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Cyanophages—Viruses Attacking Blue-Green Algae

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INTRODUCTION

Hardly a decade has passed since Safferman and Morris (106) reported the discovery of a viral agent which attacks and lyses several species of blue-green algae. These agents, now known to attack a wide range of blue-green algae, have been variously called phycoviruses (113), algophages (42), blue-green algal viruses (77, 107), and cyanophages (77, 94). The latter designation seems most apt because of their close analogies to bacteriophages and the now widely accepted close affinity of the Cyanophyta to the bacteria (143).

The significance and implications of the cyanophages have been faithfully reflected in the volume and range, both geographical and disciplinary, of the investigations undertaken of the alga-cyanophage system. In undertaking this review of cyanophages, we are well aware of the great many questions still open to inquiry and have endeavored to indicate them in the light of the results summarized from published and some as yet unpublished data.

The interest in the cyanophages stems in no small measure from the importance, both theoretical and practical, of their hosts. The blue-green algae occupy a special position in the biological world. They resemble bacteria in their prokaryotic cellular structure. However, the photoautotrophic metabolism of the blue-green algae resembles that of eukaryotic plants. The blue-green algae are among the most ancient organisms on earth, appearing in Middle Precambrian rock and, perhaps, earlier (115). Nevertheless, a monophyletic origin and limited genetic diversification during evolution of the filamentous blue-green algae are suggested by

their uniform deoxyribonucleic acid (DNA) base ratio (31).

Until the discovery of the cyanophages, photoautotrophic metabolism under viral infection could only be studied in tissues of higher plants. Although not isolated, viruses attacking eukaryotic algae have been indicated in several works, as mentioned in a recent review on algal viruses (18). Only recently a virus attacking a photosynthetic bacterium has been isolated (34). Data collected from studies of higher plant viruses on their interaction with the host were contradictory and could not be interpreted in a consistent fashion. This is because the plant consists of different tissue systems, which are difficult to isolate in tissue cultures and do not allow for homogenous infection. Cyanophages offer an advantage by providing a system that resembles that of bacteriophages. Furthermore, because the photosynthesis of blue-green algae is like that of higher plants, the alga-cyanophage system can be adopted as a model for studying plant photosynthesis under viral infection.

Blue-green algae are widespread in the aquatic environment and often occur in mass blooms related to eutrophication of water bodies. The economic and nuisance effects of blue-green algae on water quality and in causing fish intoxications have been amply documented (33, 122, 152). The probable influence of cyanophages on the geographical and seasonal distribution, as well as population dynamics, of blue-green algae, and their possible use in biological control of blue-green algae, added a further impetus to investigations of the cyanophages.

CHARACTERISTICS OF CYANOPHAGES

Typology

The cyanophages isolated so far have been named according to their known hosts. Accordingly, cyanophage groups were designated by the initials of the generic names of the hosts, to which arabic numerals have been added to designate serological subgroups (111); different isolates of similar types were marked by a suffix consisting of the first letter of the provenance (26, 37, 111).

It is accepted that the criterion of host specificity is less valid for classification of viruses than that of serological or morphological properties (82). The hazard in the host specificity criterion is obvious, because certain properties of bacteriophage systems, such as host range and types of host responses, are more subject to mutational changes than others; such frequent mutational changes occur in the cyanophage systems as well (44, 95, 131). An additional difficulty is the confusing state of the taxonomy of most blue-green algae, whose classification has been mainly determined through microscopic examination of field collections and even fixed specimens. Only recently, R. Y. Stanier and his co-workers proposed an integrated classification of unicellular blue-green algae based on morphological and physiological-biochemical properties of axenic cultures (142) and, in a like manner, they have begun to classify the filamentous blue-green algae (59). In fact, it is conceivable that cyanophages could aid in this classification of the blue-green algae (discussed below under "Host Range"). Accordingly, the presently-used nomenclature of cyanophages may be misleading. For example, the cyanophage called "SM-1" (112) was named after *Synechococcus elongatus* and *Microcystis aeruginosa* (strain NRC-1); it now seems questionable whether *M. aeruginosa* is a host since the strain NRC-1 is most probably a misclassified *Synechococcus* (142). Thus, the present cyanophage nomenclature should be regarded simply as code names without taxonomic or evolutionary significance.

Serological specificities and morphological properties (Fig. 1) of the cyanophages will be regarded here as the chief criteria for the groupings of the four main typological groups that may be defined at present (Table 1).

The LPP group is subdivided into two serologically distinct subgroups, LPP-1 and LPP-2 (111). This accords with the finding that on lysogeny of *Plectonema boryanum* with LPP-2 SPI, the alga was found to be immune only to cyanophages of the LPP-2 group and not to any of the LPP-1 strains (96). Because only one virus is known for each of the SM, N, and AS groups, they have been designated SM-1, N-1, and AS-1, respectively.

A long-tailed virus A-1, active against *Anabaena variabilis*, was observed by Kozjakov, Gromov, and Khudiakov of Leningrad University (64). A case has been reported of a cyanophage attacking *Anabaenopsis raciborskii* (130, 132), which may resemble phage trapping in bacterial spores (140, 149, 162). Reports of discoveries of other cyanophages have been published (41–43, 104, 130, 131, 150). These data were not sufficient to allow typological descriptions.

Because cyanophages are very similar to bacteriophages both in structure and infection cycle (see below), it is suggested that they should be considered a subgroup of the bacteriophages.

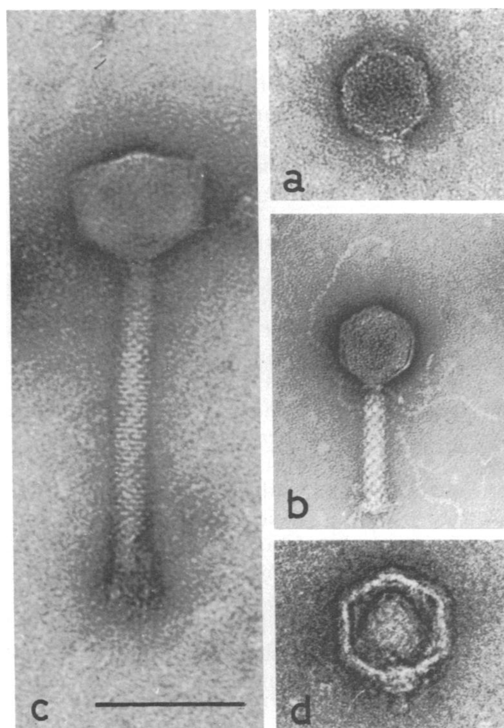

FIG. 1. *Cyanophages LPP-IG (a) and N-1 (b) (reference 4) negatively stained with 1% aqueous uranyl acetate; AS-1 (c) negatively stained with 4% aqueous uranyl acetate (from reference 106); SM-1 (d) negatively stained with saturated uranyl formate (reference 83); scale represents 100 nm.*

Host Range

LPP cyanophages attack strains of blue-green algae originally classified in the three different genera of the filamentous blue-green algae that do not form heterocysts, *Lyngbya*, *Phormidium*, and *Plectonema* (107, 109). The base ratio (46% G + C) and density (1.705 g/cm³) of DNAs of susceptible representatives of these genera are very similar, as in all members of the order Oscillatoriales (31). The striking uniformity of the base ratios of Oscillatoriales, in contrast to those of unicellular blue-green algae, led Edelman et al. (31) to suggest that this group has a common evolutionary origin and may even be conservative in having undergone limited genetic diversification during evolution.

Morphological heterogeneity allowed the separation of *Lyngbya* and *Phormidium* from *Plectonema* into distinct suborders (136). Guided by the notion that organisms with a common viral host range might be phylogenetically related, Cowie and Prager (25) investigated the DNAs of representatives of the different host genera by means of DNA-DNA-agar thermal chromatography. They found that nucleotide homologies exist among the DNAs of these algae. Moreover, a high degree of precision of base pairing was shown in the reacting nucleotide sequence. Thus, they suggested that these hosts of LPP cyanophages are closely related and may be classified in the same suborder and family (Oscillatoriaceae). A re-

TABLE 1. *Typological groups of cyanophages*

Reaction with antisera and morphological properties	Code name				
	LPP-1	LPP-2	SM-1	N-1	AS-1
LPP-1	+[a]	−[a]	−[b]		−[c]
LPP-2	−[a]	+[a]			−[c]
SM-1	−[b]	−[b]	+[b]		−[c]
AS-1	−[c]	−[c]	−[c]		+[c]
Morphological properties					
Head	Polyhedron six-sided outline[d]		Icosahedron[e]	Polyhedron six-sided outline[f]	Polyhedron six-sided outline[c]
Edge-to-edge diameter	60 ± 2 nm		67 ± 1.8 nm	55 nm	90 nm
Tail	Short, noncontractile[d]		Very short collar with thin appendage[e]	Long, contractile[f]	Long, contractile[c]
Length, width	20 nm, 15 nm			110 nm, 16 nm	243.5 nm, 22.5 nm

[a] Safferman et al. (111).
[b] Safferman et al. (112).
[c] Safferman et al. (106).
[d] Luftig and Haselkorn (77, 78).
[e] Mackenzie and Haselkorn (83).
[f] Adolph and Haselkorn (4).

classification of members of this family groups these algae into a single species, *Schizotrix calcicola* (28). Moreover, the new classification of the filamentous blue-green algae (59) based on physiological and morphological criteria also places these genera close to one another.

The blue-green alga designated *Lyngbya* sp. strain 621 is neither sensitive to LPP-1 nor does its DNA show homology with DNAs of other LPP-1 hosts (25). It is most probable that this strain might have been misclassified. Additional isolates (named either *Plectonema*, *Lyngbya*, or *Phormidium*) in different algal collections were unaffected by LPP-1 (26, 107, 109). These algae might also have been misclassified. However, it is possible that they are different variants of the host (such as resistant mutants and lysogenic strains) which are not susceptible to the virus.

Cyanophage SM-1 attacks *S. elongatus* (112), a unicellular blue-green alga that may form short filaments in different culture conditions and growth stages. The DNA of this alga has 66 to 67% G + C content (83) and a density of 1.73 g/cm^3 (142), which are the highest among the Chroococcales. Similar to other unicellular organisms, the Chroococcales exhibit a wide range of DNA base composition (35 to 70% G + C), demonstrating the heterogeneity of this group and suggesting a polyphyletic origin and/or intensive genetic diversification during evolution (31, 142).

Cyanophage AS-1 attacks strains designated *Anacystis nidulans* and *Synechococcus cedroum* (106). Although classified within two distinct genera, these algal species show marked morphological, biochemical, and physiological similarity and were classified in one typological group (142). This accords with their susceptibility to AS-1.

Cyanophage N-1 attacks the filamentous strain *Nostoc muscorum* (4) having typical differentiated cells, the heterocysts, among the vegetative cells. Other isolates assigned to *N. muscorum* are insensitive to N-1, but again these may represent either misclassification, different *Nostoc* species, or resistant variants.

It appears evident that the several cyanophage groups isolated so far each have rather narrow host ranges. Taking into account the existence of nonsusceptible variants and host-range mutants of the viruses, this specificity of the cyanophages could aid in typing algal strains and in reorganization of the blue-green algal systematics, in the same way as staphylococcus phages have been used for typing strains of *Staphylococcus aureus* and other related species (157).

Morphology

Cyanophages of the LPP group isolated so far are virions of the head-tail type and are identical in structure to one another (6, 26, 77–79, 95, 111). A typical representative of the LPP group is shown in Fig. 1a. The morphology of LPP-1 has been the most extensively studied, both on intact particles and on separate head and tail preparations. A model of LPP-1 suggested by Luftig and Haselkorn (78) is shown in Fig. 2. The viral head capsid is a polyhedron, appearing hexagonal in projection. The edge-to-edge distance of the viral head is 58.6 ± 2.1 nm (111). The detailed structure of the head has recently been studied by Adolph and Haselkorn (6) who suggested an icosahedral form and models for its construction from the capsomers. A short tail (20 nm long, 15 nm wide) is attached to one of the vertices of the head (78). Transverse and side views of isolated tails led to the suggestion that the tail is a hollow cylinder with sixfold radial symmetry made up of several (two to four) rings of six subunits. However, the orientation of the rings with respect to each other along the tail is still unknown. If the rules of symmetry apply, as in bacteriophage structure (16), then the observed sixfold radial symmetry of the tail requires similar symmetry in the head

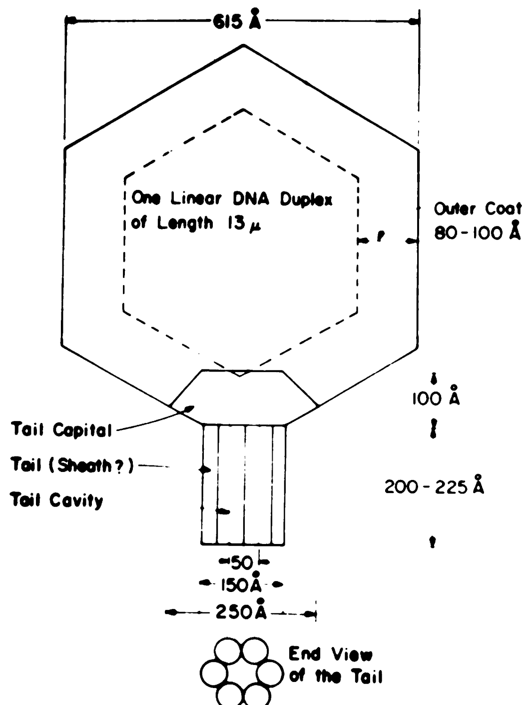

FIG. 2. *Model of LPP-1 proposed by Luftig and Haselkorn (78).*

or, as suggested by Moody (86) with reference to the T-even phages, an intermediate structure adapting the unlike head and tail symmetries. At present it is impossible to choose between these alternatives. Another unsolved problem is the role of the tail capital that protrudes into the head capsid at the head-tail junction (78). This may be either a simple mechanical joint to fix the tail to the head and/or the asymmetry adaptor suggested above.

Specimens of either LPP-1 or LPP-1G obtained from plaques and examined by negative staining (95, 137) contain long-tailed (40 nm) viral particles attached to host cell-membrane vesicles (Fig. 3). Frequently the particles also form rosettes around the vesicles. Long-tailed particles were also observed to be adsorbed onto the cell wall in sections (138, 139). Because Luftig and Haselkorn (77) observed only the short-tailed virions in isolated and purified phage preparations obtained from lysates of liquid culture or from plate lysates, it is unlikely that the long-tailed particles represent the free virions. Only heads and short-tailed viral particles were also observed inside the infected cells burst prematurely by lysozyme (described below under "Morphological Aspects of the Cyanophage Infection Process"). Although the possibility of the long tail being an artifact cannot be completely ruled out, it is most unlikely because such forms have been observed both in sections (137, 139) and in direct examination after negative staining (95, 139). It is more likely that the long-tailed particles represent a stage of the infective process which is preserved best when the infection is carried out on agar and examined directly by negative staining of plaques. Accepting the short-tailed virion as the prototype of LPP cyanophages, they may be included in group C of bacteriophage types defined by Bradley (16) as "short noncontractile tail and head with six-sided outline." Several investigators have stressed the similarity of LPP to T7 (6, 79) and T3 (40) bacteriophages, both in dimensions and morphology.

