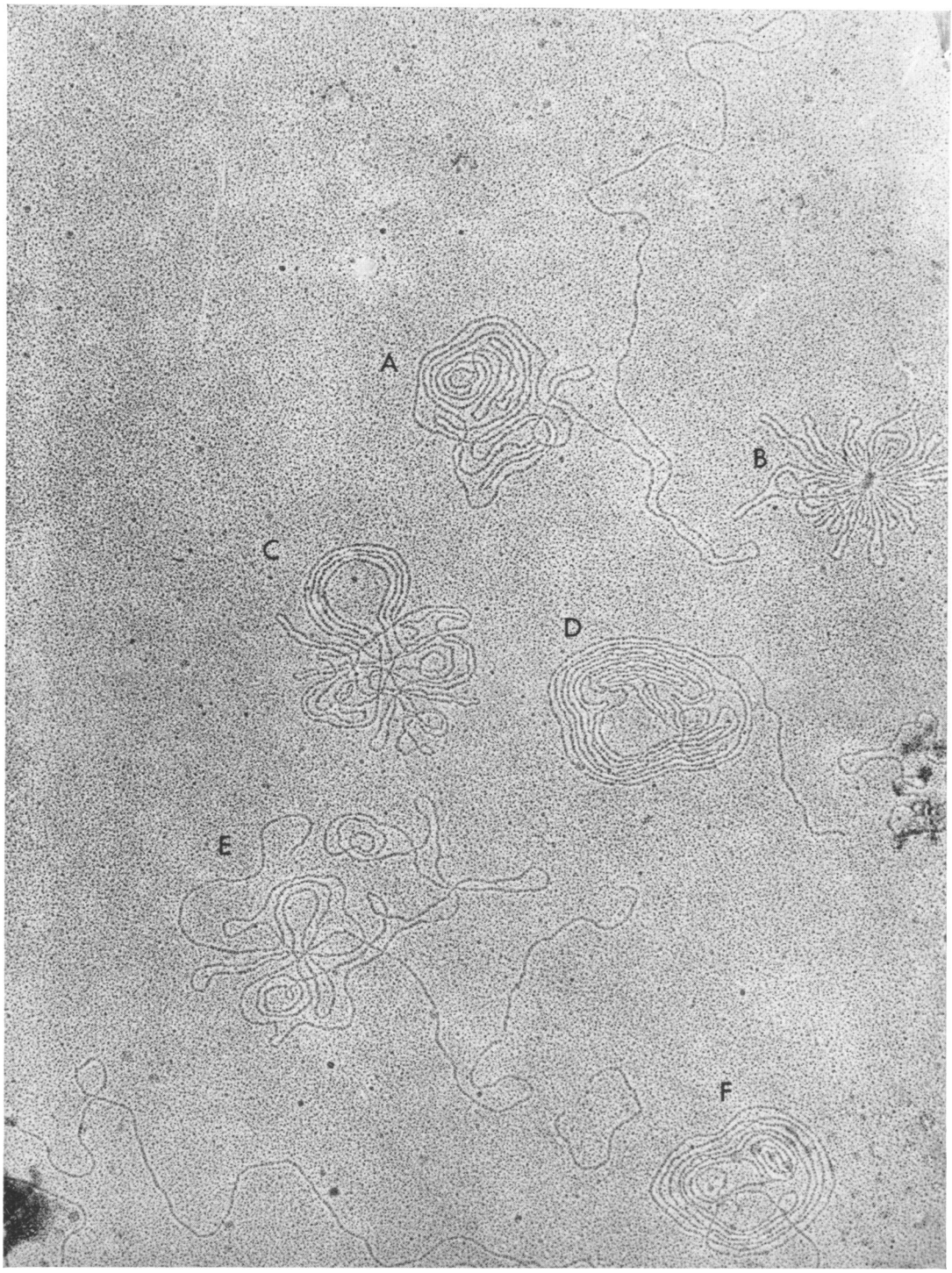

FIG. 15. *Various forms of cyanophage LPP-1 DNA spread and shadowed as in Fig. 13. A, C, D, E, and F appear to be stages in the uncoiling of the linear molecules. B is the rosette we believe to be an artifact.* × 42,000.

