Table 2. The effect of penicillamine on the concentration of amino acids in the plasma of two patients. Patient 1 (male, aged 35) received 450 mg of D-penicillamine once every 8 hours; patient 2 (female, aged 20) received 1 g of D-penicillamine daily in divided doses. Results expressed as micromoles of amino acid per 100 ml of plasma.

Amino acid	Before treatment	During treatment
Patient 1		
Alanine	31.2	35.6
Cystine	2.4	1.0
Cysteine–penicillamine disulfide		1.1
Penicillamine disulfide		0.4
Methionine	1.5	2.9
Patient 2		
Alanine	22.6	23.8
Cystine	2.1	0.8
Cysteine–penicillamine disulfide		.9
Penicillamine disulfide		.2
Methionine	2.2	1.5

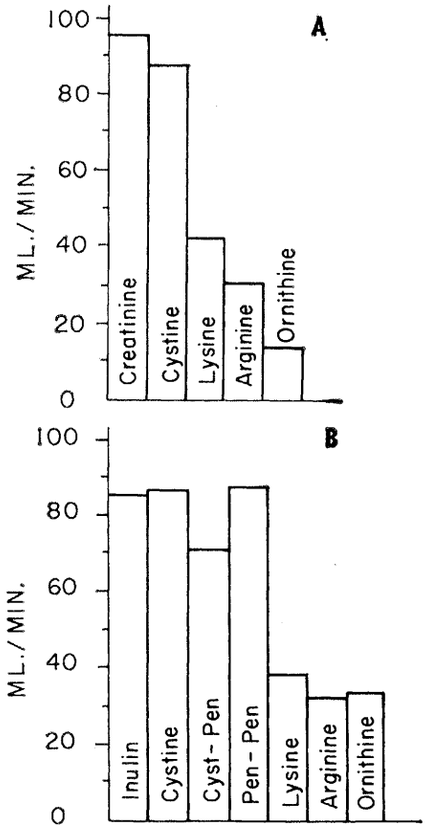

Fig. 1. Amino acids excreted in the urine of patient J.C. before (*A*) and during (*B*) the administration of penicillamine. Results (corrected to 1.73 m^2) of D-penicillamine hydrochloride administered orally at a rate of 450 mg once every 8 hours (*Cyst-pen*, cysteine–penicillamine disulfide; *pen-pen*, penicillamine disulfide).

urinary amino acid analyses were carried out, "total cysteine" excretion was reduced. The difference between these two observations depends on the large amount of cystine which is excreted in the urine of cystinuric patients. Data on renal clearance in the patients of Hartley and Walshe are not available, but it is probable that the cystine was reabsorbed normally by the renal tubule. It is possible that the mixed disulfide, which is not a naturally occurring amino acid, would not be reabsorbed by the kidney tubule and would be excreted in the urine. Calculation of "total cysteine" excreted would then show a large increase in comparison with patients not treated with penicillamine. Total recovery of penicillamine in the urine was only 30 to 40 percent of the administered dose. It is not known whether the penicillamine that was not accounted for was lost in the feces or whether it was lost in the urine in a metabolized or conjugated form.

Amino acids in the plasma were also estimated with the automatic amino acid analyzer and results for two cystinuric patients are shown in Table 2; the mixed disulfide and penicillamine disulfide are readily detectable in the plasma. The principal finding was that in both patients the concentration of cystine in the plasma was reduced during the administration of penicillamine; "total cysteine" in the plasma was also less during treatment than before treatment.

The glomerular filtration rate was measured in patient J.C., before penicillamine therapy, by clearance of endogenous creatinine (Fig. 1); the rate of clearance of amino acids was determined at the same time. The clearance of cystine was close to the glomerular filtration rate (see *4*) which was determined after penicillamine therapy had been established by measuring the rate of inulin clearance. Cystine, the mixed disulfide, and penicillamine disulfide all had clearance values close to the glomerular filtration rate. It can now be seen that the reduction in the amount of cystine excreted in the urine resulted from the lowering of the concentration of cystine in the plasma without affecting the renal clearance of that amino acid. Renal clearance of lysine and arginine remained unchanged.

Lotz and Potts (*5*) have confirmed by automatic amino acid analysis that urinary cystine is reduced by administration of penicillamine. Eldjarn and Hambraeus (*6*), using similar methods, did not observe a reduction of cystine excretion on the 2nd day after administration of D-penicillamine, but data are as yet insufficient for us to offer any explanation for this discrepancy.