Cyanophage N-1, shown in Fig. 1b, has a hexagonal head in outline with an edge-to-edge diameter of 55 nm, whose detailed structure is still unresolved (4). A long tail (110 nm long, 16 nm wide) is attached to one of the head vertices and consists of an internal core and a contractile sheath which appears striated when extended. A distinct structure at the head-tail junction

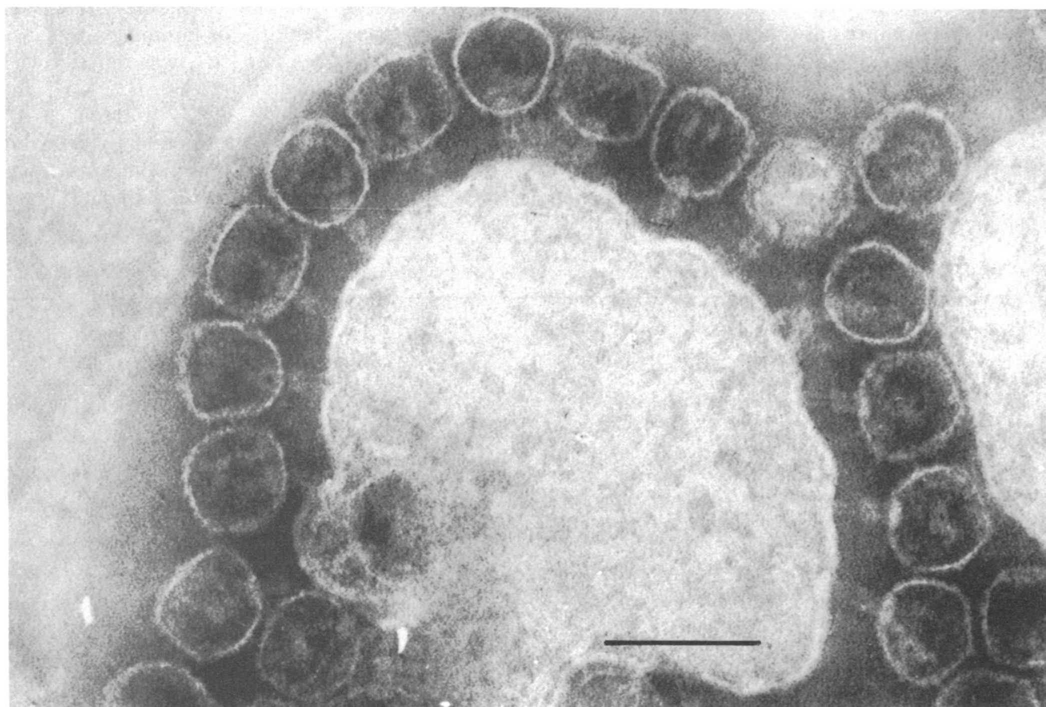

FIG. 3. *Cyanophage LPP-1G adsorbed on vesicles of lysed Plectonema cells. The viruses have long tails, empty heads, and tail capitals and were negatively stained with 1% aqueous uranyl acetate; scale represents 100 nm (from reference 95).*

(head capital) was also observed. The structure of N-1 is very similar to the basic morphological bacteriophage type A designated by Bradley (16) as "contractile tail with six-sided outlined head."

The largest cyanophage yet observed is AS-1, shown in Fig. 1c. The head is hexagonal in outline with an edge-to-edge diameter of 90 nm. A long tail (243.5 nm long, 22.5 nm wide), attached to one of the head vertices, consists of a base plate to which short pins are attached, a contractile sheath, and an internal core (106).

Cyanophage SM-1 (Fig. 1d) has an icosahedral head capsid with an edge-to-edge diameter of 67 ± 1.8 nm. A very short collar is attached to the head, from which a thin appendage, which could be a tail, protrudes (83). Particles damaged by phosphotungstic acid appear to have a thin inner membrane, in addition to the principal capsid shell (83). SM-1 particles attached to the cell wall appear to have longer tails than those of the free phage (84), similar to LPP-1G described above.

Composition

Nucleic acids. All nucleic acids of cyanophages analyzed so far are linear double-stranded DNAs. The physicochemical characteristics of the DNAs of cyanophages and their hosts are summarized in Table 2.

Because there is a good agreement in the % G + C content values calculated from buoyant density and melting points of DNA in LPP-1 (77), N-1 (4), and SM-1 (83), these viruses probably contain no odd bases as major constituents. Resolution of DNAs of host and virus during infection has already been achieved for the LPP-1-*Plectonema* system using the equilibrium density gradients (118). The difference in buoyant density values of N-1 and *Nostoc* (cf. Table 2) suggests that equilibrium density gradients can also be employed for resolution of their DNAs during infection. The very high density of *Synechococcus* DNA, similar to that of SM-1 DNA, suggests that this is not possible in this case (83).

The presence of double-stranded DNA in LPP-1, N-1, AS-1, and SM-1 conforms with Bradley's (16) prediction that head-tail bacteriophages contain only this type of nucleic acid.

Thermally denatured DNA of LPP-1 exhibits a single band in CsCl at a density 0.012 g/ml higher than the native DNA. Unfortunately no separation of the strands could be achieved by mixing the denatured DNA with poly IG (77). This behavior contrasts with T7 or λ DNA for which poly IG selectively shifts one of the strands to a higher density.

In a search for common evolutionary patterns among cyanophages and their hosts, Cowie and Prager (25) found no homology between DNAs of LPP-1 and its hosts (when using DNA-DNA-agar thermal chromatography). A similar conclusion was reached indirectly by Luftig and Haselkorn (77). It would be most interesting to study homology in the lysogenic blue-green algae systems (21, 96). No homology was evident when the DNA of LPP-1 was reacted with the DNAs of λ, 434 hybrid, φ80, P22 (25), and T7 (79).

Proteins. The major proteins of LPP-1 were characterized by sodium dodecyl sulfate-gel electrophoresis of bulk viral proteins and separate head-tail preparations (78, 119). The major

TABLE 2. *Physicochemical characteristics of the DNA of the cyanophages and typical hosts*

Physicochemical character	LPP-1	N-1[a]	SM-1[b]	AS-1[c]	*Plectonema boryanum*	*Nostoc muscorum*	*Synechococcus elongatus*	*Synechococcus cedrorum*
Molecular weight	27×10^6 [d, e]	38×10^6	$56–62 \times 10^6$		2.10^9 [f]			
Contour length (nm)	13.2[e]	17.2	24.3					
Buoyant density in CsCl (g/cm^3)	1.714 at 25 C[d, e]	1.696 at 20 C	1.725		1.706 at 25 C[f]	1.702 at 25 C[a]	1.73 at 25 C[g]	1.71[g]
Sedimentation coefficient ($s_{20, w}$)	33.4[d, e]	38.7	48.0					
G + C content (%)	53[d, e]	37	66–67	53–54	48[h, i]	43[h]	71.4[g]	55.6[g]

[a] All results from Adolph and Haselkorn (4).
[b] All results from Mackenzie and Haselkorn (83).
[c] Safferman et al. (106).
[d] Goldstein and Bendet (39).
[e] Luftig and Haselkorn (77).
[f] Sherman and Haselkorn (118).
[g] Stanier et al. (142).
[h] Edelman et al. (31).
[i] Kaye et al. (56).

head proteins are the 39,000 and 13,000 molecular weight species. The major tail protein has a molecular weight of 80,000. No internal proteins were detected. The sum of molecular weights accounts for about 35% of the coding capacity of LPP-1 DNA.

The major structural proteins of N-1, similarly characterized, are the 37,000 and 14,000 molecular weight species, whose sum accounts for 34% of the coding capacity of the DNA (4).

Mature SM-1 virions contain a dozen proteins, of which two (40,000 and 25,000 molecular weight) must be the major components of the capsid shell and contain over 80% of the sulfur atoms. The sum of the molecular weights of SM-1 structural proteins requires a coding capacity of 16% of the entire chromosome, a considerably smaller fraction than is found in viruses such as LPP-1 and T7 (83).

Physicochemical Characteristics

Table 3 summarizes physicochemical parameters of certain cyanophages. LPP-1 and, possibly, N-1 require Mg^{2+} for their stability. Luftig and Haselkorn (78) showed that LPP-1 tails separate from the heads after ethylenediaminetetraacetate DNAse treatment which bursts the head and releases DNA. Goldstein et al. (40) related this to the relatively high DNA content of these cyanophages. In agents such as T3 and T7, the Mg^{2+} requirement was related to diffusion of the polyamines out of the head during purification and their replacement by the cations, which neutralize phosphate groups of the viral DNA to maintain structural integrity (7).

Compared with bacterial viruses, which are generally stable from pH 5 to 8 (3), both LPP-1 (108) and SM-1 (112) are remarkably stable in the alkaline range (pH 7 to 11). This accords with the pH range of the blue-green algal host (see below, "ECOLOGICAL CONSIDERATIONS"). AS-1 (106) shows great stability at high pHs (up to pH 10), but can also tolerate pHs as low as 4. It would be interesting to determine the pH range of N-1 and the optimal pHs for the cyanophage growth cycles.

GROWTH CYCLE OF CYANOPHAGES

Phage Development in Filamentous Blue-green Algae

Elucidation of the growth cycle of some cyanophages is complicated by the fact that many of the algal hosts are filamentous. Most techniques and statistical analyses used in the studies of phage infection systems in unicellular hosts cannot be strictly applied in the case of multicellular hosts. Furthermore, filamentous organization may influence the pattern of viral reproduction. Cyanophage growth might be modified both by the number and character of cells in the trichome (for instance, in heterocyst or spore-containing filaments). The indications that plasmadesmata link algal cells (71) and that substrates are transferred from cell to cell in trichomes (158) seem to support this assumption. Viruses in higher plants have been indicated to spread from cell to cell via plasmadesmata (61, 72). The peculiar slow and nonlinear adsorption kinetics of LPP-1 on *Plectonema* found by Goldstein et al. (40) was related mainly to the variability in the host filament length.

To compare viral growth in filaments of different lengths, a short trichome (1 to 6 cells)

TABLE 3. *Physicochemical characteristics of cyanophage particles*

Physicochemical parameter	Cyanophage group			
	LPP-1	N-1	SM-1	AS-1[a]
Sedimentation coefficient ($s_{20,w}$)	555–548[b,c]	539[d]	1.021, 1.029[e]	
Buoyant density in CsCl (g/cm^3)	1.48[f]		1.48[g]	
Mg^{2+} requirement (M)	0.001[b,h,i]		Not required[e]	Not required
Temperature of inactivation (C)	55[i]		55[e]	60
Temperature range of greatest stability	4–40[i]		4–40[e]	
pH range of greatest stability	5–11[i]		5–11[e]	4–10

[a] All results from Safferman et al. (106).
[b] Goldstein et al. (40).
[c] Schneider et al. (113).
[d] Adolph and Haselkorn (4).
[e] Safferman et al. (112).
[f] Luftig and Haselkorn (79).
[g] Mackenzie and Haselkorn (83).
[h] Luftig and Haselkorn (77).
[i] Safferman and Morris (108).

mutant of *P. boryanum* was selected from a wild-type population consisting of trichomes of 50 cells or more (94). The growth rate of the mutant is identical to that of the wild type, and LPP-1G grows on both strains with equal efficiency.

The adsorption kinetics of LPP-1G on the short trichrome mutant was found to be linear with a rate constant of 3 to 5 × 10^{-11} cm^3/s. A similar constant was found for adsorption on the long-filamented *Nostoc* (5) and for bacteriophages on bacteria (124). The adsorption of AS-1 (106) to its unicellular host apparently also follows the kinetics of a first-order reaction; however, the rate was slow, similar to that of LPP-1 on wild-type *Plectonema* (40). These results may indicate that filament length has no effect on adsorption.

The one-step growth curve and internal growth curve of LPP-1G in *Plectonema* are shown in Fig. 4. These growth curves in the short trichome mutant are identical to those of the wild type, indicating that the length of the filament has no great influence on the reproductive cycle of the virus. The general pattern of the growth cycle of LPP-1G resembles that of the productive bacteriophages, although it is much slower. The latent period lasts for 6 to 6.5 h, the first 3 h of which are the elipse period. The rise period is completed only 6 h after the latent period. The average burst size is 350 plaque-forming units (PFU)/cell. Similar growth curves have been found for LPP-1 by Sherman and Haselkorn (117), who also showed that neither the burst size nor the latent period are dependent on input multiplicity over the range from 1 to 50 PFU/cell.

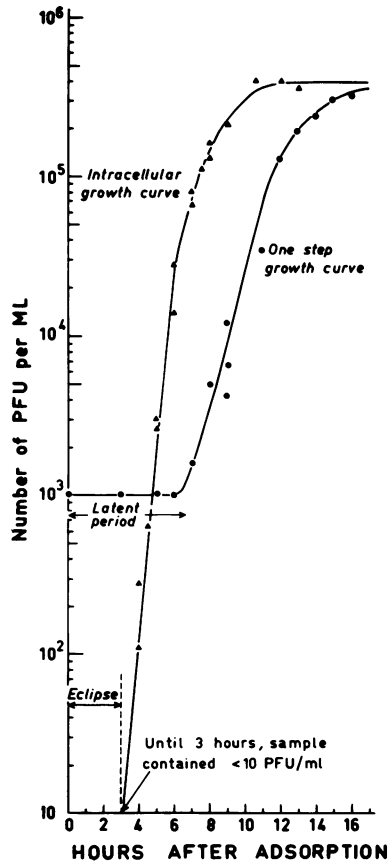

FIG. 4. *One-step growth* (●) *and intracellular growth* (▲) *curves of cyanophage LPP-1G. Infection with an input multiplicity of 0.1 PFU/trichome. Latent period was considered to begin at 0 h (after 1 h adsorption time when unadsorbed viruses were removed) (from reference 91).*

Similar to the LPP cyanophages, N-1 has a latent period of 7 h, followed by a rise period to the 13th h after infection (5). The burst size of N-1 is 100 PFU per infected cell. AS-1 has a latent period of 8.5 h, a rise period of 7.5 h, and an average burst size of 50 PFU per infected cell (106). SM-1 has a latent period of 30 h, a rise period of 20 h, and an average burst size of 100 PFU per infected cell (84).

The single-burst experiment (3) was easily accomplished with the short trichome *Plectonema* mutant infected with LPP-1G. The bursts obtained after 13 h in 50 samples are tabulated in Table 4 according to size. The distribution of different phage yields in the samples is in complete agreement with the distribution of singly infected trichomes expected from the Poisson formula for an average number of 0.45 singly infected trichome per sample; 32 samples yield no PFU, whereas 14 samples cluster around 350 PFU, 3 samples around 700 PFU, and there is one of 1,000 PFU. The samples with the phage yield clustering around 350 PFU, which is the average burst size, most probably received a singly infected trichome. A degree of variation exists between the burst sizes of the infected cells in individual trichomes, but this does not exceed half a burst size. In any case, it is not likely to be due to the variation of one to six cells in the filament length. An even greater variation has been observed in bacteriophages and was ascribed to physiological factors in the infected cells that influence the viral yield (3).

Even though each infected cell in the trichome behaves like a unicellular host, neighboring cells might be more susceptible to infection by nascent phages than cells located remotely in the trichome or in other trichomes because of a local density effect. We resolved this question

by incubating the highly diluted single-burst experimental systems for an additional growth cycle period of 13 h. The number of samples showing a second-cycle yield of 700 to 1,200 PFUs was within the range expected for singly infected trichomes. This is what might be expected from infection of neighboring cells at the moment of the original burst. Thus the juxtaposition of the algal cells in the trichome and not the plasmodesmata expedites the spread of a single infection along the whole length of the filament.

