Table 1. Synthesis of homoarginine and homocitrulline after the injection of lysine and uniformly labeled lysine-C^{14}. Each rat was injected with 15 μc of uniformly labeled lysine-C^{14} per 100 grams of body weight and 1.5 mmole of lysine per 100 grams of body weight. Specific activity of uniformly labeled lysine-C^{14} was 209 mc/mmole. Values are per gram of tissue, wet weight. In the 2.0-ml flow cell, 0.01 μc produced 50,000 counts.

Time (min)	Liver		Kidney	
	Count/ μmole	μmole	Count/ μmole	μmole
	Homocitrulline			
0		0.032		0.00
10	5,000	.090	15,000	.273
60	34,000	.538	76,000	.680
120	25,000	.330	107,000	1.35
	Homoarginine			
0		0.00		0.00
10	150	.002	4,000	.090
60	1,300	.031	10,000	.246
120	6,000	.090	10,500	.300

Table 2. Homocitrulline and homoarginine in adult human urine after the ingestion of lysine. Values are total milligrams excreted during the 6- to 9-hour period after ingestion of lysine. Analyses were made on 5 to 10 ml of urine.

Homocitrulline		Homoarginine	
Before lysine	After lysine	Before lysine	After lysine
0.00	2.67	1.03	1.13
1.12	1.78	1.10	2.38
1.59	2.14		
0.61	1.92	0.00	0.20
.75	7.23	.00	12.0
.34	1.28	.00	0.00
.51	2.94	.00	1.30

3.25 buffer (*2*). The lysine employed in these experiments was analyzed chromatographically and found to contain less than 0.003 μmole of homocitrulline and homoarginine in 200 μmole of lysine.

After the injection of lysine, the rats develop a marked thirst and drink almost continuously for an hour. The amount of drinking permitted causes considerable variation in the concentrations of homocitrulline and homoarginine, particularly in the kidney. Therefore, in subsequent experiments the drinking water was removed when the lysine was injected.

In order to establish the assumed relationship between lysine, homocitrulline, and homoarginine, nine rats (male, white, Holtzman, 120 to 150 grams) were injected with 1.5 mmole of L-lysine per 100 grams of body weight which contained 15 μc of uniformly labeled DL-lysine-C^{14} per 100 grams of body weight. The animals were killed at 10 minutes, 1 hour, and 2 hours thereafter. The tissues were prepared and analyzed for homocitrulline and homoarginine (*7*) (Table 1). The results provide evidence that the homocitrulline and homoarginine are derived from lysine.

In these experiments, radioactivity was also observed in the glutamic acid, glutamine, and α-aminoadipic acid peaks. The presence of C^{14} in all of these amino acids would be expected from studies of Borsook (*8*) and Rothstein and Miller (*9*) who have described the metabolic path of lysine.

Since milk is an excellent source of lysine, it appears probable that the homocitrulline found in the urine of infants by Gerritsen may be the result of the ingestion of a diet rich in lysine. In addition, infants have lower renal thresholds for amino acids (*10*). It thus seemed probable that the addition of extra lysine to the diet of normal human adults would result in an increased excretion of homocitrulline and homoarginine.

To test our hypothesis, 4 grams of lysine monohydrochloride were ingested by each of seven normal adults; this dose was about 2.5 times the tentative minimum requirement value of Rose (*11*). The lysine was dissolved in orange juice and ingested before retiring. The urines were collected the following morning, the volumes were measured, and homocitrulline and homoarginine were determined chromatographically. This same procedure, with the exception of the ingestion of lysine, was used to obtain the normal values of homocitrulline and homoarginine in adult urine. The results of this experiment are presented in Table 2. It is apparent from the values obtained that the ingestion of lysine leads to an increased excretion of homocitrulline and homoarginine.

