

Fig. 2. Turbidity profiles according to twilight measurements at 20° elevation during the winter of 1959-60 (Blue Hill Observatory) and the winter of 1963-64. Abscissa:  $\sigma/\sigma_R$ , ratio of observed scattering to Rayleigh scattering;  $\Sigma_D / \Sigma_R = ratio$ of attenuation coefficient by dust and air. Wavelength, 6600 Å.

1962 (8) were definitely higher, from 30 to 45 km. The dust layer producing the crimson glow may have extended to about 22 km. Its elevation curve fits rather well to the computed curves.

Fortunately, twilight measurements which were made at Blue Hill Observatory, Harvard University, near Boston, Mass., during the undisturbed period from September 1959 to December 1961 (9) could be continued at Weissenau with similar equipment. The twilight course on 9 October 1963 seemed to be as usual; on 12 October the red intensity at 4.5 deg sun depression and 20 deg elevation was already 1.5 times larger than previously and about 2.8 times larger during January 1964. In Fig. 2, turbidity profiles derived from two typical twilight measurements of the end of 1963 are compared with average profiles of the previous normal period and indicate considerable amounts of new dust in the northern stratosphere. However, the turbidity increase was much smaller than over the Southern Hemisphere; measurements of solar radiation at Zugspitz Observatory (2960 m) near Garmisch indicated a dust attenuation by less than 0.02 magnitudes.

There seems to be no doubt that the dust came from the eruption of Agung volcano. In the Northern Hemisphere September and October are the months during which there is a strong exchange of air between low and high latitudes in the stratospheric circulation. At the Krakatoa eruption in 1883, at the same time of year, similar observations of dust were made in the United States and Europe (10). The dust stripes may perhaps be explained as undulations of the upper borders of dust-laden, rather laminar, layers, or as undulations of the borders of inversions.

According to observations after earlier volcanic eruptions and to the results of radioactive fallout studies of atomic bomb tests, the present stratospheric dust disturbance may slowly fade in the course of about 2 years.

It is unlikely that the submarine volcano at 63°N, 20°36'W, which has been emitting ash and dust since 14 November up to an altitude of 8 km, produced any of the aforementioned phenomena. However, specks in the twilight arch, and areas of slightly different brightness of about 7 deg extension, were noticed at Weissenau on some evenings around 20 November for about 20 minutes after sunset. The specks may well be attributed to diffuse dust clouds produced by this volcano within the upper troposphere.

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## Homocitrulline and Homoarginine Synthesis from Lysine

Abstract. After the injection of Llysine and uniformly labeled L-lysinecarbon-14 into the rat, labeled homocitrulline and homoarginine are found in the liver and kidney. The ingestion of lysine by seven normal adults results in an increased urinary excretion of homocitrulline and homoarginine. These data suggest the occurrence of an unreported metabolic pathway for lysine in the rat and man.

A quantitative study of the free amino acids of tumor tissues revealed the presence of the amino acid homocitrulline in human tumors and in the C<sub>3</sub>HBA mammary adenocarcinoma (1). This amino acid had not previously been described in normal or malignant tissues.

Gerritsen et al. have reported homocitrulline in the urine of infants and young children but not in adults (2). Studies have provided evidence that the homocitrulline was of dietary origin (3). The imide of homocitrulline, homoarginine, was not found in any of the urine samples examined (4).

Stevens and Bush (5) observed a limited growth response in lysine-deficient rats fed homoarginine, indicating conversion of homoarginine to lysine. Our study was initiated to determine if lysine is the metabolic precursor of homocitrulline and homoarginine.

Homoarginine was determined chromatographically by using the 50-cm column and the buffer (pH 5.28, 0.7N) described by Kominz (6). After arginine is eluted, the addition of 69 ml of buffer results in the elution of homoarginine. The identity of the homoarginine was established by the addition of L-homoarginine, guanido-C<sup>14</sup>  $(0.005 \ \mu c)$ , to the unknown samples. The eluent from the column was pumped through a 2.0-ml flow cell in a scintillation counter and then returned to the reaction coil of the Spinco Amino Acid Analyzer. The shape and position of the radiograph curves were compared with the colorimetric curves of the unknown. Since labeled homocitrulline was not available, the samples were analyzed again after the addition of known amounts of homocitrulline to establish the identity of the homocitrulline peak. Homocitrulline is eluted from the 150-cm column after the addition of 400 ml of pH

SCIENCE, VOL. 144

Synthesis of homoarginine and Table 1. homocitrulline after the injection of lysine and uniformly labeled lysine-C<sup>14</sup>. Each rat was injected with 15  $\mu$ c of uniformly labeled lysine-C14 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<sup>14</sup> was 209 mc/mmole. Values are per gram of tissue, wet weight. In the 2.0-ml flow cell, 0.01  $\mu$ c produced 50,000 counts.

Time (min)	Liver		Kidney	
	Count/ µmole	[µmole]	Count/ µmole	μmole
	Ha	mocitrull	ine	
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
	$H_{i}$	omoargini	ine	
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  $\mu$ mole of homocitrulline and homoarginine in 200  $\mu$ mole 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  $\mu$ c of uniformly labeled DL-lysine-C<sup>14</sup> 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  $\alpha$ -aminoadipic acid peaks. The presence of C14 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 bluegreen 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<sup>s</sup> plaque-forming units (PFU) per milliliter] that had been filtered through sintered glass. A gas mixture of 5-percent carbon dioxide in air