Sequence of Synthesis of Cyanophage Macromolecules

Biosynthesis of DNA and functional and structural proteins and production of LPP-1 virions are summarized in Fig. 5 in correlation with the time sequence of host symptoms of infection.

DNA. The bulk of LPP-1 DNA synthesis occurs between 6 to 8 h after infection, although incorporation of exogenous ^{3}H-adenine into viral DNA was detected very early after infection (2 to 8 h) (118). Because the majority of the infective particles appear in the infected cells between 7 to 11 h after infection, the replication of viral DNA is close in time to the maturation

TABLE 4. *"Single-burst experiment" of LPP-1G on short trichome mutant of Plectonema boryanum*[a]

Vial no.	No. of plaques
33	410
34	520
35	300
36	300
37	380
38	470
39	380
40	210
41	100
42	380
43	72
44	326
45	180
46	274
47	772
48	684
49	600
50	1,000

[a] The short trichome mutants were infected with an input multiplicity of 0.1 PFU per trichome. After completion of adsorption and removal of free phages, the culture was diluted so that each sample contained, on the average, 0.45 infected trichomes (B. Raboy and E. Padan, unpublished data). Vials 1 to 33 had no plaques.

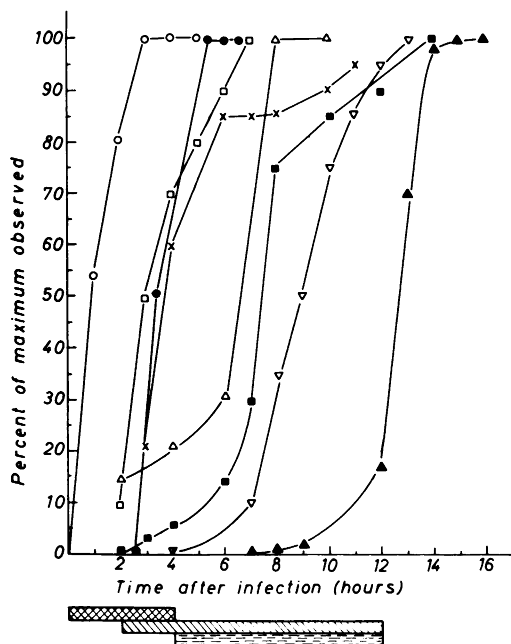

FIG. 5. *Time course of functions in the Plectonema cell infected with LPP-1 or LPP-1G cyanophages. The data presented in this figure are based on published results indicated in parenthesis. Host DNA breakdown (□) and DNA-breakdown capacity (○); viral DNA synthesis (■) and DNA-synthesis capacity (△) (118); intracellular infective particles (▽) and extracellular infective particles (▲) (93); cells with invaginated lamellae (×) (117); and inhibition of CO_2 photoassimilation (●) (37). Groups of viral proteins synthesized are shown in bars below the graph; earliest function (■); early functional proteins (▩); late structural proteins (▤) (119).*

process occurring almost at the end of the rise period. Sherman and Haselkorn (118) claimed that the incorporation of the label into viral DNA early in infection, prior to the abundant DNA synthesis, cannot be explained entirely by precocious synthesis in an asynchronously infected population. This active prereplication incorporation of the label is, in their view, possible either through recombination and/or repair. The high input multiplicity of 10 makes recombination likely. Repair was suggested because blue-green algae are very resistant to both ultraviolet and X rays and contain very active repair systems (160). Protein synthesis is required from the 2nd to 8th h after infection for synthesis of viral DNA. These experiments also showed that the protein synthesis is necessary for both replication of LPP-1 DNA and its stabilization, for some of the DNA synthesized in the presence of chloramphenicol

is labile. This probably reflects the fate of unpackaged viral DNA (118).

Assuming that mitomycin C inhibits DNA replication in blue-green algae as in bacteria, A. Rimon in our laboratory tested its effect on the LPP-1G infection cycle. Because the inhibitor is light sensitive, the infective process was carried out in the dark with *Plectonema* cells preincubated in the light to enhance viral synthesis (see "Photosynthesis and respiration" below). It was found that mitomycin C (2 μg/ml) inhibits cyanophage development when administered at any time during the growth cycle (103). Hence, DNA synthesis is required throughout the cyanophage growth cycle for full yield. This accords with the findings that LPP-1 DNA synthesis continues up to the end of the cycle (118).

Protein synthesis. Sherman and Haselkorn (119) characterized the virus-induced proteins and followed the course of their synthesis during LPP-1 infection. Three classes of viral proteins were distinguished (cf. Fig. 5). Synthesis of the earliest class (three proteins) begins soon after infection and is shut off by the 4th h. The early class (another three proteins) begins to form around the 2nd h and continues forming until lysis, but is not found in the mature particles. Finally, the late class includes viral structural proteins whose synthesis begins at about the 4th h and continues until lysis. It is significant that the synthesis of structural proteins begins 2 h before the bulk synthesis of viral DNA. A total of 17 distinct, viral-induced proteins were detected after infection. They account for about 65% of the coding capacity of the viral genome. Sherman and Haselkorn found no evidence for the synthesis of large precursors of any structural proteins during LPP-1 infection, such as noted for T4 (70) and poliovirus (146). The pattern of viral protein synthesis in the LPP-1-*Plectonema* system is very similar to that of the T7-*Escherichia coli* system (119).

At any time during infection, chloramphenicol (25 μg/ml) and erythromycin (5 μg/ml) inhibit the formation of new, infective viral particles (103). Hence, protein synthesis is required throughout the infective process, corroborating the conclusion of Sherman and Haselkorn (119).

Assuming that actinomycin D and rifampin inhibit DNA-dependent ribonucleic acid (RNA) synthesis, as they do in bacteria, they were used to determine the sequence of RNA synthesis in LPP-1G infection. As these inhibitors are sensitive to light, the dark reaction system was employed (see below under "Photosynthesis and respiration"). Rifampin (50 μg/ml) and actinomycin D (30 μg/ml) inhibit cyanophage development until the end of the infection cycle, implying that mRNA synthesis required for cyanophage development continues up to the end of the infection process.

It is apparent that the pattern of the infection cycle of the cyanophage is very similar to that of bacteriophages. However, it is much slower, lasting 13 h at 26 C in LPP-1G or 50 h in SM-1, whereas most bacteriophages complete their reproduction in tens of minutes. This may be related to the slower generation time of blue-green algae as compared with most bacteria.

Morphological Aspects of the Cyanophage Infective Process

The cell in which the cyanophage develops is a highly organized prokaryont (71). In many blue-green algae, such as *Plectonema*, the photosynthetic lamellae are organized in sheaths at the periphery and compartmentalize the cell to a certain degree (117, 137). The DNA is located in the central cytoplasm (nucleoplasm) where polyphosphate granules and the enigmatic polyhedral bodies are also found. Additional inclusion bodies, such as α- or polyglucoside granules (22), β-granules which are probably lipids (71), and peptide-containing cyanophycin granules (127), are seen mainly between or outside the lamellae (156). Thus, the topology of the cyanophage development cycle is no less interesting than its morphological aspects.

The cyanophage LPP-1 adsorbs to the algal cell wall by the distal end of the tail (138). The exact depth to which the tail penetrates has not been determined, although Smith et al. (138, 139) found that it appears to penetrate the cell wall. The cyanophage injects its DNA into the host cell, most probably through its tail as suggested for bacteriophages, and empty ghosts adhere to the host cell. It must be noted, however, that the classical experiment of Hershey and Chase (47) to demonstrate DNA injection has not yet been conducted in this system. The existence of a resistant *Plectonema* mutant (95) that does not adsorb the cyanophage LPP-1G indicates that specific receptor sites exist on the surface of susceptible host cells, such as those shown in bacteria (154).

By studying sections of *Plectonema boryanum* cells infected with LPP-1, Smith and his co-workers (19, 137) observed that the first sign of infection is invagination of the photosynthetic lamellae. The space between the folded lamellae and the plasma membrane in which viral particles later appear was called the virogenic stroma. Similar symptoms appear in other hosts of LPP-1 (139). Sherman and Ha-

selkorn (117) established the precise, visible sequence of LPP-1 development in infected *Plectonema* cells by using an improved fixation technique. The invagination is first seen 3 h after infection and is distinct by the 4th h, corresponding to the end of the eclipse period. By the end of the latent period (7 h), almost all of the cells show invaginated lamellae and virogenic stroma containing phage particles (Fig. 6). These authors noted sections of infected cells in which the lamellae are invaginated in three places with viral particles in all three stromata. However, they suggest that it is possible that the lamellar invagination is asymmetric in three dimensions, and that what appears to be three separate stromata in a single section is actually continuous. Serial sections should resolve this problem. Sherman and Haselkorn suggest also that the photosynthetic lamellae collapse is due to breakdown of host DNA (see below under "Host DNA and protein synthesis during infection").

Bacteriophage development is usually not accompanied by any gross cytological changes in the host cell (except for the DNA) until just before lysis (16). Among the few exceptions to this are the proliferations of the cytoplasmic membrane and, possibly also, of the cell wall noted during the growth cycle of filamentous coliphage (17).

Smith et al. (138, 139) suggested that after infection the cyanophage DNA reaches the nucleoplasm where it replicates and then migrates into spaces between the photosynthetic lamellae where long helices (18 nm in diameter) are formed. Leaving "holes" between the lamellae, these helices then migrate into the virogenic stroma where assembly occurs. Sherman and Haselkorn (117) argue, however, that the intralamellar particles, thought by Smith et al. (138) to be viral precursors, are also observed in uninfected cells and are most probably α-granules, and the "holes" are probably artifacts caused by loss of α-granules during preparation. They further suggested that cyanophage DNA replication occurs only in the virogenic stroma. Direct proof for this suggestion would be the detection of a pool of viral DNA in the virogenic stroma only. If DNA synthesis is confined to the virogenic stroma, then the possibilities for recombination should be investigated. Whether the infectious DNA attaches to the photosyn-

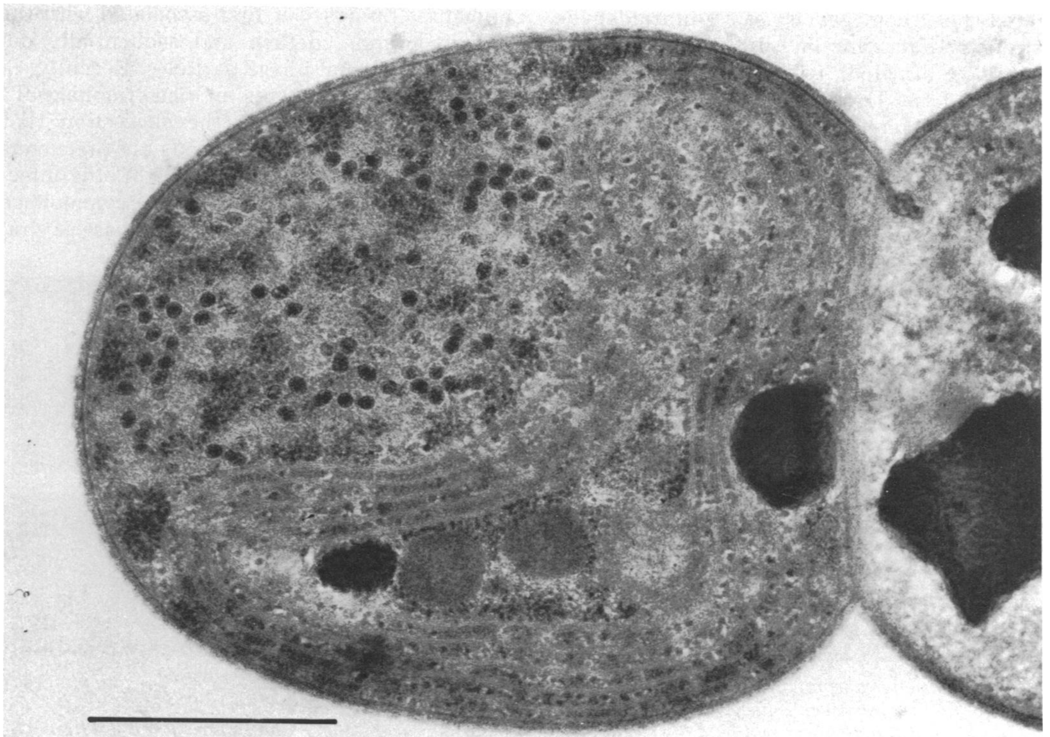

FIG. 6. *Plectonema cell 7 h after infection with LPP-1G cyanophage. Note the invaginated photosynthetic lamellae and the virogenic stroma containing the viral heads. Cells were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer at pH 6.9 and postfixed in 1% osmium tetraoxide in the same buffer; scale represents 1 μm.*

thetic lamellae and/or to the cell membrane also remains to be investigated.

In preliminary work in our laboratory (35) on the localization of protein synthesis and viral assembly, attempts were made to locate the viral antigens in sections of infected cells by using ferritin-labeled antibodies prepared against the purified, whole-virus particle. These phage antigens appear to be localized only in the virogenic stroma similar to the viral heads (117). Therefore, cyanophage particles seem to assemble only in a virogenic stroma separated from the nucleoplasm by the photosynthetic lamellae.

The time of appearance of the viral heads, as seen by electron microscope (35, 117), is similar to the rate of appearance of infective viral particles in infected cells (cf. Fig. 5). Until 4 h after infection (end of eclipse period), viral heads were very rarely seen in the virogenic stroma. By the end of the latent period (7 h) many cells contain viral heads; by 10 h viral assembly appears to have begun in almost every cell.

Sherman and Haselkorn (117) noted viral heads with various configurations of DNA together with heads which were empty and interpreted this inhomogeneity as a glutaraldehyde-fixation artifact. Similar observations in T7 led Zagury et al. (163) to suggest inhomogenous packing of the DNA. Such particles were detected in bacteria infected with other phages (126).

