involved in inducing the hippocampal rather than the neocortical rhythms of deep sleep, the electroencephalographic patterns characterizing this stage of sleep can hardly result from activity limited to the specific pontolimbic connections which we investigated. Whether the conduction system from the rhomboencephalic pacemaker of deep sleep to the more rostral mechanisms regulating cortical activity is restricted to small regions of the brain stem, other than those projecting to the rhinencephalon, or is widely scattered through the midbrain, cannot be determined as yet, but the latter hypothesis is supported by the persistence of desynchronized sleep patterns after such large lesions as those shown in Fig. 1*C*.

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Algal Virus: Isolation

Abstract. *Freshwater blue-green algae of the genera* Lyngbya, Plectonema, *and* Phormidium *are susceptible to a virus recently isolated from a waste-stabilization pond. Electron micrographs of a partially purified preparation show that the viral particle has an icosahedral structure about 66 mμ in diameter.*

Rapid decomposition of algae is often observed under circumstances that suggest the presence of a factor not generally associated with degeneration. From studies on the phytoplankton populations of shallow lakes, Tucker (*1*) reported that inhibitory agents of unspecified origin were responsible for fluctuations in the development of these organisms. Krauss (*2*) suggested that the solution to many of these unresolved incidences might be explained by the presence of viral agents.

In view of the ubiquitous nature and distribution of viruses, it seems unlikely that the biological characteristics of the algal cell are such that the entire group would be immune to viral infections. With this in mind, emphasis was placed on determining the susceptibility of blue-green algae to these agents. Screening for the virus was carried out with samples collected from environments having a dense and somewhat unstable algal population. The present report describes the isolation of a virus-like agent which, to the authors' knowledge, is the first definite evidence that freshwater algae (*3*) may be infected by such agents.

The agent was isolated from samples collected at a waste-stabilization pond used exclusively for the treatment of domestic sewage. The pond, approximately 4 acres in size, is located in southeastern Indiana and supports a considerable variety of algal forms. Composite samples taken from the pond were added to algae-enriched media, incubated for one week, and then centrifuged. The supernatant was treated with chloroform (*4*), and the final preparation was screened for viral activity. Lysis of one of the blue-green algal test organisms, *Plectonema boryanum* IU 594 (*5*), in liquid medium and demonstration of plaque development in solid medium indicated the presence of a virus-like agent in the assayed sample. Examination of the lytic agent showed that it could readily be passed through an ultrafine sintered-glass filter and that exposure to high temperatures (90° to 100°C) completely inactivated it. Bacteria-free filtrates recovered from lysed cultures of *P. boryanum* have been transferred through 35 subculturings, the final subculture being equivalent to a 10^{-70} dilution of the original material. Additions of small numbers of this agent to growing algal cultures resulted in total lysis of the algae and a 5- to 6-log increase in the titer of the agent. It is, therefore, evident that the agent possesses those characteristics that are generally ascribed to a virus.

To insure the purity of the viral strain, the virus was picked and replated twice from single plaques selected at random. The isolated strain was examined for its capacity to lyse algae, actinomycetes, and bacteria. Of the 78 organisms tested, the virus lysed 11 filamentous algal strains belonging to the class Myxophyceae. Included in these forms were members of three genera—*Lyngbya, Plectonema,* and *Phormidium*. The first letters in the names of these three genera have been used to classify the blue-green algal (BGA) virus as strain LPP-1. The algal strains from the Indiana University (IU) culture collection which are susceptible to the BGA virus are *Phormidium luridum* var. *olivace* IU 426, *Phormidium* species IU 485, *Phormidium fareolarum* IU 427, *Plectonema boryanum* IU 581, 594, 597, and 790, *Plectonema calothricoides* IU 598, *Plectonema notatum* IU 482, *Lyngbya* species IU 487 and 488.

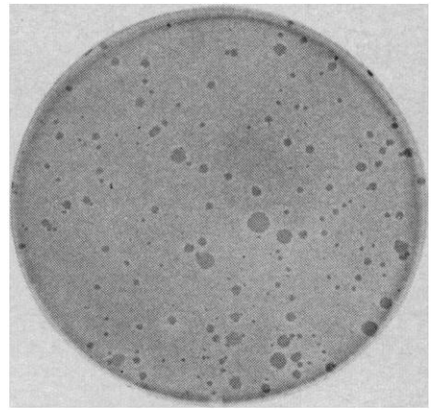

Fig. 1. Plaques of BGA virus strain LPP-1 on *Plectonema boryanum* culture plate.

The viral agent was propagated under static conditions with *Plectonema boryanum* IU 594 as the host and a modified Chu No. 10 broth (*6*) as the medium. Cultures were incubated at 20°C under a light intensity of 160 to 180 lu/ft^2. After an incubation period of 2 to 3 days, the titer was generally about 10^8 viral particles per milliliter of broth. On solid medium the virus and the host cells were incubated 3 to 4 days before plaque counts were made. Additional incubation of the virus often resulted in lysis of the entire culture plate. Figure 1 shows relatively clear plaques with a well-defined border that is somewhat frayed by the filamentous nature of the host cells. Plaques produced by this strain differ considerably in size and vary from less than 0.1 mm to more than 8 mm in diameter.