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Blue-Green Algal Virus LPP-1: Purification and Partial Characterization

Abstract. *The blue-green algal virus LPP-1 was concentrated by ultrafiltration and purified by density-gradient and differential centrifugation. The virus contains DNA and has a sedimentation coefficient of 548S. Electron micrographs of purified viral preparations show that the polyhedral particles have short tails, which are approximately one-fourth as long as the diameter of the head. Data presented in this report indicate that the blue-green algal virus more closely resembles bacteriophages than viruses infecting higher plants.*

The recent isolation of a virus lysing certain blue-green algae is of considerable interest since it is apparently the first example of a virus infecting an algal cell (*1*). Some taxonomists have placed the Cyanophyceae closer to bacteria than to other algae, and one question facing virologists is whether the newly discovered virus more closely resembles bacterial viruses or viruses of higher plants. In this report, we describe the purification of the blue-green algal virus LPP-1 and some of its properties.

Large-scale production of the blue-green algal virus was carried out in a fermentor drive assembly (*2*) with two 14-liter fermentors. Each contained 10 liters of a modified Chu No. 10 broth (*3*) and was sterilized in batches by steam pressure at 1.3 atm for 1 hour. The fermentors, placed in a 30°C water bath, were inoculated with 300 ml of a 3-week-old culture of *Plectonema boryanum* IU 594 (Indiana University culture collection) and 1 ml of virus preparation [10^8 plaque-forming units (PFU) per milliliter] that had been filtered through sintered glass. A gas mixture of 5-percent carbon dioxide in air

was bubbled through the agitated medium (300 rev/min), and continuous illumination of 5400 to 6500 lu/m^2 was provided by a central light source composed of six vertical "cool white" fluorescent tubes. After a 5-day incubation period, the cultures were clarified in a continuous-flow centrifuge (4°C, 80 to 90 ml/min) at 10,000*g*. All subsequent operations were also performed at 1° to 4°C.

Under these culturing conditions, virus titers of 10^9 to 10^{10} PFU per milliliter were regularly obtained (*4*). Initial attempts to concentrate culture lysates by ultracentrifugation proved unsuccessful. Ultrafiltration was therefore used in the concentration of lysed cultures (*5*). The 10-liter suspensions were reduced to volumes ranging from 25 to 50 ml. By this method, titers of 10^{11} to 10^{12} PFU per milliliter were obtained if the large amounts of virus adsorbed to the walls of the ultrafiltrator bags were recovered. The resulting concentrates, which were turbid and contained an orange pigment, were then dialyzed against a solution containing 0.1*M* NaCl and 0.001*M* $MgCl_2$ (saline magnesium solution) (*4*). When the components of this solution were studied, we found that Mg^{++} is essential for maintaining the biological activity of the virus.

Purification was achieved by density-gradient centrifugation followed by one or more cycles of differential centrifugation. Portions of 1 to 4 ml of dialyzed concentrate were layered onto linear gradients (0.2 to 0.8*M* sucrose in the saline magnesium solution). Preliminary experiments showed that the virus was stable for 24 hours in the presence of 0.7*M* sucrose but lost appreciable activity in the presence of 1*M* sucrose. The tubes were centrifuged at 20,000 rev/min for 75 minutes in the SW 25.1 rotor (*2*). Examination of the centrifuged gradients for light scattering revealed a well-defined zone in the lower third of the tube, two or three minor zones in the upper half of the tube, and a large orange pellet.

The gradients were fractionated into 1-ml portions with a density-gradient fractionator (*2*). Figure 1 shows a typical recorder tracing and infectivity data. Two major ultraviolet absorbing components were found; one in the first 4 ml (upper component) and the other, corresponding to the lower visible zone, at approximately 19.5 ml (lower component). Most of the infectivity (10^{10} PFU per optical density unit) coincided with the lower component. The fractions containing the upper component were associated with the least infectivity (10^5 PFU per optical density unit) found in the gradient. The faint zones visible in the upper portion of the tubes had slight or no ultraviolet absorption and low biological activity.

Fractions comprising the lower component were pooled, dialyzed against the saline magnesium solution to remove sucrose, concentrated in an ultrafiltrator, and then centrifuged a second time in an identical sucrose gradient. Most of the optical-density and biological activity was again located in the same position in the tube. Fractions containing the bulk of the virus were again pooled, dialyzed against the saline magnesium solution, and concentrated. Insoluble portions were removed by centrifugation for 15 minutes at 1000*g*.