Further investigations of the morphological aspect of cyanophage development were undertaken in our laboratory (35). For this purpose, Kellenberger's in situ lysis technique (57), by which intracellular phages can be studied directly after staining, was adapted to study burst spheroplasts of *Plectonema* cells (see legend of Fig. 7). During the eclipse period, no forms resembling viral structures could be observed. At 4 h after infection, a few cells, amounting to about 1% of the population, contained various morphological forms not observed in uninfected cells. By the end of the latent period, almost 80% of the cells contain such forms (Fig. 7), including doughnut-shaped forms, empty rounded and hexagonal structures and tail-like structures in the monomer and trimer tail configuration proposed by Luftig and Haselkorn (78), and complete viral particles. Reaction of the in situ preparations with ferritin-labeled antibodies against mature viral particles showed that the empty headlike formations contain viral antigens (Fig. 8). No ghosts were observed with this technique at any time during the infection. In crude lysates, however, ghosts were observed, in addition to the above forms. In a study of normal T4 development, Simon (126) was able to demonstrate in thin section that what had previously been considered aberrant forms are true stages in phage-head development. "Lumps," at first associated with the cell membrane, detach and sequentially develop into normal phage particles. Recently, in vivo tracer experiments in bacteriophage T4 (80) have substantiated the suggestion that empty or partially empty heads are precursors of the complete head. In this light, the infection-related forms observed in the cyanophage system may represent a precursor phase of viral

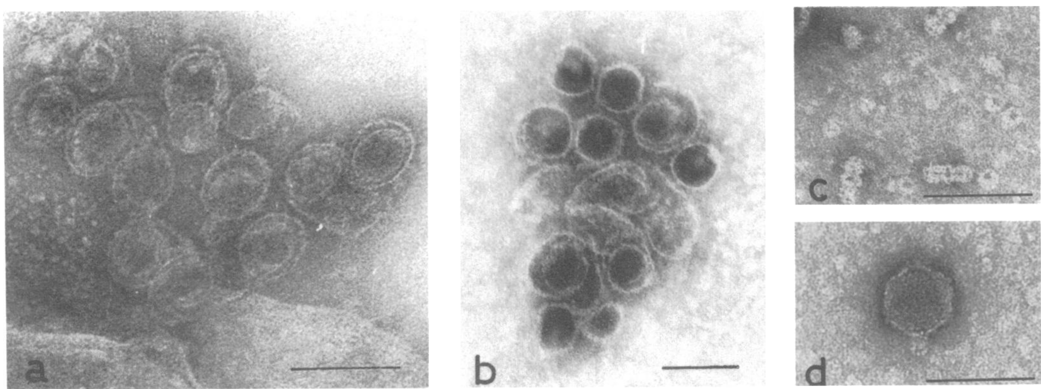

FIG. 7. *Various morphological forms observed in Plectonema cells infected with LPP-1G cyanophage by using the in situ lysis technique. Spheroplasts of the infected cells were prepared according to Ginzberg and Padan (36) and lysed on the grid by addition of 1% aqueous uranyl acetate or 1% ammonium molybdate in the presence of 10 mM magnesium chloride which insures that the viral particles remain intact (77). Various forms include doughnut-shaped (a), empty rounded and hexagonal (b), taillike structures (c), and mature viral particles (d). Scales represent 100 nm.*

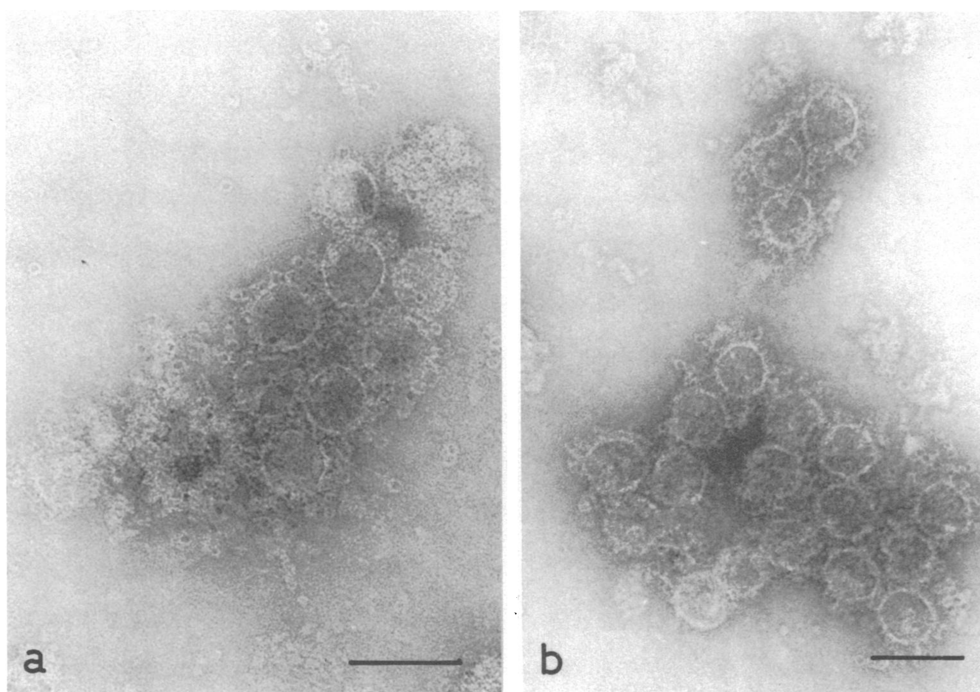

FIG. 8. *Infection-related forms after reaction with ferritin-conjugated antibodies to purified LPP-1G virions (35). Prepared by the in situ lysis technique (see legend to Fig. 7). Empty rounded (a) and empty hexagonal (b) forms; scales represent 100 nm.*

assembly, although the possibility that they are aberrant aggregates is not excluded. Nevertheless, even the latter may be considered reserve precursors and their possible reuse for viral assembly is suggested, as for bacteriophages (69). The kinetics of appearance of viral-related structures generally agrees with that obtained for complete viral heads by Sherman and Haselkorn (117) in sections of the infected cells. The in situ lysis technique has better resolution powers and therefore may allow the detection of additional structures. Considering the prolonged duration of the cyanophage reproductive cycle compared with that of the bacteriophage, it is not surprising that intermediates of cyanophage viral assembly can be detected.

LPP-1G is a thermosensitive cyanophage that does not produce mature particles above 31 C (97). However, the early signs of infection (invagination of the lamellae and cessation of CO_2 photoassimilation) are expressed at 37 C. Examination of spheroplasts burst 4 to 5 h after infection at the restrictive temperature reveals significant amounts of doughnut-shaped particles like those observed at 26 C. These particles also react with the ferritin-labeled antibody active against mature phage, further stressing the possibility that they may indeed be intermediate structures in viral assembly.

By using the in situ lysis technique, LPP-1G cyanophage particles are frequently observed being released from the cell as a single large aggregate. This is to be expected if the virions are assembled in a single site in the cell, the virogenic stroma. Counts of full-headed intact particles in the burst stroma gave numbers close to the burst size (Fig. 9).

When preparations are made directly from plaques, characteristic crystalline arrays are seen (Fig. 10). These appear almost exclusively in relation to the lamellae and may be seen either in surface view as hexagonal crystal forms, whose basic units are 10 nm hexamers, or in side view as bridging two adjacent lamellae and appearing as microtubules 4 nm in diameter. These crystalline forms were thought to be phycocyanin by Luftig and Haselkorn (77). However, these structures are not found in uninfected *Plectonema* lawns lysed by lysosyme and do not react with ferritin-labeled antibodies prepared against phycocyanin. It is noteworthy that antibodies prepared against mature virus particles also do not react with these forms.

Release of mature LPP-1G particles (at 26 C)

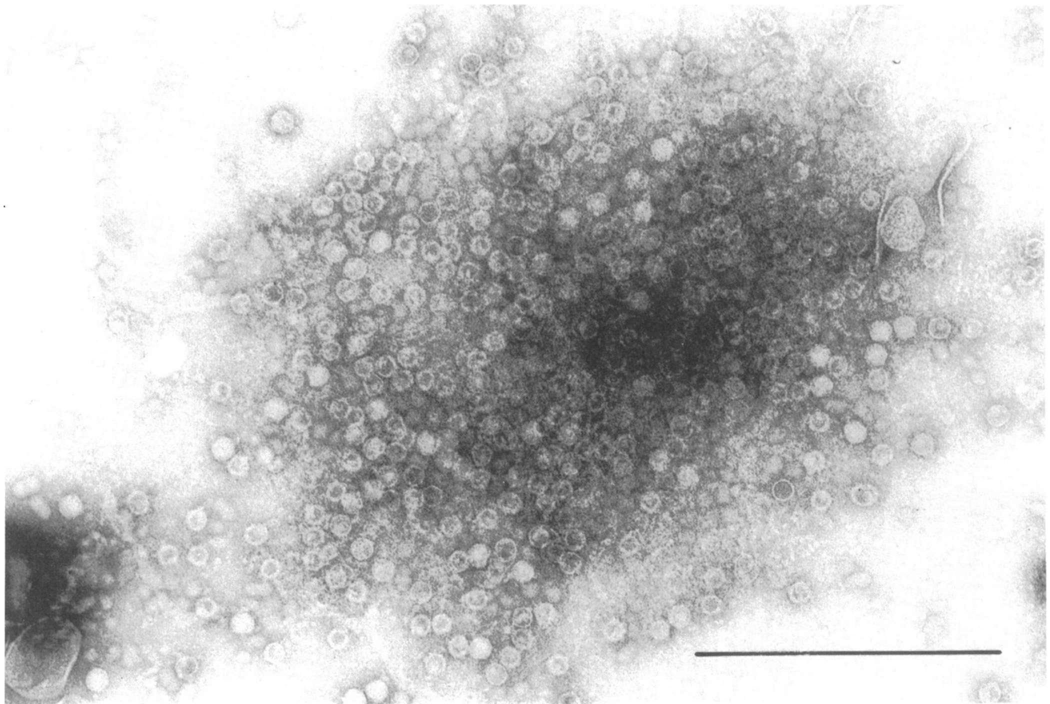

FIG. 9. *Aggregate of LPP-1G viral particles released from burst Plectonema cell. Prepared by in situ lysis technique (see legend to Fig. 7); scale represents 1 μm.*

is accomplished by cell lysis. Cell debris remaining after lysis includes remnants of isolated vesicles of different size and shape which may be torn lamellae, cell membranes, and even cell walls (95, 137, 139). Viral particles were seen both in sections and with negative staining, either embedded in or adsorbed on these structures, by the distal end of their long tails (cf. Fig. 3). The attachment of the cyanophages to all the different structures remaining after lysis might well reflect a common component in the membranes acting as viral receptor. The presence of related antigen on the photosynthetic vesicles and on the cell wall has, in fact, been demonstrated in *Rhodospirillum rubrum* by Newton (88).

Lysis of various phage-infected bacteria is accompanied by the formation of numerous vesicles consisting of cell membrane and wall material (16). Different bacteriophages adsorb onto such structures after lysis (89).

The morphology of unicellular blue-green algae (*Synechococcus*) infected with virus SM-1 has been studied in thin sections prepared at various times during the 50 h required for one cycle of virus growth (84). In contrast to LPP systems, no changes in the photosynthetic lamellae are seen prior to lysis. Furthermore, SM-1 virions accumulate in the nucleoplasm. After 24 h, mature virions start appearing, and by 32 h the entire space interior to the photosynthetic lamellae is packed with virus particles (Fig. 11). Similar to our results with LPP-1G, forms that could be either precursors of SM-1 particles or artifacts have been identified (84); these include apparently empty heads and condensed "cores."

The morphological aspects of N-1 and AS-1 infection cycles have not yet been studied. In view of the localization of viral assembly processes in a special site in the cell, the virogenic stroma, in LPP, but not in SM-1, such investigations would be most interesting, especially because the assembled virions in most bacteria infected with DNA-bacteriophages are evenly distributed throughout the cell or are formed in the nucleoplasm itself (16).

INTERACTION BETWEEN CYANOPHAGE DEVELOPMENT AND THE PHOTOAUTOTROPHIC METABOLISM OF THE BLUE-GREEN ALGAL HOST

The biochemistry and metabolism of blue-green algae have been recently summarized (30,

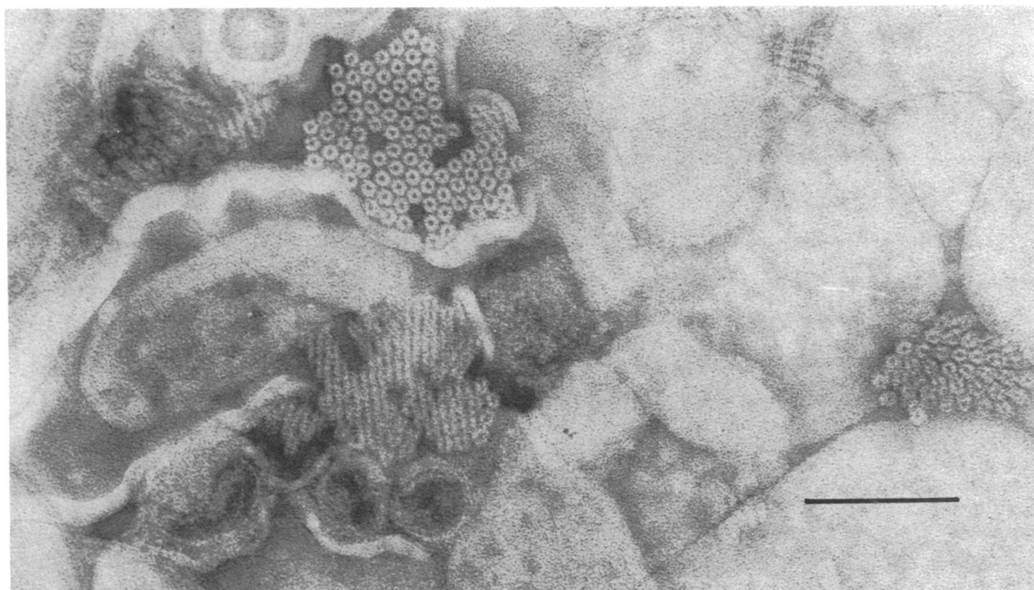

FIG. 10. *Crystalline forms in preparations made directly from plaques of LPP-1G on Plectonema lawns and negatively stained with 1% ammonium molybdate; scale represents 100 nm.*

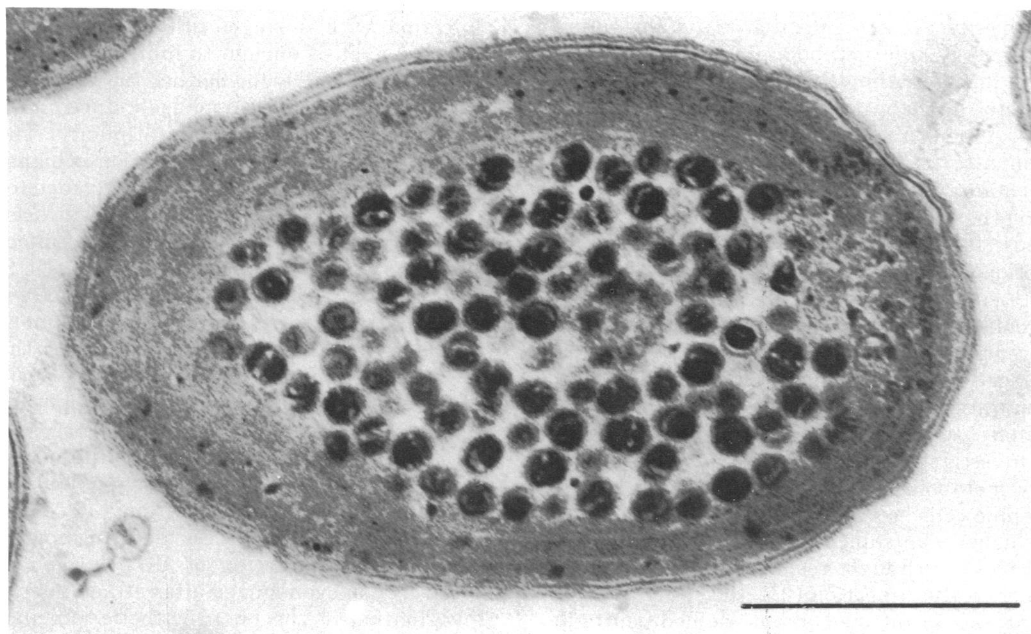

FIG. 11. *Synechococcus (NRC-1) cell 48 h after infection with SM-1. Thin section; scale represents 500 nm (from reference 84).*

50, 69, 142), so only topics and new data relevant to our discussion of the cyanophages will be reviewed here.

Blue-green algae are photoautotrophic prokaryonts whose photosynthetic system resembles that of eukaryotic plants. These algae contain chlorophyll a and β-carotene, as well as the accessory pigment c-phycocyanin in common

with some eukaryotic algae (30). Both photosystems I and II and related reactions, including the oxygen-evolution mechanism (14, 29, 147), are operative in blue-green algae, yielding electron flow from H_2O to triphosphopyridine nucleotide (oxidized form) and the accompanying noncyclic photophosphorylation. It is possible to inhibit photosystem II by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in blue-green algal cell extracts and to obtain only cyclic photophosphorylation (147). This reaction has been inferred indirectly in intact blue-green algal cells (91) and may perhaps be identical with that found in plants (148).