Because of the symbiotic and tenacious relationships between bacteria and blue-green algae, it is not inconceivable that a bacteriophage could indirectly inhibit algal development. Algal degeneration under such circumstances would undoubtedly be concomitant with a nutritional deficiency, but microscopic examination of unialgal cultures showed that the algal cells are

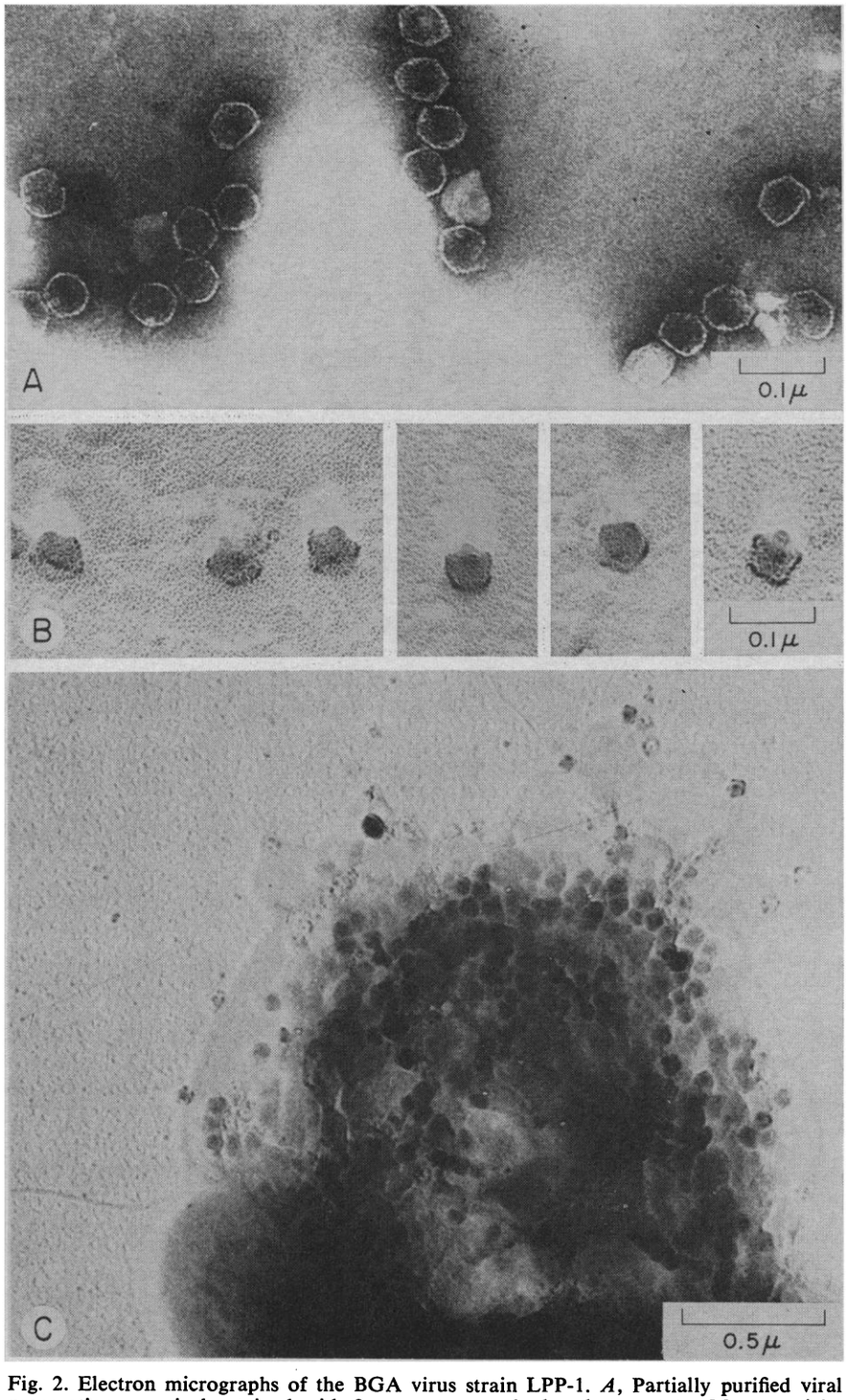

Fig. 2. Electron micrographs of the BGA virus strain LPP-1. *A*, Partially purified viral preparation negatively stained with 2 percent neutral phosphotungstate. Most particles appear to be empty shells filled with this stain. An angular appearance, usually six-sided, is frequently observed. There is also a suggestion that the particles are made up of a large number of subunits, possibly six per edge. When this same preparation was shadowed with chromium, the particles were often associated with long fine threads that appeared to be nucleic acid strands. *B*, Selected particles of an infected algal culture shadowed with chromium. The accentuation of the points lying on the five-fold symmetry axes may have resulted from shrinkage of the particles on drying. *C*, A partially disintegrated algal cell prepared from an infected culture (edge of a plaque) and shadowed with chromium. Of the numerous particles which appear to be attached to the algal membrane, several clearly show a pentagonal outline.

randomly lysed by the virus. As a result the algal filaments fragment into progressively smaller units until only scattered cells can be observed. Disintegration of the algal culture was invariably accompanied by a rapid rise in the density of the bacterial population and in no instance did the bacteria isolated from these algae give any indication of being susceptible to the virus.

Lysed algal cultures partially purified by differential centrifugation contained polyhedral particles that were relatively homogeneous in appearance. The electron micrographs in Fig. 2 show the outlines of hexagonal and pentagonal particles having an average diameter of about 66 mμ. The hexagonal shape of the negative stained shells (Fig. 2*A*) appears to coincide with the two- and three-fold axes seen in icosahedral structures, whereas the five-fold axes are evident in the metal shadowed particles (Fig. 2*B*). No tails could be distinguished in these preparations. The algal virus is morphologically similar to the adenovirus and wound-tumor virus as well as to a large number of other animal and plant viruses, and at least one insect virus (*7*). No attempt has been made to identify the virus with either the plant or bacterial viral groups (*8*).

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