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7. We are grateful to E. F. Scowen for his encouragement and advice. We thank A. G. Spencer and P. Purkis for advice and assistance in the renal-clearance measurements, and Elisabeth Saunders for technical assistance.
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Blue-Green Algae: Fine Structure of the Gas Vacuoles

Abstract. *The gas vacuoles seen in several species of blue-green algae under the light microscope are shown by electron microscopy to correspond to packed arrays of cylindrical, electron-transparent vesicles. Single vesicles average 75 millimicrons in diameter, range from 0.2 micron to 1.0 micron in length, have conical ends, and are bounded by a single membrane 2 millimicrons wide. The reversible disappearance of gas vacuoles induced by sudden application of pressure is accompanied by a reversible collapse of the individual gas vesicles.*

Conspicuous refractile bodies, resembling air bubbles, are revealed by light microscopy in the cells of a number of species of blue-green algae. Although these inclusions are characteristic of the planktonic species in "water blooms," similar structures are found in a few sedentary blue-green algae, as well as in several species of bacteria. On the basis of studies in which specific gravity was measured, Klebahn called these structures "gas vacuoles"

(*1*) and showed that they disappeared when cells were subjected to sudden pressure of the order of 2 to 3 atmospheres (*2*). The concept that these bodies represent inclusions of gas and probably are a flotation mechanism in planktonic forms has been supported by most workers (*3*). The organelles are unique in that they are somehow able to collect or secrete a gas and subsequently prevent its diffusion and consequent dissipation.

In the course of a survey of many species of blue-green algae, we have noted three-dimensional packed arrays of electron-transparent cylindrical vesicles in electron micrographs of cells from several samples, which included species of *Aphanizomenon*, *Anabaena*, and *Gleotrichia*, and occasionally in samples including some species of *Oscillatoria* and *Nostoc*. Since occurrence of these structures in our electron micrographs always coincided with the occurrence of gas vacuoles in cells observed with the light microscope, we decided to compare the fine structure of such cells with that of cells whose gas vacuoles had been removed by pressure.

We subjected freshly collected cells of *Aphanizomenon flos-aquae* (L.) Ralfs and *Anabaena spiroides* Klebahn to sudden pressure of the order of 7 bars in a simple brass piston, O-ring, and cylinder device. After examination of both treated and control cells in the light microscope, cells were fixed for 3 hours at room temperature in 1 percent osmic acid buffered at *p*H 6.1 with Veronal-acetate to which traces of calcium and amino acids were added (*4*). Cells were embedded in Epon and sectioned with a diamond knife. Some cells subjected to pressure were placed in lake water under constant illumination (approximately 0.3 lumen/cm^2) for 9 or 24 hours after which they were fixed in osmic acid as before.

Control cells, not subjected to pressure, showed conspicuous gas vacuoles under the light microscope (Fig. 1*A*); in electron micrographs, ordered, parallel arrays of electron-transparent cylindrical vesicles were visible in interthylakoidal (interlamellar) regions of these cells (Fig. 2*A*). Individual vesicles ranged from 0.1 μ to 1.0 μ in length, were about 75 mμ in diameter, had conical ends, and were bounded by a membrane which appeared in profile as a single electron-dense line 2 mμ wide.

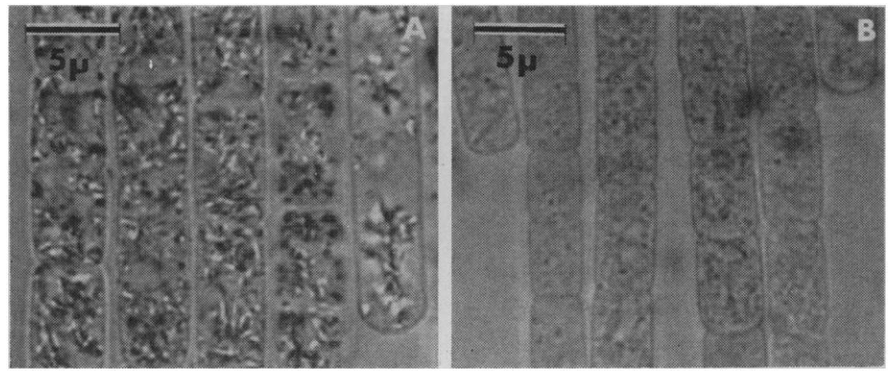

Fig. 1. Light micrographs of living trichomes of *Aphanizomenon flos-aquae* (× 2500). *A*, Control cells showing refractile gas vacuoles. *B*, Cells shortly after being subjected to pressure. Gas vacuoles have disappeared.