Many strains of blue-green algae, considered obligate autotrophs, were recently shown to have heterotrophic metabolism (23, 32, 48, 49, 59, 60, 62, 73, 98, 101, 134, 151, 153). This heterotrophic potency is also manifested in light growth of these strains in the presence of DCMU (59).

Effect of Cyanophage Development on Host Metabolism

Photosynthesis during infection. The study of the effect of viral infection on systems supplying energy and metabolic intermediates in the host has been almost neglected. Such a study in heterotrophic bacteria was conducted by Cohen and Anderson (24), who found that the respiration rate of *E. coli* cells infected with T2 is constant throughout the latent period, and oxygen uptake only stops at the time of cell lysis. It was mainly from this work that the notion emerged that phages do not directly influence the energetic and intermediary metabolism of the host cell. However, it was found recently that the bacteriophage directly affects the cell membrane, thereby inhibiting bacterial respiration at the end of the latent period and before lysis (55, 87).

Investigation of the energetic and intermediary metabolism during viral infection in autotrophic cells was restricted to studies of tissues of higher plants until the isolation of the cyanophages. Although respiration generally increased after infection (27), the study of photosynthesis in infected plants yielded contradictory results. In chloroplasts isolated from Chinese cabbage infected with turnip yellow mosaic virus (38), an increase in photophosphorylation was observed. In tobacco leaves infected with tobacco mosaic virus, a decrease in photophosphorylation occurred 1 h after inoculation (90). Zaitlin and Jagendorf (164), however, found that both photophosphorylation and the Hill reaction are normal in chloroplasts isolated from the tobacco mosaic virus-infected plant if the nitrogen supply is sufficient. Contradictory results have also been obtained concerning CO_2 photoassimilation and other metabolic pathways (12). The confusing findings may stem from experimental difficulties due to the complicated structure of higher plants and the almost complete impossibility of homogenic infection.

The behavior of the photosynthetic system of *Plectonema* during LPP-1G infection was studied by following the CO_2-fixation reaction (37). It was found that cyanophage infection effects a rapid and complete cessation of CO_2 photoassimilation. The cyanophage-induced inhibition starts 2.5 to 3 h after infection and becomes complete by the 5th h (Fig. 12), which is distinctly before lysis of the cells (7 to 13 h). These results were later confirmed for LPP-1 as well (120).

Adolph and Haselkorn (5) recently noted similar inhibition of CO_2 photoassimilation by *Nostoc* cells after infection with N-1. Although the effect is less immediate than that found with LPP-1G-infected *Plectonema*, it precedes lysis of the host cells.

Infection with a single LPP-1G virion per *Plectonema* cell is enough to inhibit CO_2 fixation completely (37), for neither the onset nor the magnitude of the cyanophage-induced inhibition depend on the input multiplicity. The effect of nitrogen on photoassimilation in higher plants, mentioned above (164), was excluded for the LPP-1G infection system, because nitrogen supplementation both before and during infection did not influence the inhibition (37).

Light is required during the eclipse period for the completion of the cyanophage development cycle at maximal burst size and during the entire latent period for completion of the cycle at the maximal rate (91). By excluding light for different time periods and at different stages, it was established that a fixed period of illumination (1.5 to 2 h) after adsorption is required for the cyanophage-induced inhibition (37). This implies that an active process requiring energy and leading to inhibition of CO_2 fixation is induced by the cyanophage after attachment.

Invagination of the photosynthetic lamellae of *Plectonema* cells appearing from 3 to 8 h after LPP-1 infection requires protein synthesis during the first 3 h of infection (117). The coincidence in time of invagination and cessation of the CO_2 photoassimilation is evident (cf. Fig. 5). The relationship between these symptoms is further emphasized in the infection cycle at 35 C of the temperature-sensitive strain LPP-1G in

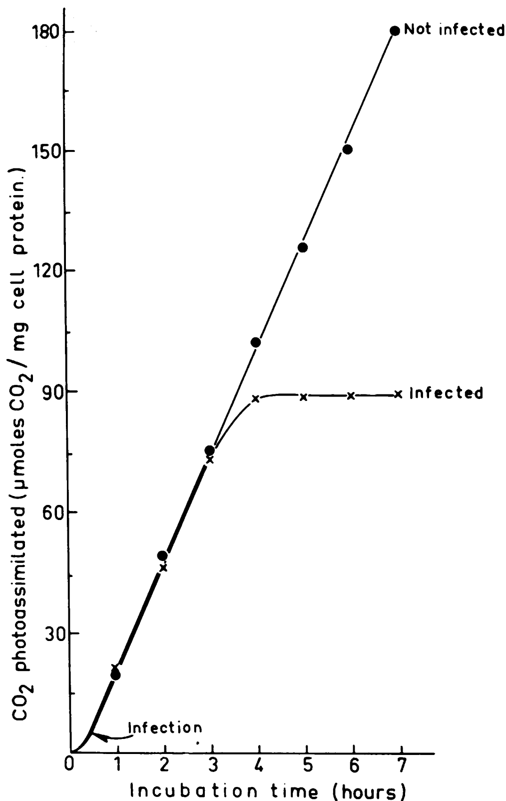

FIG. 12. *Rate of CO_2 photoassimilation of infected* (×) *and uninfected* (●) *Plectonema cultures under LPP-1G infection (input multiplicity of 10 PFU per cell). CO_2 fixation was determined by incorporation of $NaH^{14}CO_3$ into the cells (adapted from reference 37).*

Plectonema (97). Both invagination of the photosynthetic lamellae and the inhibition of CO_2 photoassimilation are expressed at the elevated temperature. It remains to be demonstrated whether a causal relation exists between these two symptoms.

Portions of the photosynthetic system were studied to determine the site of inhibition. Wu and Shugarman (161) investigated photosynthetic O_2 evolution and respiration in LPP-1-infected *Plectonema*. They found that the rate of oxygen evolution did not change until the infected cells ruptured. In the extracts of LPP-1G-infected host cells, no inhibition of phenazine metasulfate-cyclic photophosphorylation was found, the algal pigments (chlorophyll a and c-phycocyanin) showed no difference in quantity and fluorescence (D. Berns and E. Padan, unpublished data), and ribulosediphosphate carboxylase activity was unaffected (D. Ginzberg, unpublished data). Thus, the inhibition does not seem to be due to any major damage to the polysynthetic cycle.

Sherman and Haselkorn (118) found that DNA breakdown coincides in time with invagination of photosynthetic lamella in LPP-1-infected *Plectonema* (cf. Fig. 5). They proposed that the degradation of DNA may cause the invagination simply on a mechanical basis. The raised level of nucleotides in the cell may explain the cessation of CO_2 photoassimilation, since these are known to inhibit this reaction in bacterial systems (54).

An additional possibility is that CO_2 fixation ceases as a result of competition with viral synthesis for adenine triphosphate. It may be of significance in this respect that the synthesis of viral proteins and the bulk of the DNA take place at the stage when CO_2 fixation has ceased (cf. Fig. 5).

In SM-1 infection of *Synechococcus*, no cessation of CO_2 photoassimilation occurred, and there was no invagination of the photosynthetic lamellae (85), in contrast to the LPP systems.

Host DNA and protein synthesis during infection. Sherman and Haselkorn (118) showed that approximately 50% of the *Plectonema* DNA is degraded to acid-soluble material between 3 to 7 h after infection with LPP-1 (cf. Fig. 5). Most of the acid-soluble product is then reincorporated into viral DNA. This breakdown of host DNA requires protein synthesis during the first 3 h after infection and shows a superficial similarity to T7 in its effect on *E. coli* DNA. In spite of the extensive degradation of *Plectonema* DNA induced by the 6th h after infection, a significant amount of labeled nucleotides are still incorporated into host DNA as late as 7 h after the infection. It thus seems that cyanophage infection does not arrest DNA synthesis of its host.

Host protein synthesis is depressed soon after infection of *Plectonema* by LPP-1 (119) and appears to be entirely shut off by the 5th h.

Dependence of Cyanophage Development on the Photoautotrophic Metabolism of the Host

Photosynthesis and respiration. Under standard photosynthetic conditions, the growth curves of the cyanophage LPP-1G in *Plectonema* cells in the presence or absence of CO_2 are identical (91). Hence, the CO_2 photoassimilation reaction is not required in the infection process. The inhibition of the CO_2 fixation by the cyanophage at an early stage of infection (see above "Photosynthesis during infection") obviously accords with this conclusion.

In the presence of DCMU, the burst size and rate of virion release were the same as in normal infection. Only the eclipse period is somewhat prolonged. Thus, it appears that neither noncyclic photophosphorylation nor photoreduction of triphosphopyridine nucleotide (oxidized form) are absolute requirements during the infective process of the virus and that they only accelerate the eclipse processes. Similar results were obtained in another LPP-1 strain (120); the slightly decreased burst size in the latter experiments may be due to the exceedingly high concentration of DCMU used.

Because darkness depresses the burst size by 90% (Table 5), most of the adenosine triphosphate required for viral synthesis under these conditions must be derived from photophosphorylation. Moreover, it is evident that cyclic photophosphorylation can supply (in the presence of DCMU) all the adenosine triphosphate requirements of the virus. Accordingly, the uncoupler of phosphorylation at the electron transfer level, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (147), was found to inhibit viral synthesis completely. Thus, substrate level phosphorylation or the existence of an intracellular adenosine triphosphate pool do not seem to be significant under autotrophic conditions. Furthermore, anaerobiosis in the light does not seem to influence the cyanophage infection cycle (cf. Table 5), indicating that oxidative phosphorylation under these conditions is quantitatively insignificant.

TABLE 5. *Yield of cyanophages in Plectonema cells incubated under different experimental conditions*[a]

Conditions	Yield[b] (PFU/ infected cell)	Percent of maximal burst size under standard conditions
Dark		
Aerobic	36	10
Anaerobic	8	2
Aerobic + CCCP (10^{-4} M)	0	0
Light		
Aerobic	300-400	100
Anaerobic	300-400	100
Aerobic + DCMU (10^{-6} M)	300-400	100
Anaerobic + DCMU (10^{-6} M)	300-400	100
Aerobic + CCCP (10^{-4} M)	0	0

[a] Table reproduced from reference 93. CCCP, Carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

[b] Yield of cyanophage was determined from intracellular growth curves for each experimental condition.

To determine when photophosphorylation is required in the cyanophage infection cycle, the infection cycle of LPP-1G was followed in *Plectonema* cells illuminated for different periods of time from the beginning of the latent period (91). Illumination during the entire eclipse period is sufficient to produce the maximal yield, although at a slower rate. Shorter illumination periods within the eclipse period caused decreases in yield and rate of formation of the infective particles in the cells. Continuation of illumination beyond the eclipse period up to the end of the latent period progressively accelerated the rate of intracellular synthesis of the infective virions. Light must also affect release of the viral particles, for only part of the change in the rate of release can be explained by its effect on intracellular synthesis. Illumination after free phages begin to appear does not affect the cycle.

The dependence of the cyanophage LPP-1 development cycle on photophosphorylation is not absolute (92, 161). We have seen that 10% of the viral synthesis proceeds in the dark (cf. Table 5). No virus is produced in the dark under anaerobic conditions or in the presence of CCCP (92). Thus, viral multiplication in the dark must depend for its energy on oxidative phosphorylation. Indeed, oxidative phosphorylation has been demonstrated in intact cells and extracts of several blue-green algae (15, 74, 75, 114). Although the endogenous respiration of blue-green algae preincubated in standard light conditions is low, it can be enhanced progressively by increasing the period of preincubation in optimal photoassimilation conditions (high light intensities and bicarbonate medium). This enhancement of endogenous respiration in infected cells is fully correlated with an increased burst size up to 50% of light yield and rate of viral synthesis (92). Similar cyanophage yields are obtained when *Plectonema* is grown heterotrophically (on glucose substrate) and infected in the dark (D. Ginzberg and B. Raboy, unpublished data).

Sherman and Haselkorn (120) recently measured the infection cycle of LPP-1 in *Plectonema* cells kept in the dark for various times before and after infection. Although maintenance of cells in the dark for 10 h after infection delayed the viral synthesis, the final yield was about twice that of the control, and the rate of release of virions was accelerated. These authors assumed that incomplete viral particles accumulating in the postinfection dark period are rapidly completed upon illumination. They further inferred that early viral functions proceed as efficiently in the dark as in the light. How-

ever, two early functions, the capacities to synthesize DNA and degrade host DNA, are delayed in the dark. It seems equally plausible that the algal cells must be in a specific physiological state for replication of the cyanophage. Synchronization of the algal population (by introduction of dark periods) could contribute to more efficient infection cycles, as was recently demonstrated in another phage system (155). Synchrony of the infection seems to be the explanation for the finding that dark periods before and after infection markedly accelerated phage-induced inhibition of CO_2 photoassimilation (37).

In general, the dependence of the N-1 infection cycle on the energy metabolism of *N. muscorum* resembles the situation found in LPP-1- and LPP-1G-infected *Plectonema*, although some differences may be noted (5). DCMU reduces yields in N-1 infections to 25% of the control, whereas it had no effect on LPP-1 yields. As expected, addition of CCCP in the light drastically reduces the viral yield to 2% of the control. Because N-1 multiplication is not completely abolished in the presence of this inhibitor, it may be inferred that limited adenosine triphosphate pools or substrate level phosphorylation may be present in *Nostoc* cells and contribute in a limited way to N-1 multiplication in the light. N-1 appears to be more dependent on photosynthesis than LPP (5). However, this finding should be reevaluated in the light of our results that the respiration is closely linked to the growth history and physiological stage of the algae.

In contrast to LPP and N-1 cyanophages in which the dependence on photosynthesis is not absolute, SM-1 development in the infected *Synechococcus* is completely stopped by DCMU, darkness, or absence of CO_2. SM-1 is considerably more dependent upon host photosynthetic metabolism than any previously studied cyanophage (85). In SM-1 infection, CO_2 photoassimilation is not inhibited, photosynthetic lamellae do not invaginate, and viruses develop in the nucleoplasm.

Building blocks. Sherman and Haselkorn (118) showed that approximately 50% of the host DNA is degraded 6 to 7 h after LPP-1 infection, and most of the acid-soluble products are reincorporated into viral DNA.