Virus preparations were purified further by one or two cycles of differential centrifugation. Virus was collected as pellets at 17,500 rev/min in 2-ml tubes in the 40 rotor of the Spinco Model L centrifuge for 30 minutes followed by resuspension in the saline magnesium solution and a low-speed clarification.

Purified virus had an absorption spectrum (*2*) characteristic of nucleoprotein (maximum at 258 mμ; minimum at 240 mμ; maximum/minimum ratio, 1.35 to 1.38; 260/280 mμ ratio, 1.50 to 1.53).

Partially purified virus (two cycles of density-gradient centrifugation) was subjected to analytical ultracentrifuga-

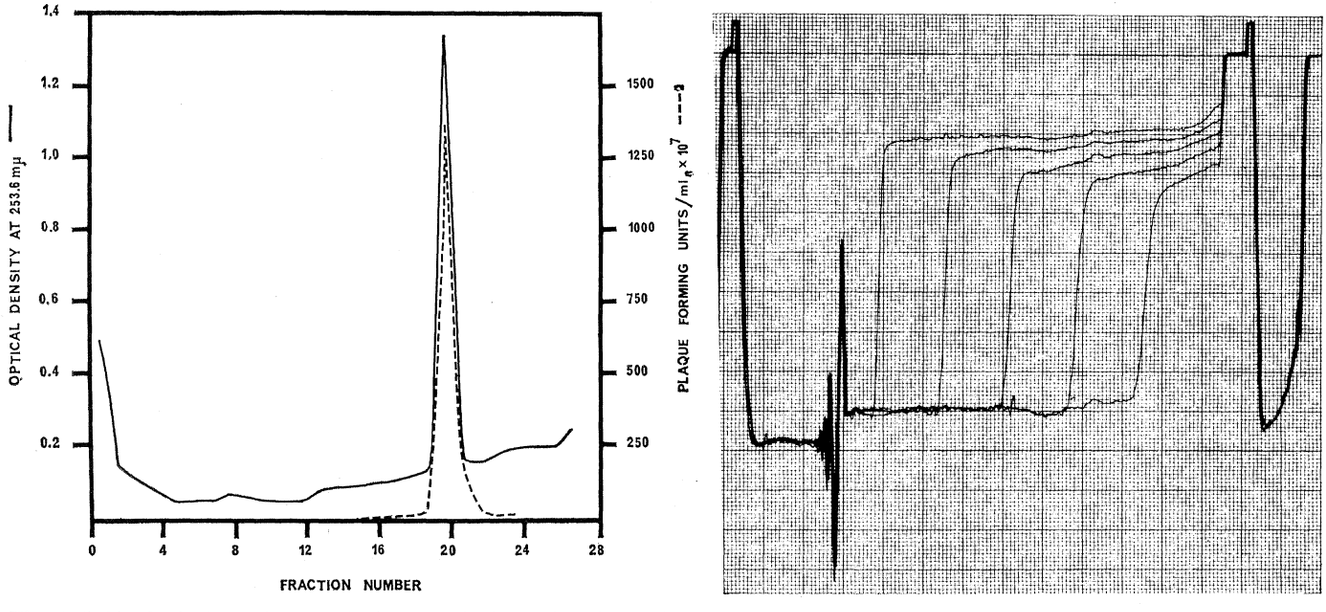

Fig. 1 (left). Optical density at 253.6 mμ and plaque-forming units of consecutive 1-ml fractions taken from a centrifuged density gradient column containing a concentrated lysate of *Plectonema boryanum* infected with blue-green algal virus. Fig. 2 (right). Microdensitometer tracing of the sedimentation patterns of blue-green algal virus as recorded with ultraviolet optics. Speed was 17,980 rev/min and temperature was 5.5°C. Pictures were taken at 4-minute intervals immediately after speed was reached. Sedimentation is left to right.

tion at about 5°C (*2*). At 17,980 rev/min, a single component sedimented completely in about 25 minutes. However, approximately 32 percent of the ultraviolet-absorbing material had not begun to sediment at this speed. No evidence of sedimentation was observed when centrifugation was continued for 32 minutes at 59,780 rev/min. Thus, this material is much smaller than the sedimenting component. Most biological activity was found in the pellet, indicating that the sedimenting component is the virus. The presence of nonsedimenting material is surprising at this stage of purification. However, ultraviolet-absorbing material was also regularly detected in the uppermost portion of the second density gradient. Apparently, this material is identical with the upper component found after the first density gradient centrifugation (see Fig. 1). The material may have been initially adsorbed to the virus or may be a degradation product of the virus.