1962. The action of lysozyme on several blue-green algae. Biochem. Biophys. Res. Commun. **9**:569-572.

7. DAVISON, P. F., AND D. FREIFELDER. 1962. The physical properties of T7 bacteriophage. J. Mol. Biol. **5**:635-642.
8. DAVISON, P. F., AND D. FREIFELDER. 1962. The physical properties of the deoxyribonucleic acid from T7 bacteriophage. J. Mol. Biol. **5**:643-649.
9. FERNANDEZ-MORAN, H., E. F. J. VAN BRUGGEN, AND M. OHTSUKI. 1966. Macromolecular organization of hemocyanins and apohemo-

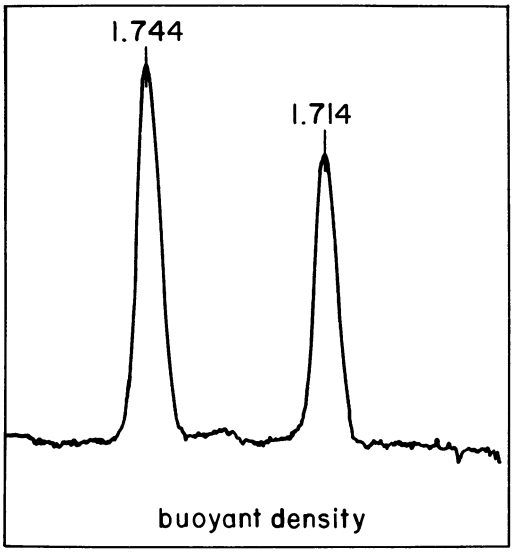

FIG. 16. *Equilibrium centrifugation of cyanophage LPP-1 DNA in CsCl, with an SP01 DNA density marker, corrected to 25 C.*

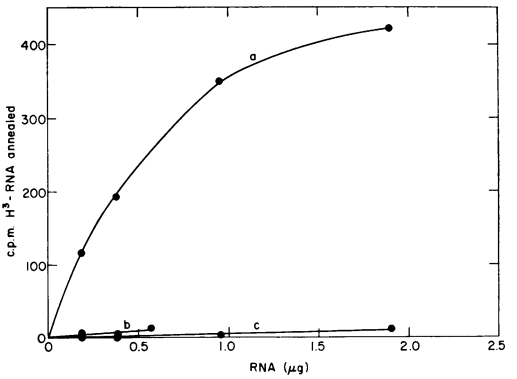

FIG. 18. *Hybridization of ^{3}H-RNA synthesized in vitro on a cyanophage LPP-1 DNA template, with (a) cyanophage LPP-1 DNA, (b) Plectonema boryanum DNA, and (c) bacteriophage T7 DNA. Each point in (a) and (c) represents hybridization with 0.25 μg of DNA; in (b), with 0.5 μg of DNA.*

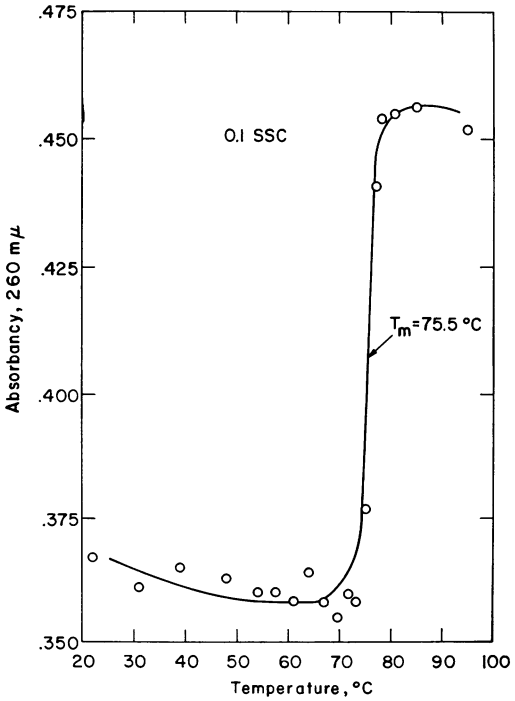

FIG. 17. *Thermal denaturation of cyanophage LPP-1 DNA in 0.1 SSC.*

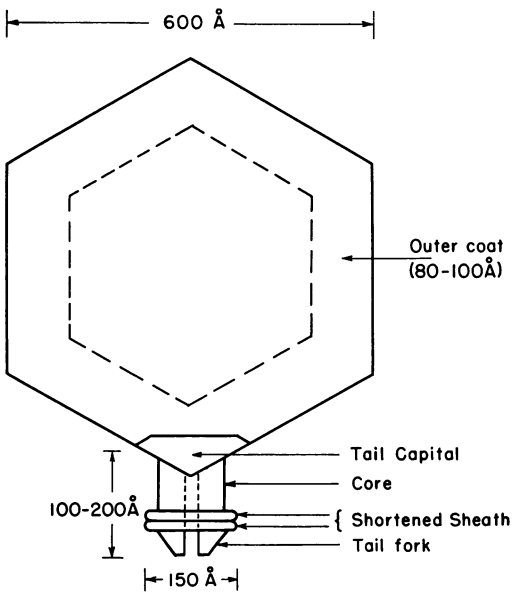

FIG. 19. *Schematic diagram of the structure of cyanophage LPP-1. DNA has been omitted from the head cavity. The tail sheath is shown compressed toward the distal end, a condition believed to be abnormal, but useful in revealing the tail core attached to the capital.*