Preliminary results of A. Rimon in our laboratory indicate that proteins of the algal host, synthesized before the infection, contribute to the synthesis of cyanophage proteins (103). In an experiment in which sulfate-starved *Plectonema* cells were uniformly labeled with ^{35}S, by the method of Sherman and Haselkorn (119), about 90% of the host label was recovered in proteins; the residual 10% was probably bound to polysaccharides. Because almost no cold-acid-soluble label was recovered after sulfate labeling, it was inferred that the sulfur-amino acid pool in the cells is very small. The labeled cells were washed, infected, and divided into the three experimental systems described in Table 6. After completion of the infection cycle, the cyanophages were purified, and their radioactivity was determined. The phage proteins produced in the control system became highly labeled, and their computed specific activity reached that of the host proteins. This similarity of specific activities indicates that algal proteins are the major precursors of phage proteins and that CO_2 photoassimilation does not contribute significantly, even before it is inhibited in the infected cell (see above "Photosynthesis during infection"). This is also supported by similar specific activities of cyanophage proteins produced in the presence or absence (due to DCMU addition) of CO_2 photoassimilation. When the experiment was conducted with methionine and Na_2SO_4, the computed specific activity of cyanophage proteins decreased to 15% of that of the host proteins. After infection, proteins of the host cells probably undergo degradation to amino acids which are then reincorporated into the phage proteins. The residual labeling of the cyanophage in the presence of the methionine chase may be due to labeled sulfate-containing polysaccharides that also may contribute to phage proteins. There

TABLE 6. *Contribution of host proteins to cyanophage LPP-1G proteins*[a]

Experimental system	^{35}S label of cyanophage (counts/min)	Cyanophage yield (PFU)	Specific activity (counts per min per mg of viral proteins)[b]	Ratio of specific activities of viral proteins to host proteins (%)
Na_2SO_4 + methionine	5,790	2.73×10^9	4.25×10^7	15.0
DCMU	50,800	4.44×10^9	2.45×10^8	91.0
Control	30,260	2.92×10^9	2.14×10^8	79.2

[a] Proteins of *Plectonema* cells were labeled with ^{35}S according to Sherman and Haselkorn (119) and incubated under the standard experimental infective conditions (91) at an input multiplicity of 10 PFU/cell in the above systems. After a 9-h incubation, the cells were ruptured, virions were purified by differential centrifugation and in CsCl gradients (103), and the amount of isotopes in the cyanophages was determined. DCMU, 3-(3,4-Dichlorophenyl)-1,1-dimethylurea.

[b] For determination of viral specific activity, 52% of the virus particle weight was assumed to be protein with a molecular weight of 30×10^6 (40).

are indications that polysaccharides break down during infection (118).

In the light of these preliminary indications, several interesting problems remain to be examined. One problem is whether all or only selected host proteins are utilized by the cyanophage. Another is whether mainly host proteins are required for cyanophage protein synthesis or whether other cellular constituents can contribute building blocks. Finally, there is the question of how different physiological conditions, such as starvation in the dark or loading the cells with reserve materials, affect the cyanophage protein synthesis.

Since cyanophage (LPP-1G) can multiply without an exogeneous carbon source and most probably without external nitrogen as well, host components must supply all the building blocks for viral synthesis. Furthermore, growth of the host cells is not required for LPP multiplication; this differs from that of most bacteriophage which only develop efficiently in growing cells. Synthesis of bacteriophages attacking heterotrophic hosts depends on exogeneous substances (125), except for host DNA in some cases (82).

The unique dependence of the LPP-1G cyanophage on host cellular constituents for building blocks represents an extreme degree of host-parasite interaction. Such extreme parasitism could be visualized in a photoautotrophic host in which the photosynthetic system supplies adenosine triphosphate, and minimal breakdown of host proteins together with host DNA utilization suffice to satisfy phage synthesis. This extreme dependence even seems to offer selective advantages, because the infective process would thus be less dependent on environmental factors. The lack of strict control of enzyme synthesis found for blue-green algae (51, 52, 76, 99, 101) may allow a spontaneous flood of intermediates without disturbing the state of the cell. Heterotrophic bacteria, on the other hand, have biosynthetic and energy metabolism depending on substrate supply, and a strict control mechanism governs the steady states of the cell. In such hosts, the agent that disturbs the host metabolism least is the one with the selective advantage. A study of other cyanophage systems and other viral infections of other photoautotrophic and chemoautotrophic prokaryonts, such as *Rhodopseudomonas spheroides* (34) and *Hydrogenomonas* (63), is needed to evaluate these suggestions. It would also be interesting to identify the precursors of LPP synthesis in dark conditions as compared with those in photosynthesizing cells, as well as those of SM-1 which shows extreme dependence on CO_2 photoassimilation.

ECOLOGICAL CONSIDERATIONS

The hosts of cyanophages, the blue-green algae, are widely spread in the aquatic environment, although some terrestrial species are also known (50). In freshwaters, the blue-green algae exhibit characteristic cyclic growth; they bloom in large masses usually during the summer, and then often spontaneously die off and lyse (122). Although this decomposition causes enormous economic damage by deteriorating water quality and by causing mass mortality of fish populations, neither the causes of the extensive growth of the blue-green algae nor the factors leading to their rapid decomposition are thoroughly understood.

Ever since the discovery by Safferman and Morris of a virus attacking blue-green algae, particular attention was paid to the ecology of the cyanophages and to studies of their relationships with the algal host in the ecosystem and their possible role in the fluctuations of blue-green algae populations (109, 123).

Systematic searches for cyanophages were therefore undertaken in all parts of the world. Safferman and his group showed that the LPP cyanophages represent a steady population in waste stabilization ponds (110). Strains of LPP-1 and LPP-2 were found in all seasons in a wide variety of locations in the United States (111). Shane (116) initially screened the Christina River area of the Delaware River and found LPP-type cyanophages in all waters tested (including natural lakes, quarries, farm and residential ponds, industrial storage tanks, stabilization lagoons, and streams and rivers). Water areas near urban settlements gave higher viral counts. Routine collections from sewage oxidation ponds gave the highest viral concentrations. Safferman analyzed these isolates of Shane and found them all to be identical or similar to LPP-1.

A variety of LPP types, including LPP-1 and LPP-2 types, and a temperature-sensitive strain (97) have been isolated in Israel from brackish and freshwater ponds and from Lake Kinneret (93).

Viruses attacking *Plectonema*, *Phormidium*, and *Lyngbya* were also isolated in a number of sewage ponds in Scotland (26). One isolate was shown to be serologically identical with LPP-1. These isolates showed some variability in plaque size, indicating that different viral strains are present. The cyanophages were consistently detected throughout the year in one of

the ponds. Findings of LPP-type cyanophages also have been reported from India in sewage ponds (130) and rice fields (128) and in the Soviet Union (44).

Other types of cyanophages have also been isolated in very divergent geographical and habitational locations. Cyanophages active against vegetative cells of *A. raciborskii* and *Cylindrospermum* were reported from India (130). SM-1 cyanophage was isolated from a waste stabilization pond in Indiana (112), and AS-1 was also reported from the United States (106). A group of Swedish investigators (43) and a group in the Soviet Union (64) described viruses active on *A. variabilis*. Two cyanophages active against different species of *Microcystis* were reported by a Kiev-based group of investigators (41, 42). Cyanophage N-1 was isolated from Lake Mendota in Wisconsin (4).

It is now clearly evident that cyanophages are ubiquitously distributed in freshwater bodies. Systematic screenings of Israeli fishponds (93) and oxidation ponds in the United States (110) for cyanophages attacking *Plectonema* yielded cyanophages in 70 to 90% of the samples. Safferman et al. (110) found numbers of 4 to 270 PFU per ml throughout the year. The cyanophage yields obtained are comparable to bacteriophage titers recorded in sewage (8). Numbers of these viruses in selected fishponds in Israel over a year generally ranged between a few to several tens of PFU per liter; a drastic increase in number to several thousands occasionally occurs during the summer when intensive blue-green algal blooms are noted (93). The fact that LPP cyanophages maintain their numbers in habitats with appreciable flow rates (up to 1.5×10^6 gallons/day) indicates that the virus is being replicated rapidly in nature (26).

An instructive observation, made by Safferman and Morris (110) and in our laboratory (93), was the fact that the susceptible algal genera were never dominant in ponds where the appropriate cyanophages appeared in significant amounts. This possibly may be due to continuous lysis of sensitive algae by proliferating cyanophages, so that population equilibrium among them is established. The worldwide distribution of LPP-type cyanophages and the fact that *Lyngbya*, *Phormidium*, and *Plectonema* are not known to form blooms suggest that such an equilibrium indeed exists in many natural water bodies.

The predominance of finds of LPP-type cyanophages the world-over is striking, but not surprising if one considers that their host genera were described by Drouet (28) as ecophenes of a single species, *Schizotrix calcicola*, which seems to be "the most widely distributed and most frequently encountered blue-green alga on earth." In addition, the hosts of LPP viruses (in most cases, *P. boryanum*) have been the species most widely used in isolation by direct plating of samples (110) or of concentrates (93), or have been the host organism for selective enrichments (93, 110) and baiting of the cyanophages in situ (26). The limited success in isolation may also be due to the use of blue-green algal strains kept for years in culture collections and readily available to the investigators, but not necessarily the most suitable hosts for the cyanophages from the natural environment. At present, fresh isolation of prevalent blue-green algal forms and their use as hosts are underway in our laboratory.

The interaction of cyanophage with its host is markedly influenced by environmental conditions. The requirement for high (0.001 M) magnesium ion concentration in the milieu for maintenance of activity of LPP-1 (40, 113) and possibly N-1 (4) has been demonstrated. On the other hand, AS-1 (106) and SM-1 (112) cyanophages show stable activity in water lacking the cation. Although the salinity range of cyanophages has not been rigidly determined, LPP phages have been isolated from brackish fish ponds with salinities reaching 2,000 mg of Cl^-/liter (110). Interestingly, NaCl exerts a stabilizing effect on attachment in the AS-1 system (106).

The tolerance of the cyanophages (see "Physicochemical Characteristics") to alkalinity accords with the alkaline range (7 to 11) of *Plectonema*, which shows little or no growth below neutrality. Most other blue-green algae prefer the alkaline range, which prevails in aquatic systems where these algae thrive (illustrated in the extreme in several African equatorial lakes, such as Lake Aranguadi, the "green lake" near Debra Zet in Ethiopia, which have permanently high alkaline pHs and continuous blooms of *Spirulina platensis*). Survival of cyanophages at high pHs must have great ecological significance as a selective factor in conditions common to a great many blue-green algal blooms.

Additional environmental factors influencing the host-phage interaction are temperature effects on the survival of free phage and on cyanophage development in hosts. LPP and SM-1 cyanophages show similar temperature sensitivity: 85% of the free phage particles remain infective at temperatures up to 40 C, whereas only 55% attack at 45 C, and less than 0.001% at 50 C (108, 112). Two LPP isolates from nature (LPP-1 and LPP-1G) are tempera-

ture sensitive (97). They multiply normally in the 26 to 29 C range, but produce only early symptoms of infection in hosts (invagination of the photosynthetic lamellae and cessation of CO_2 photoassimilation) above 31 C. The stability of the different cyanophages to temperatures up to 50 C is also consistent with the blue-green algal temperature range in nature which is between 20 to 50 C, and is even higher for special thermophilic types.

Provided that adenosine triphosphate supply is ensured, certain cyanophages (LPP) can grow and replicate in the dark and under conditions inhibiting host growth (such as lack of any external carbon sources and presence of DCMU), whereas bacteriophages require actively multiplying hosts. This wide tolerance of the LPP cyanophages makes them quite suited to succeed in surviving and reproducing in natural conditions. The natural life cycle of a virus whose replication demands photosynthetic CO_2 fixation must be extremely hazardous. Incubation of SM-1 for 12 h in the dark is enough for irreversible lose of 20% of plated, infectious centers. Mackenzie and Haselkorn (85) expect to find rather striking circadian phenomena characteristic of SM-1 populations in nature.

All of the cyanophages isolated until recently showed characteristics of virulent phages. However, LPP-1D, LPP-2SPI, and other cyanophages were shown to be temperate phages (20, 21, 96, 132). Attempts at induction of the SPI prophage with ultraviolet, X rays, or mitomycin C proved relatively unsuccesful. However, when a thermosensitive mutant of SPI was prophage, its induction was accomplished by transferring the lysogenic culture from 25 to 35 C (96). Factors, such as temperature, ultraviolet, or unbalanced growth conditions, which are known to influence formation of lysogenic cultures and the lytic cycle, might have considerable significance on the algal population balance in nature. The lytic cycle could be induced if a thermosensitive, lysogenic algal population in nature is exposed to elevated temperatures, as was seen experimentally.

The role of cyanophages in determining the cyclic blooms of blue-green algae in nature has been suggested ever since these viruses were discovered. Daft et al. (26) consider it possible that viral and microbial pathogens of algae may be even as important in determining bloom formation and disappearance in nature as are conventional factors such as light intensity and nutrient level. Indeed, an effect on certain species is indicated (as illustrated by the fact that *Plectonema* blooms never form in nature). However, it is likely that blue-green algal population fluctuations cannot be solely related to cyanophage attack. Die-off has been observed in many different blue-green algal species, some of which are not yet known to be attacked by cyanophages. In certain other cases, sensitization of blue-green algal populations to photooxidation in conditions of CO_2 limitation (1, 2) seems to be the cause of the algal die-offs.

The possible use of cyanophages for biological control has been suggested (105). By simulating conditions of a *Plectonema* bloom in the laboratory, Safferman et al. (109) were able to prove the feasibility of bloom control with LPP cyanophages. Jackson and Sladecek (53) studied the interaction of LPP-1 with its host *P. boryanum* on a large scale in 5,000-gallon tanks. They concluded that algal viruses can prevent growth of their blue-green algal hosts under natural conditions, because all attempts to grow the algae in effluents of a sewage treatment plant failed, but excellent growth of *Plectonema* was supported by autoclaved samples of the sewage water. Russian investigators have claimed success in using the virus to clear *Microcystis* blooms in an experiment in a large water reservoir in the Ukraine (L. A. Sirenko, personal communication). These experiments were based on observations of V. Goriushyn and others (42, 104) that the Kremenchug reservoir had clear areas even at the height of a *Microcystis* bloom. He found that within these clear patches the algae had been destroyed by a virus which had not previously been characterized.

An important consideration in establishing the role of cyanophages and their potential as control agents in nature is the rapid appearance of resistant host mutants. Algal mutants resistant to LPP-1 cyanophages were found at a frequency of 10^7. However, such resistant hosts are still susceptible to suitable host-range phage mutants (44, 95). One of the resistant algal clones isolated by Gromov and Kozykov (44) appeared to be refractive in all their tests. Resistance to phages among the algal mutants tested by us seems to involve a change in the algal cell envelope which prevents phage adsorption (95).

The high degree of host specificity, the selection of resistant host mutants, and the dependence on environmental factors indicate the complexity of the alga, cyanophage interaction, whose outcome depends not only on inherent properties of both phage and host, but on fluctuations in external conditions as well. The length of the phage developmental cycle which varies over a large range is still another, perhaps

critical, factor in the choice of the phage promising for control. Deliberate manipulation, both of viral genetic material and of external conditions, could serve as the key to prospective biological control of undesirable blue-green algal blooms.

GENETICS OF CYANOPHAGES

Apart from several spontaneous mutants, neither mutagenesis nor recombination have been reported so far for cyanophages, although complementation and recombination in the temperature-sensitive mutants (LPP-2-SPI) have been indicated in the most recent work of A. Rimon in our laboratory. The spontaneous mutations, reported for LPP cyanophages, include acriflavine resistance (131) and rapid lysis rate (108, 131). Host-range mutants of LPP cyanophages have also been isolated (95, 131).

Recently Cannon et al. (20, 21) reported on the lysogeny of *P. boryanum*. Addition of mitomycin C induced a 100-fold increase in virus production between 4 and 5 h after treatment. When the lysogenic cultures were grown with antivirus serum, all free virus was eliminated. A lysogenic strain of *Anabaenopsis* has also been indicated (132). We have shown that LPP-2-SPI is a temperate cyanophage (96). Immunity is rapidly conferred on *Plectonema* cells 90 min after infection with LPP-2-SPI. About 80% of the *Plectonema* cells become lysogenic; cultures of such cells always contain virulent phages, probably produced by occasional induction. The frequency of induction can be increased only fivefold after ultraviolet or X-ray irradiation. When a temperature-sensitive mutant is present as a prophage, efficient induction can be accomplished by heat treatment of the lysogenic culture. The mechanism of lysogenization of *Plectonema* by LPP-2-SPI thus seems similar to that of lysogenic bacteriophages and consists of a proteinous repressor. The lysogenic strain P1(SPI) is also immune to infection by all other phages of the LPP-2 group, indicating that all LPP-2 strains are homoimmune (96). This further justifies the serological classification of LPP cyanophages into LPP-1 and LPP-2 groups (111). The ecological importance of lysogenic strains has been discussed.