The following experiment indicated that the contamination of virus with presumed upper component could be reduced by repeated centrifugation. A preparation of virus with a concentration equivalent to 2.4 optical density units per milliliter was centrifuged in the analytical ultracentrifuge at 17,980 rev/min for 25 minutes, during which time all the virus was pelleted. After removal of the supernatant (containing 32 percent of the ultraviolet-absorbing material), the virus was resuspended and subjected to ultracentrifugation at the same speed and for the same length of time. Figure 2 shows a densitometer tracing (*2*) of the second ultracentrifugation. Only 11 percent ultraviolet-absorbing material remained in the supernatant, while the bulk of the material sedimented as a single sharp boundary. The corrected sedimentation coefficient ($S_{20,w}$) was 548*S*.

Purified virus reacted positively with diphenylamine (*6*), indicating that the virus contains DNA. The amount of color produced accounted for most of the nucleic acid known to be present from the ultraviolet absorption of the sample when compared with salmon sperm DNA as standard. Orcinol tests were also positive (*7*); but the color produced, if considered to be RNA, accounted for no more than 10 percent of the nucleic acid known to be present (standard: yeast RNA).

Correlation of ultraviolet absorption and biological activity showed that a virus concentration corresponding to about 40 μg of DNA per milliliter resulted in 10^{11} PFU per milliliter. The ultraviolet absorption spectrum of the virus indicates that it contains roughly 20 percent nucleic acid. If a molecular weight of 10^7 is assumed—because of the similarity of the blue-green algal virus in its size and sedimentation constant to wound-tumor virus (*8*)—the number of particles required to produce one plaque is within a magnitude of 10^2.

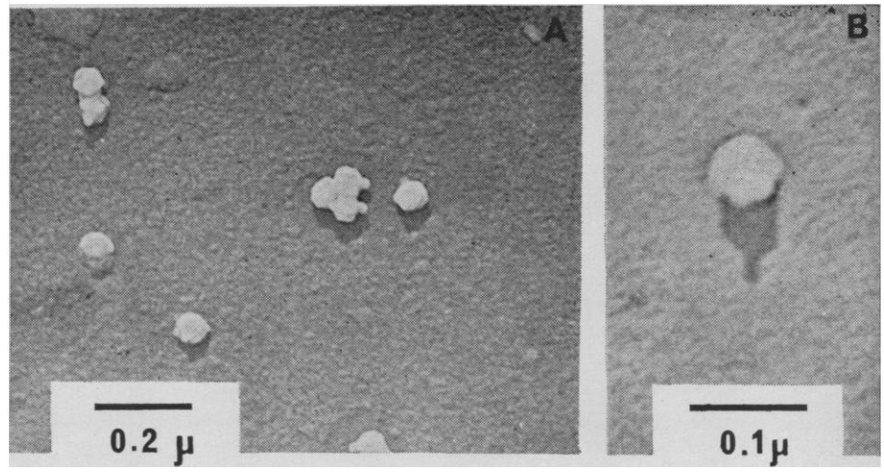

Fig. 3. Electron micrographs of blue-green algal virus shadowed with chromium. *A*, Air-dried virus in the saline magnesium solution. Grids were prepared as described by Steere and Faust (*12*). *B*, A selected virus particle from a frozen-dried preparation (*13*).

Electron micrographs of the blue-green algal virus (Fig. 3) revealed polyhedral particles as before (*1*). However, an average diameter (center to center spacing) of about 56 mμ was determined, and a tail approximately one-fourth as long as the diameter of the head could clearly be discerned in purified preparations.