cyanins as revealed by electron microscopy. J. Mol. Biol. **16**:191–207.
10. FREIFELDER, D., 1965. A rapid technique for the preparation of purified bacteriophage DNA from crude lysates. Biochim. Biophys. Acta **108**:318–319.
11. FREIFELDER, D., AND A. K. KLEINSCHMIDT. 1965. Single-strand breaks in duplex DNA of coliphage T7 as demonstrated by electron microscopy. J. Mol. Biol. **14**:271–278.
12. GEIDUSCHEK, E. P. 1961. "Reversible" DNA. Proc. Natl. Acad. Sci. U.S. **47**:950–955.
13. GEIDUSCHEK, E. P. 1965. Some aspects of RNA synthesis on a DNA template. Bull. Soc. Chim. Biol. **47**:1571–1577.
14. GREEN, M. H. 1963. Complementarity between lambda (λ) phage and *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **50**:1177–1184.
15. HEARST, J. E. 1962. The specific volume of various cationic forms of deoxyribonucleic acid. J. Mol. Biol. **4**:415–417.
16. HOAGLAND, D. R., AND D. ARNON. 1950. Calif. Agr. Expt. Sta. Circular No. 347.
17. HUXLEY, H. E., AND G. ZUBAY. 1961. Preferential staining of nucleic acid containing structures for electron microscopy. J. Biophys. Biochem. Cytol. **11**:273–296.
18. KLEINSCHMIDT, A. K., S. J. KASS, R. C. WILLIAMS, AND C. A. KNIGHT. 1965. Cyclic DNA of Shope papilloma virus. J. Mol. Biol. **13**:749–756.
19. NYGAARD, A. P., AND B. D. HALL. 1963. A method for the detection of RNA-DNA complexes. Biochem. Biophys. Res. Commun. **12**:98–104.
20. PADAN, E., M. SHILO, AND N. KISLEV. 1967. Isolation of phycoviruses from freshwater ponds and their interaction with *Plectonema boryanum*. Virology, *in press*.
21. RIS, H., AND B. L. CHANDLER. 1963. The ultrastructure of genetic systems in prokaryotes and eukaryotes. Cold Spring Harbor Symp. Quant. Biol. **28**:1–8.
22. SAFFERMAN, R. S., AND M. MORRIS. 1963. Algal virus: isolation. Science **140**:679–680.
23. SAFFERMAN, R. S., AND M. MORRIS. 1964. Growth characteristics of the blue-green algal virus LPP-1. J. Bacteriol. **88**:771–775.
24. SCHNEIDER, I. R., T. O. DIENER, AND R. S. SAFFERMAN. 1964. Blue-green algal virus LPP-1: purification and partial characterization. Science **144**:1127–1130.
25. SMITH, K. M., R. M. BROWN, JR., D. A. GOLDSTEIN, AND P. L. WALNE. 1966. Culture methods for the blue-green alga *Plectonema boryanum* and its virus, with an electron microscope study of virus-infected cells. Virology **28**:580–591.
26. SMITH, K. M., R. M. BROWN, JR., P. L. WALNE, AND D. A. GOLDSTEIN. 1966. Electron microscopy of the infection process of the blue-green alga virus. Virology **30**:182–192.
27. STOECKENIUS, W. 1961. Electron microscopy of DNA molecules stained with heavy metal salts. J. Biophys. Biochem. Cytol. **11**:297–310.
28. STUDIER, F. W. 1965. Sedimentation studies of the size and shape of DNA. J. Mol. Biol. **11**: 373–390.
29. VINOGRAD, J., AND J. E. HEARST. 1962. Equilibrium sedimentation of macromolecules and viruses in a density gradient. Progr. Chem. Org. Nat. Prod. **20**:372–422.
30. VINOGRAD, J., AND R. GREENWALD. 1965. Effect of temperature on the buoyant density of bacterial and viral DNA in CsCl solutions in the ultracentrifuge. Biopolymers **3**:109–114.
31. WILKINS, M. H. F. 1963. Molecular configuration of nucleic acid. Science **140**:941–950.