The genetics of blue-green algae is poorly known. Some cases of mutagenesis have been reported (9–11, 66–68, 129, 131, 141, 144, 145). Recombination has been claimed by some authors (13, 65, 133), while these results have been questioned by others (102). Recently, transformation was also reported (45, 46, 121). The temperate cyanophages may allow for transduction studies, which can lead to extensive genetic investigations of the blue-green algae and cyanophages. Searches are currently underway in our laboratory for LPP-2-SPI-mediated transduction. Singh et al. (132) claim transduction of streptomycin resistance in *P. boryanum* by LPP-1.

CONCLUDING REMARKS

A general conception of the sequence of the developmental cycle of several groups of cyanophages is now available to us. Nevertheless, details of phage synthesis, assembly, and the nature of the interaction with the host system and of regulatory processes involved at the molecular level have yet to be unraveled.

The absolute dependence of SM-1 cyanophage on the photosynthetic activity of its unicellular host is in sharp contrast to the well-documented action of LPP and N-1 cyanophages on their filamentous hosts. The infection cycle of the latter groups of cyanophages not only can proceed independently of host photosynthesis, but disrupts (structurally and physiologically) the host's photosynthetic apparatus. These different patterns of interaction suggest divergent evolutionary trends in the development of these blue-green algae. In the light of their unique position, further studies of the infection cycle may aid in clarifying the phylogenetic development of this ancient group.

Use of phage material to induce transduction should become a valuable tool in opening up the field of genetical studies of the blue-green algae. Among the more promising lines of study would be a genetical approach to studies of the structure and function of the cyanophytic photosynthetic system.

The ubiquitous distribution of the LPP cyanophages and the fact that their host algae, though widespread, never form blooms serve as indications of the importance of cyanophages in determining the dynamics of algal populations in nature. Nevertheless, much has yet to be learned of the role of the cyanophages in different ecosystems. Finally, the use of cyanophages in manipulating and controlling algal blooms remains an open but still promising question.

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LITERATURE CITED

1. Abeliovich, A., and M. Shilo. 1972. Photooxidative reactions of c-phycocyanin. Biochim. Biophys. Acta **203**:483-491.
2. Abeliovich, A., and M. Shilo. 1972. Photooxidative death in blue-green algae. J. Bacteriol. **111**:682-689.
3. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, New York.
4. Adolph, K. W., and R. H. Haselkorn. 1971. Isolation and characterization of a virus infecting the blue-green alga *Nostoc muscorum*. Virology **46**:200-208.
5. Adolph, K. W., and R. H. Haselkorn. 1972. Photosynthesis and the development of blue-green algal virus N-1. Virology **47**:370-374.
6. Adolph, K. W., and R. H. Haselkorn. 1972. Comparison of the structures of blue-green algal viruses LPP-1M and LPP-2 and bacteriophage T7. Virology **47**:701-710.
7. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**:769-775.
8. Anderson, E. S. 1957. The relations of bacteriophages to bacterial ecology, p. 189-217. Symp. Soc. Gen. Microbiol. 7th
9. Asato, Y. 1972. Isolation and characterization of ultraviolet light-sensitive mutants of the blue-green alga *Anacystis nidulans*. J. Bacteriol. **110**:1058-1064.
10. Asato, Y., and C. E. Folsome. 1969. Mutagenesis of *Anacystis nidulans* N-nitro-N-nitrosoguanidine and UV irradiation. Mutat. Res. **8**: 531-536.
11. Asato, Y., and C. E. Folsome. 1970. Temporal genetic mapping of the blue-green alga *Anacystis nidulans*. Genetics **65**:407-419.
12. Baur, J. R., B. Richardson, R. S. Halliwell, and R. Langston. 1967. Effect of tobacco mosaic virus infection on glucose metabolism in *Nicotiana tabacum* var. Samsun. Virology **32**:580-588.
13. Bazin, M. J. 1968. Sexuality in a blue-green alga: genetic recombination in *Anacystis nidulans*. Nature (London) **218**:282-283.
14. Biggins, J. 1967. Photosynthetic reactions by lysed protoplasts and particle preparations from the blue-green alga, *Phormidium luridum*. Plant Physiol. **42**:1447-1456.
15. Biggins, J. 1969. Respiration in blue-green algae. J. Bacteriol. **99**:570-575.
16. Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. **31**:230-314.
17. Bradley, D. E., and C. A. Dewar. 1967. Intracellular changes in cells of *Escherichia coli* infected with a filamentous bacteriophage. J. Gen. Virol. **1**:179-188.
18. Brown, R. M., Jr. 1972. Algal viruses. Advan. Virus Res. **17**:243-277.
19. Brown, R. M., Jr., K. M. Smith, and P. L. Walne. 1966. Replication cycle of the blue-green algal virus LPP-1. Nature (London) **212**:729-730.
20. Cannon, R. E., and M. S. Shane. 1972. The effect of antibiotic stress on protein synthesis in the establishment of lysogeny of *Plectonema boryanum*. Virology **49**:130-133.
21. Cannon, R. E., M. S. Shane, and V. N. Bush. 1971. Lysogeny of a blue-green alga, *Plectonema boryanum*. Virology **45**:149-153.
22. Chao, L., and C. C. Bowen. 1971. Purification and properties of glycogen isolated from a blue-green alga, *Nostoc muscorum*. J. Bacteriol. **105**:331-338.
23. Cheung, W. Y., and M. Gibbs. 1966. Dark and photometabolism of sugars by a blue-green alga: *Tolypothrix tenuis*. Plant Physiol. **41**:731-737.
24. Cohen, S. S., and T. F. Anderson. 1946. Chemical studies of host-virus interactions. I. The effect of bacteriophage adsorption on the multiplication of its host *Escherichia coli* B. J. Exp. Med. **84**:511-521.
25. Cowie, D. B., and L. Prager. 1969. Cyanophyta and their viruses, p. 391-397. *In* Carnegie Institution of Washington Year Book, 1968-69. Carnegie Institution of Washington, Washington, D.C.
26. Daft, M. J., J. Begg, and W. D. P. Stewart. 1970. A virus of blue-green algae from fresh water habitats in Scotland. New Phytol. **69**:1029-1038.
27. Diener, T. O. 1963. Physiology of virus-infected plants. Annu. Rev. Phytopathol. **1**:197-218.
28. Drouet, F. 1963. Ecophenes of *Schizothrix calcicola* (oscillatoriaceae). Proc. Nat. Acad. Sci. U.S.A. **115**:261-281.
29. Duane, W. C., M. C. Hohl, and D. W. Krogman. 1965. Photophosphorylation activity in cell-free preparations of a blue-green alga. Biochim. Biophys. Acta **109**:108-116.
30. Echlin, P., and I. Morris. 1965. The relationship between blue-green algae and bacteria. Biol. Rev. (Cambridge) **40**:143-187.
31. Edelman, M., D. Swinton, J. A. Schiff, H. T. Epstein, and B. Zeldin. 1967. Deoxyribonucleic acid of the blue-green algae (cyanophyta). Bacteriol. Rev. **31**:315-331.
32. Fay, P. 1965. Heterotrophy and nitrogen fixation in *Chlorogloea fritschii*. J. Gen. Microbiol. **39**:11-20.
33. Fogg, G. E. 1969. The physiology of an algal nuisance. Proc. Roy. Soc. Ser. B. Biol. Sci. **173**:175-189.
34. Freund-Mölbert, E., G. Drews, K. Bosecker, and

B. Schubel. 1968. Morphologie und Wirstkreis eines neu isolierten *Rhodopseudomonas palustris*-phagen. Arch. Mikrobiol. **64**:1-8.

35. Ginzberg, D. 1973. The interaction between cyanophage LPP-1G and the photosynthetic system of the blue-green alga *Plectonema boryanum*. Ph.D. thesis, Hebrew University, Jerusalem.
36. Ginzberg, D., and E. Padan. 1972. Light effect on the internal osmotic pressure of the blue-green algae *Plectonema boryanum* and *Phormidium luridum*. Arch. Mikrobiol. **87**:181-183.
37. Ginzburg, D., E. Padan, and M. Shilo. 1968. Effect of cyanophage infection on CO_2 photoassimilation in *Plectonema boryanum*. J. Virol. **2**:695-701.
38. Goffeau, A., and J. M. Bove. 1965. Virus infection and photosynthesis. 1. Increased photophosphorylation by chloroplasts from Chinese cabbage infected with turnip yellow mosaic virus. Virology **27**:243-252.
39. Goldstein, D. A., and I. J. Bendet. 1967. Physical properties of the DNA from the blue-green algal virus LPP-1. Virology **32**:614-618.
40. Goldstein, D. A., I. J. Bendet, M. A. Lauffer, and K. M. Smith. 1967. Some biological and physicochemical properties of blue-green algal virus LPP-1. Virology **32**:601-613.
41. Goriushyn, V. A., and S. M. Chaplinskyaya. 1966. Existence of viruses of blue-green algae. Mykrobiol. Zhurn. (Kiev) **28**:94-97.
42. Goriushyn, V. A., and S. M. Chaplinskyaya. 1968. The discovery of viruses lysing blue-green algae in the Dneprovsk reservoirs. Tsvetenie Vody, Dumka, Kiev.
43. Granhall, U., and A. V. Hofsten. 1969. The ultrastructure of a cyanophage attack on *Anabaena variabilis*. Physiol. Plant. **22**:713-722.
44. Gromov, B. V., and S. Y. Koz'yakov. 1970. Study of the properties of the interrelationship between populations of the blue-green alga *Plectonema boryanum* GOM, and the cyanophage LPP-1. Vestn. Leningrad Univ. Ser. Biol. **1**:128-135.
45. Herdman, M., and N. G. Carr. 1971. Recombination in *Anacystis nidulans* mediated by an extracellular DNA/RNA complex. J. Gen. Microbiol. **68**:14.
46. Herdman, M., and N. G. Carr. 1972. The isolation and characterization of mutant strains of the blue-green alga, *Anacystis nidulans*. J. Gen. Microbiol. **70**:213-220.
47. Hershey, A. D., and M. Chase. 1952. Independent function of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36**:39.
48. Hoare, D. S., S. L. Hoare, and A. J. Smith. 1969. Assimilation of organic compounds by blue-green algae and photosynthetic bacteria, p. 1540-1573. *In* H. Metzner (ed.), Progress in photosynthetic research III.
49. Hoare, D. S., L. O. Ingram, E. L. Thurston, and R. Walkup. 1971. Dark heterotrophic growth of an endophytic blue-green alga. Arch. Mikrobiol. **78**:310-321.
50. Holm-Hansen, O. 1968. Ecology, physiology and biochemistry of blue-green algae. Annu. Rev. Microbiol. **22**:47-70.
51. Hood, W., and N. G. Carr. 1971. Apparent lack of control by repression of arginine metabolism in blue-green algae. J. Bacteriol. **107**:365-367.
52. Hood, W., A. G. Leaver, and N. G. Carr. 1969. Extracellular nitrogen and the control of arginine biosynthesis in *Anabaena variabilis*. Biochem. J. **114**:12P.
53. Jackson, D. F., and Sladecek, V. 1970. Algal viruses—eutrophication control potential. Yale Sci. Mag. **44**:16-22.
54. Johnson, E. J. 1966. Occurrence of the adenosine monophosphate inhibition of carbon dioxide fixation in photosynthetic and chemosynthetic autrophs. Arch. Biochem. Biophys. **114**:178-183.
55. Josslin, R. 1970. The lysis mechanism of phage T_4: mutants affecting lysis. Virology **40**:719-726.
56. Kaye, A. M., R. Salomon, and B. Fridlander. 1967. Base composition and presence of methylated bases in DNA from a blue-green alga, *Plectonema boryanum*. J. Mol. Biol. **24**:479-483.
57. Kellenberger, E., F. A. Eiserling, and E. Boy de la Tour. 1968. Studies on the morphopoiesis of the head of phage T-even. III. The cores of head-related structures. J. Ultrastruct. Res. **21**:335-360.
58. Kellenberger, E., J. Sechaud, and A. Ryter. 1959. Electron microscopical studies of phage multiplication. IV. The establishment of the DNA pool of vegetative phage and the maturation of phage particles. Virology **8**:478-498.
59. Kenyon, C. N., R. Rippka, and R. Y. Stanier. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch. Mikrobiol. **83**:216-236.
60. Khoja, T., and B. A. Whitton. 1971. Heterotrophic growth of blue-green algae. Arch. Mikrobiol. **79**:280-282.
61. Kitajima, E. W., and J. A. Lauritis. 1969. Plant virions in plasmodesmata. Virology **37**:681-684.
62. Kiyohara, T., Y. Fujita, A. Hattori, and A. Watanabe. 1960. Heterotrophic culture of a blue-green alga, *Tolypothrix tenuis* I. J. Gen. Appl. Microbiol. **6**:176-182.
63. Korant, B. D., and F. P. Pootjes. 1968. Bacteriophage producing by autotrophically growing *Hydrogenomonas facilis*. J. Virol. **2**:87-88.
64. Kozjakov, S. Y., B. V. Gromov, and I. Y. Khudiakov. 1972. A-1 (L)-cyanophage of the blue-green alga *Anabaena variabilis*. Mikrobiologiya **41**:555-559.
65. Kumar, H. D. 1970. Apparent genetic recombination in a blue-green alga. Nature (London) **196**:1121-1122.
66. Kumar, H. D. 1963. Effects of radiations on blue-green algae. I. The production and characterization of a strain of *Anacystis nidulans*

resistant to ultraviolet radiation. Ann. Bot. **27**:723–733.