Algal strains reported susceptible to the blue-green algal virus (*1*) yielded protoplasts when treated with lysozyme (*9*) under conditions which were essentially the same as those employed by Crespi, Mandeville, and Katz (*10*). In view of these results and the recent report that muramic acid and mucopolysaccharides are important components in the cell wall of *Phormidium uncinatum* (*11*), it appears that with respect to cell wall composition, these strains are more closely related to bacteria than to higher plants. Similarly, the high specific infectivity, the presence of DNA, and the presence of a tail indicates that the blue-green algal virus more closely resembles bacteriophages than viruses infecting higher plants. Undoubtedly, other viruses will be detected that infect algae. We propose that the name Phycovirus be adopted as a collective term for all these viruses.

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3. $Ca(NO_3)_2 \cdot 4H_2O$, 0.232 g; K_2HPO_4, 0.01 g; $MgSO_4 \cdot 7H_2O$, 0.025 g; Na_2CO_3, 0.02 g; $Na_2SiO_3 \cdot 5H_2O$, 0.044 g; ferric citrate, 0.0035 g; citric acid, 0.0035 g; distilled water, 1000 ml.
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Polarographic Investigation of Conjugated Fat-Soluble Vitamins

Abstract. *The half-wave potentials of all-*trans *β-carotene, all-*trans *retinol, 13-*cis *retinol, all-*trans *retinyl acetate, all-*trans *retinal, and vitamins D_2 and D_3 were related to the number of double bonds in conjugation. A minimum of three double bonds in conjugation was required before reduction took place at the dropping-mercury electrode. As the number of conjugated bonds increased in the fat-soluble vitamins, the initial reduction took place at a lower half-wave potential. All of the waves were linearly proportional to the concentration of the vitamins in the concentration range studied.*

The electrochemical reductions at the dropping-mercury electrode have been applied to a wide series of water-soluble vitamins. The mechanism of reduction or the conditions necessary for the determination of thiamine, riboflavin, pyridoxine, and vitamin B_{12}, as well as ascorbic acid, niacin, pantothenic acid, and folic acid, in biological samples have been reviewed (*1*). The fat-soluble vitamin E has been investigated by reduction of the quinone or the oxidation of the hydroquinone (*2*). As with vitamin E, vitamin K in the quinone form is reduced in aqueous (*3*) potassium chloride solution and nonaqueous solutions (*4*).

Vitamin A (*5*) and β-carotene (*6*) in plants were separated and then converted to the iodinated derivatives, which gave anodic waves in methanolic solutions. In a more direct method, Salah and Heyrovski (*7*) showed that no polarographic wave was given by vitamin A. However, the aldehyde at low *p*H gave two reduction waves, and in alkaline media, a third wave. Conjugated hydrocarbons are known to be reduced at the dropping-mercury electrode (*8–11*) in protonic solvents, which on addition of hydrogen ions or water shift the reduction to more negative potentials. Although 10 percent aqueous dioxane with tetra-butyl ammonium hydroxide would not be considered a protonic solvent, reproducible polarograms were obtained for all the conjugated fat-soluble vitamins in this solvent. In this report I describe the polarographic reduction and determination of various fat-soluble vitamins and give reasons for associating the increase in conjugation with greater ease in placing the first electrons to form a conjugated anion.

The polarograms were obtained by using a jacketed polarographic cell (*12*) maintained at a constant temperature of 25° ± 0.2°C; the inner compartment contained a 1.1 cm^2 mercury anode. The resistance of the 10 percent aqueous dioxane containing either 0.1*N* tetra-butyl ammonium hydroxide or the chloride, as measured by a conductivity bridge (*13*), was 9800 and 23,200 ohms, respectively. At −2.50 volts the capillary constant $m^{\frac{2}{3}}t^{\frac{1}{3}}$ in tetra-butyl ammonium hydroxide was 1.82, and in tetra-butyl ammonium chloride, 3.03 (*14*).