67. Kumar, H. D. 1966. Some experiments on the induction of nutritionally deficient mutants in the blue-green alga, *Anacystis nidulans*. Advan. Front. Plant Sci. **14**:133–142.
68. Kunisawa, R., and G. Cohen-Bazire. 1970. Mutations of *Anacystis nidulans* that affect cell division. Arch. Mikrobiol. **71**:49–59.
69. Kushner, D. J. 1969. Self-assembly of biological structure. Bacteriol. Rev. **33**:302–345.
70. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) **227**:680–685.
71. Lang, N. J. 1968. The fine structure of blue-green algae. Annu. Rev. Microbiol. **22**:15–46.
72. Lawson, R. H., and H. Hearon. 1970. Subcellular localization of chrysanthemum aspermy virus in tobacco and chrysanthemum leaf tissue. Virology **41**:30–37.
73. Lazaroff, N., and W. Vishniac. 1964. The relationship of cellular differentiation to colonial morphogenesis of the blue-green alga, *Nostoc muscorum* A. J. Gen. Microbiol. **35**:447–457.
74. Leach, C. K., and N. G. Carr. 1969. Oxidative phosphorylation in an extract of *Anabaena variabilis*. Biochem. J. **112**:125–126.
75. Leach, C. K., and N. G. Carr. 1970. Electron transport and oxidative phosphorylation in the blue-green alga *Anabaena variabilis*. J. Gen. Microbiol. **64**:55–70.
76. Leach, C. K., J. M. Old, and N. G. Carr. 1971. Aspects of macromolecular synthesis in the blue-green alga *Anabaena variabilis*. J. Gen. Microbiol. **68**:14.
77. Luftig, R., and R. Haselkorn. 1967. Morphology of a virus of blue-green algae and properties of its deoxyribonucleic acid. J. Virol. **1**:344–361.
78. Luftig, R., and R. Haselkorn. 1968. Studies on the structure of blue-green algae virus LPP-1. Virology **34**::644–674.
79. Luftig, R., and R. Haselkorn. 1968. Comparison of blue-green algae virus LPP-1 and the morphologically related viruses G_{111} and coliphage T7. Virology **34**:675–678.
80. Luftig, R. B., W. B. Wood, and R. Okinaka. 1971. Bacteriophage T_4 head morphogenesis on the nature of gene 49-defective heads and their role as intermediates. J. Mol. Biol. **57**:555–573.
81. Lund, J. W. G. 1965. Ecology of freshwater phytoplankton. Biol. Rev. **40**:231–293.
82. Luria, S. E., and J. E. Darnell, Jr. 1967. General virology. John Wiley & Sons, Inc., New York.
83. Mackenzie, J. J., and R. Haselkorn. 1972. Physical properties of blue-green algal virus SM-1 and its DNA. Virology **49**:497–504.
84. Mackenzie, J. J., and R. Haselkorn. 1972. An electron microscope study of infection by the blue-green algal virus SM-1. Virology **49**:505–516.
85. Mackenzie, J. J., and R. Haselkorn. 1972. Photosynthesis and the development of blue-green algal virus SM-1. Virology **49**:517–521.
86. Moody, M. F. 1965. The shape of the T-even bacteriophage head. Virology **26**:567–576.
87. Mukai, F., G. Streisinger, and B. Miller. 1967. The mechanism of lysis in phage T_4-infected cells. Virology **33**:398–404.
88. Newton, J. W. 1960. Macromolecular variation in the chromatophores of the photosynthetic bacterium *Rhodospirillum rubrum*. Biochim. Biophys. Acta **42**:34–43.
89. Niblack, J. F., R. W. McDaniel, P. Killmer, and I. C. Gunsalus. 1966. Density heterogeneity in *Pseudomonas putida* bacteriophage preparations. J. Bacteriol. **92**:1254–1255.
90. Owen, P. C. 1957. The effects of infection with tobacco mosaic virus on the photosynthesis of tobacco leaves. Ann. Appl. Biol. **45**:456–461.
91. Padan, E., D. Ginzburg, and M. Shilo. 1970. The reproductive cycle of cyanophage LPP1-G in *Plectonema boryanum* and its dependence on photosynthetic and respiratory system. Virology **40**:514–521.
92. Padan, E., B. Raboy, and M. Shilo. 1971. Endogenous dark respiration of the blue-green alga, *Plectonema boryanum*. J. Bacteriol. **106**:45–50.
93. Padan, E., and M. Shilo. 1969. Distribution of cyanophages in natural habitats. Verh. Int. Verein. Limnol. **17**:747–751.
94. Padan, E., and M. Shilo. 1969. Short trichome mutant of *Plectonema boryanum*. J. Bacteriol. **97**:975–976.
95. Padan, E., M. Shilo, and N. Kislev. 1967. Isolation of "cyanophages" from freshwater ponds and their interaction with *Plectonema boryanum*. Virology **32**:234–246.
96. Padan, E., M. Shilo, and A. B. Oppenheim. 1972. Lysogeny of the blue-green alga *Plectonema boryanum* by LPP2-SPI cyanophage. Virology **47**:525–526.
97. Padan, E., A. Rimon, D. Ginzberg, and M. Shilo. 1971. A thermosensitive cyanophage (LPP1-G) attacking the blue-green alga *Plectonema boryanum*. Virology **45**:773–776.
98. Pan, P., and W. W. Umbreit. 1972. Growth of mixed cultures of autotrophic and heterotrophic organisms. Can. J. Microbiol. **18**:153–156.
99. Pearce, J., and N. G. Carr. 1969. The incorporation and metabolism of glucose by *Anabaena variabilis*. J. Gen. Microbiol. **54**:451–462.
100. Pearce, J., C. K. Leach, and N. G. Carr. 1969. The incomplete tricarboxylic acid cycle in the blue-green alga *Anabaena variabilis*. J. Gen. Microbiol. **55**:371–378.
101. Pearce, J., and N. G. Carr. 1967. The metabolism of acetate by the blue-green algae *Anabaena variabilis* and *Anacystis nidulans*. J. Gen. Microbiol. **49**:301–313.
102. Pikalek, P. 1967. Attempt to find genetic recombination in *Anacystis nidulans*. Nature (London) **215**:666–667.
103. Rimon, A. 1971. The source of the LPPIG cyanophage proteins in the blue-green alga *Plectonema boryanum* and the study of the relations between events in the infected host

and the multiplication of the phage. M. S. thesis, Hebrew University, Jerusalem.
104. Rubenchik, L. I., O. I. Bershova, N. S. Novikova, and Z. P. Koptyeva. 1966. Lysis of blue-green alga *Microcystis pulvera*. Mikrobiol. Zh. Akad. Nauk. Ukr. RSR. **28**:88-91.
105. Safferman, R. S. 1968. Virus diseases in blue-green algae. *In* Daniel F. Jackson (ed.), Algae, man and the environment. Syracuse University Press, New York.
106. Safferman, R. S., T. O. Diener, P. R. Desjardins, and M. E. Morris. 1972. Isolation and characterization of AS-1, a phycovirus infecting the blue-green algae *Anacystis nidulans* and *Synechococcus cedrorum*. Virology **47**:105-113.
107. Safferman, R. S., and M. E. Morris. 1963. Algal virus: isolation. Science **140**:679-680.
108. Safferman, R. S., and M. E. Morris. 1964. Growth characteristics of the blue-green algal virus LPP-1. J. Bacteriol. **88**:771-775.
109. Safferman, R. S., and M. E. Morris. 1964. Control of algae with viruses. J. Amer. Water Works Ass. **56**:1217-1224.
110. Safferman, R. S., and M. E. Morris. 1967. Observations on the occurrence, distribution, and seasonal incidence of blue-green algal viruses. Appl. Microbiol. **15**:1219-1222.
111. Safferman, R. S., M. E. Morris, L. A. Sherman, and R. Haselkorn. 1969. Serological and electron microscopic characterization of a new group of blue-green algal viruses (LPP-2). Virology **39**:775-780.
112. Safferman, R. S., I. R. Schneider, R. L. Steere, M. E. Morris, and T. O. Diener. 1969. Phycovirus SM-1: a virus infecting unicellular blue-green algae. Virology **37**:386-395.
113. 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.
114. Scholes, P., P. Mitchell, and J. Moyle. 1969. The polarity of proton translocation in some photosynthetic microorganisms. Eur. J. Biochem. **8**:450-454.
115. Schopf, J. W. 1970. Precambrian microorganisms and evolutionary events prior to the origin of vascular plants. Biol. Rev. **45**:319-352.
116. Shane, M. S. 1971. Distribution of blue-green algal viruses in various types of natural waters. Water Res. **5**:711-716.
117. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga *Plectonema boryanum*. I. Electron microscopy. J. Virol. **6**:820-833.
118. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga *Plectonema boryanum*. II. Viral deoxyribonucleic acid synthesis and host deoxyribonucleic acid breakdown. J. Virol. **6**:834-840.
119. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga *Plectonema boryanum*. III. Protein synthesis. J. Virol. **6**:841-846.
120. Sherman, L. A., and R. Haselkorn. 1971. Growth of the blue-green algae virus LPP-1 under conditions which impair photosynthesis. Virology **45**:739-746.
121. Shestakou, S. U., and N. T. Khyon. 1970. Evidence for genetic transformation in blue-green alga *Anacystis nidulans*. Mol. Gen. Genet. **107**:372-375.
122. Shilo, M. 1967. Formation and mode of action of algal toxins. Bacteriol. Rev. **31**:180-193.
123. Shilo, M. 1971. The ecology of cyanophages. Vehr. Int. Verein. Limnol. **21**:(in press).
124. Shlesinger, M. 1932. Adsorption of bacteriophages to homologous bacteria. II. Quantitative investigations of adsorption velocity and saturation. *In* G. S. Stent (ed.), Papers on bacterial viruses. Little, Brown and Co., Boston.
125. Siddiqi, M. S., L. M. Kozloff, F. W. Putnam, and E. A. Evans, Jr. 1952. Biochemical studies of virus reproduction. IX. Nature of the host cell contributions. J. Biol. Chem. **199**:165-176.
126. Simon, L. D. 1972. Infection of *Escherichia coli* by T_2 and T_4 bacteriophages as seen in the electron microscope: T_4 head morphogenesis. Proc. Nat. Acad. Sci. U.S.A. **69**:907-911.
127. Simon, R. D. 1971. Cyanophycin granules from the blue-green alga *Anabaena cylindrica*: a reserve material consisting of copolymers of aspartic acid and arginine. Proc. Nat. Acad. Sci. U.S.A. **68**:265-267.
128. Singh, P. K. 1973. Occurrence and distribution of cyanophages in ponds, sewage and rice fields. Arch. Mikrobiol. **89**:169-72.
129. Singh, R. N., and H. N. Singh. 1964. Ultra-violet induced mutants of blue-green algae. II. *Anabaena doliolum* Bharadwaja. Arch. Mikrobiol. **48**:118-121.
130. Singh, R. N., and P. K. Singh. 1967. Isolation of cyanophages from India. Nature (London) **216**:1020-1021.
131. Singh, R. N., P. K. Singh, A. K. Kashyap, T. A. Sarma, B. Dhar, I. J. Chaubey, and K. I. D. Choudhury. 1971. Isolation and characterization of new cyanophages and mutations of LPP-1 and host alga *Plectonema boryanum*. 12th Ann. Congr. Ass. Microbiol. India.
132. Singh, R. N., P. K. Singh, and P. K. Varanasi. 1969. Lysogeny and induction of lysis in blue-green algae and their viruses, p. 272. Proc. 56th Int. Sci. Congr.
133. Singh, R. N., and R. Sinha. 1965. Genetic recombination in blue-green alga, *Cylindrospermum majus* Kuetz. Nature (London) **207**:782-783.
134. Smith, A. J., J. London, and R. Y. Stanier. 1967. Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J. Bacteriol. **94**:972-983.
135. Smith, A. J., and C. Lucas. 1971. Propionate resistance in blue-green algae. Biochem. J. **124**:23.
136. Smith, G. 1950. Freshwater algae of the United States. McGraw-Hill Book Co. Inc., New York.
137. 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.

138. Smith, K. M., R. M. Brown, Jr., and P. L. Walne. 1967. Ultrastructural and time-lapse studies on the replication cycle of the blue-green algal virus LPP-1. Virology **31**:329-337.
139. 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.
140. Sonenshein, A. L., and D. H. Roscoe. 1969. The course of phage ϕe infection in sporulating cells of *Bacillus subtilis* strain 3610. Virology **39**:265-276.
141. Srivastava, B. S. 1969. Ultraviolet induced mutations to growth factor requirement and penicillin resistance in a blue-green alga. Arch. Mikrobiol. **66**:234-238.
142. Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae. (order *Chroococcales*). Bacteriol. Rev. **35**:171-205.
143. Stanier, R. Y., and C. B. Van-Niel. 1962. The concept of a bacterium. Arch. Mikrobiol. **42**:17-35.
144. Stevens, S. E., and C. Van Baalen. 1969. N-methyl-N-nitro-N-nitrosoguanidine as a mutagen for blue-green algae: evidence for repair. J. Phycol. **5**:136-139.
145. Stevens, S. E., Jr., and C. Van Baalen. 1970. Growth characteristics of selected mutants of a coccoid blue-green alga. Arch. Mikrobiol. **72**:1-8.
146. Summers, D. F., and J. V. Maizel. 1968. Evidence for large precursor proteins in poliovirus synthesis. Proc. Nat. Acad. Sci. U.S.A. **59**:966-971.
147. Susor, W. A., and D. W. Krogmann. 1964. Hill activity in cell-free preparations of a blue-green alga. Biochim. Biophys. Acta **88**:11-19.
148. Tagawa, K., H. Y. Tsujimoto, and D. I. Arnon. 1963. Role of chloroplast ferredoxin in the energy conversion process of photosynthesis. Proc. Nat. Acad. Sci. U.S.A. **49**:567-572.
149. Takahashi, I. 1964. Incorporation of bacteriophages genome by spores of *Bacillus subtilis*. J. Bacteriol. **87**:1499-1502.
150. Ueda, K. 1965. Virus-like structures in the cells of the blue-green alga, *Oscillatoria princeps*. Exp. Cell Res. **40**:671-673.
151. Van Baalen, C., D. S. Horre, and E. Brandt. 1971. Heterotrophic growth of blue-green algae in dim light. J. Bacteriol. **105**:685-689.
152. Walsby, A. E. 1970. The nuisance algae: curiosities in the biology of planktonic blue-green algae. Water Treat. Exam., no. 4.
153. Watanabe, A., and Y. Yamamoto. 1967. Heterotrophic nitrogen fixation by the blue-green alga *Anabaenopsis circularis*. Nature (London) **214**:738.
154. Weidel, W., and E. Kellenberger. 1955. The *E. coli* B receptor for the phage T_5. II. Electron microscopic studies. Biochim. Biophys. Acta **17**:1-9.
155. Weppelman, R. M., and C. C. Brinton. 1971. The infection of *Pseudomonas aeruginosa* by RNA pilus phage PP_7: the adsorption organelle and the relationship between phage sensitivity and the division cycle. Virology **44**:1-17.
156. Whitton, B. A., N. G. Carr, and I. W. Craig. 1971. A comparison of the fine structure and nucleic acid biochemistry of chloroplasts and blue-green algae. Protoplasma **72**:325-357.
157. Williams, R. E. O., and J. E. Rippon. 1953. Bacteriophage typing of strains of *Staphlyococcus aureus* from various sources. Lancet **264**(1):510-514.
158. Wolk, C. P. 1968. Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. J. Bacteriol. **96**:2138-2143.
159. Wu, J. H., G. L. Choules, and R. A. Lewin. 1968. Early stages of the infection process in a blue-green algal virus system, as affected by KCN and light, p. 153-160. *In* Biochemical regulation in diseased plants or injury. Phytopathological Society of Japan, Tokyo.
160. Wu, J. H., R. A. Lewin, and H. Werbin. 1967. Photoreactivation of UV irradiated blue-green algal virus LPP-1. Virology **31**:657-664.
161. Wu, J. H., and P. M. Shugarman. 1967. Effect of virus infection on rate of photosynthesis and respiration of a blue-green alga, *Plectonema boryanum*. Virology **32**:166-167.
162. Yehle, C. O., and R. H. Doi. 1967. Differential expression of bacteriophage genomes in vegetative and sporulating cells of *Bacillus subtilis*. J. Virol. **1**:935-947.
163. Zagury, D., P. Model, and G. D. Pappas. 1967. A simplified technique for preparing thin sections of bacterial and viral suspensions: application to *Escherichia coli* and bacteriophage T_2. Virology **33**:347-352.
164. Zaitlin, M., and A. T. Jagendorf. 1960. Photosynthetic phosphorylation and Hill reaction activities of chloroplasts isolated from plants infected with tobacco mosaic virus. Virology **12**:477-486.