All the conjugated fat-soluble vitamins studied were purchased commercially (*15*) and considered to be of high chemical purity. Vitamins D_2 and D_3, all-*trans* retinyl acetate, all-*trans* retinol, 13-*cis* retinol, and all-*trans* retinal were soluble in 10 percent aqueous dioxane containing 0.1*N* tetra-butyl ammonium chloride or hydroxide as a supporting electrolyte at $10^{-3}M$ concentrations or higher. Limited solubility of β-carotene made it necessary to run polarograms starting at a maximum concentration of $10^{-4}M$ in tetra-butyl ammonium chloride.

As shown in Fig. 1, the polarograms of vitamin D_2, 13-*cis* retinol, and β-carotene gave stepwise reduction waves depending on the degree of conjugation in 10 percent aqueous dioxane containing 0.1*N* tetra-butyl ammonium hydroxide. The first reduction wave of each of these fat-soluble vitamins shows β-carotene to be the easiest to reduce, followed by 13-*cis* retinol and vitamin D_2. Similar polarographic reduction waves were found in tetra-butyl ammonium chloride with half-wave potentials slightly more negative. This is in agreement with the polarographic reduction of a series of increasingly conjugated polyenes (*9, 10*) where the first reduction wave appeared at more positive half-wave potentials.

Several fat-soluble vitamins with increasing unsaturation with known geometrical isomers were investigated in 10 percent aqueous dioxane containing tetra-butyl ammonium chloride and hydroxide as supporting electrolytes (Table 1). A minimum of three double bonds in conjugation was needed before a reduction wave could be observed. This minimum was confirmed by alkaline isomerization of linolenic acid (purity > 99 percent), which showed a reduction wave at half-wave potential of −1.95 volts in tetra-butyl ammonium hydroxide. Alkaline isomerized linoleic acid showed no reduction wave even at concentrations greater than $10^{-3}M$. In tetra-butyl ammonium chloride and hydroxide, a linear relationship existed between the diffusion current and the concentrations shown

Table 1. Observed half-wave potentials ($E_{\frac{1}{2}}$) for various fat-soluble vitamins in basic and neutral solvents.

Double bonds * (No.)	$E_{\frac{1}{2}}$ †	$\frac{i_d}{Cm^{\frac{2}{3}}t^{\frac{1}{3}}}$	$E_{\frac{1}{2}}$ ‡	$\frac{i_d}{Cm^{\frac{2}{3}}t^{\frac{1}{3}}}$
		Vitamin D_2		
3	−2.01	2.57	−2.25	1.50
		Vitamin D_3		
3	−2.01	2.76	−2.22	1.66
		Retinyl acetate (*all*-trans)		
5	−1.53	2.51	−1.44	1.87
	−1.71	1.65	−1.92	1.82
	−2.16	1.19	−2.39	1.07
		Retinol (*all*-trans)		
5	−1.53	2.64	−1.86	1.60
	−1.77	1.85	−2.10	0.99
	−2.34	1.58	−2.58	0.92
		Retinol (*13*-cis)		
	−1.50	2.51	−1.74	1.72
	−2.18	1.52	−2.37	1.19
		Retinal (*all*-trans)		
5	−0.78	1.31	−0.96	1.11
	−1.08	1.31	−1.26	1.11
	−1.65	0.26	−2.37	1.67
	−2.19	2.04		
		β-Carotene (*all*-trans)		
11	−1.14	1.58	−1.35	0.11‖
	−1.50	0.73	−1.74	0.10‖
	−1.77	1.50	−1.96	0.14‖
	−2.10	1.32	−2.22	—

* Number of double bonds in conjugation. † Half-wave potential with reference to mercury pool anode in 10 percent aqueous 0.1*N* tetra-butyl ammonium hydroxide as supporting electrolyte. ‡ Half-wave potential with reference to mercury pool anode in 10 percent aqueous 0.1*N* tetra-butyl ammonium chloride as supporting electrolyte. ‖ Apparent diffusion current calculated in the concentration range of 10^{-4} to $10^{-5}M$, due to limited solubility; all other vitamins 10^{-3} to $10^{-4}M$.