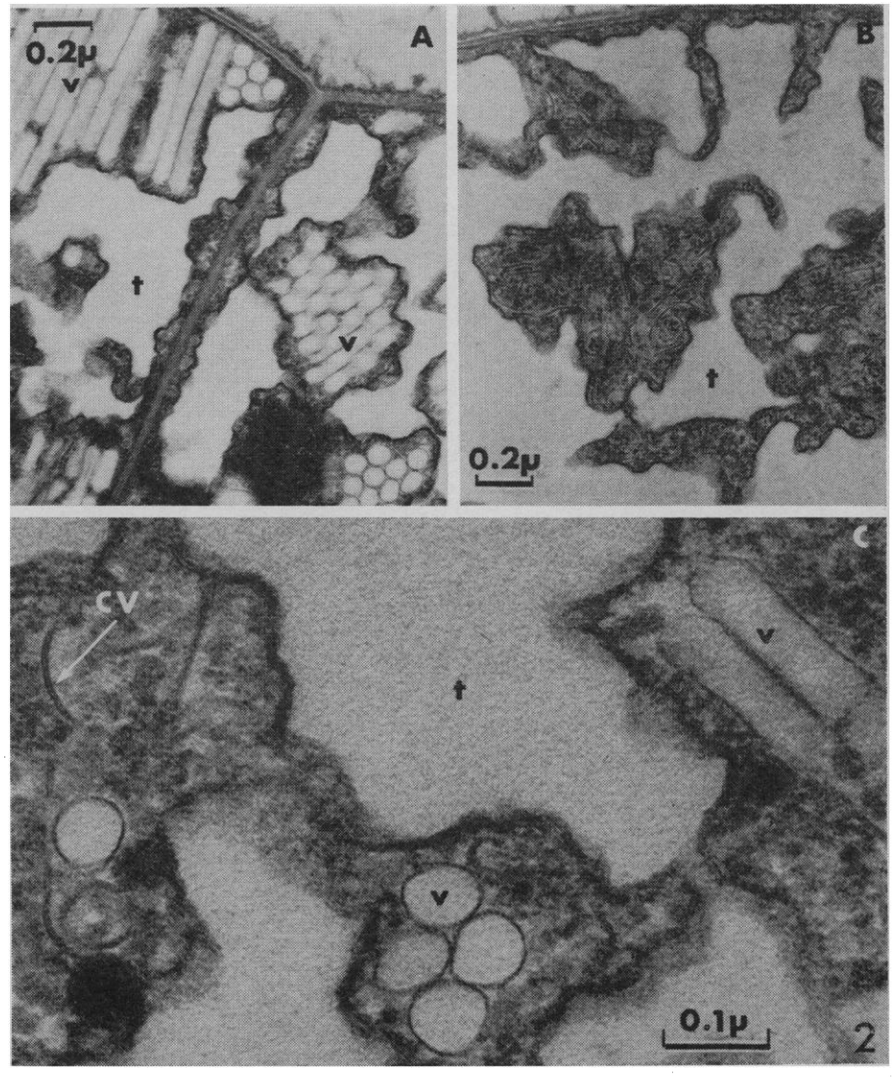

Fig. 2. Electron micrographs of portions of cells of *Aphanizomenon flos-aquae*. Large "vacuolate" regions (*t*) are intrathylakoidal (intralamellar) spaces typical of this species. *A*, Control cell (× 38,000) showing profiles of expanded gas vesicles in both cross and longitudinal section (*v*). *B*, Cell (× 38,000) fixed shortly after pressure treatment, showing collapsed gas vesicles. *C*, Cell (× 145,000) fixed 24 hours after pressure treatment, showing reexpanded gas vesicles (*v*) as well as collapsed gas vesicles (*cv*).

Cells that were examined shortly after being subjected to pressure had a significantly smaller diameter than they had prior to treatment, and no gas vacuoles were seen under the light microscope (Fig. 1*B*). The arrays of electron-transparent vesicles seen in electron micrographs of control material were conspicuously absent in cells subjected to pressure (Fig. 2*B*). Instead, many short membranous elements, each 6 mμ wide, 200 mμ or more in length, and resembling a typical "unit membrane," were seen in the regions of the treated cell where vesicles appeared in control cells.

Gas vacuoles were visible by 9 hours and were extensive after 24 hours of recovery from pressure treatment. Electron micrographs of cells allowed to recover for 9 hours showed some scattered electron-transparent vesicles in the locality of the 6-mμ membranous elements, and cells allowed to recover for 24 hours showed fairly extensive arrays of such vesicles (Fig. 2*C*).

We conclude that the vesicular aggregates we see with the electron microscope can be identified with the gas vacuoles observed by the light microscopist, and that individual gas vesicles can reversibly collapse and expand. The membrane of the collapsed gas vesicle has the dimensions and configuration of a unit membrane, and of a half-unit membrane when the vesicle is expanded by gas (Fig. 2*C*). However, unlike typical unit membranes, membranes of gas vesicles are not preserved by fixation with $KMnO_4$.

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21 December 1964

Structure of a Ribonucleic Acid

Abstract. *The complete nucleotide sequence of an alanine transfer RNA, isolated from yeast, has been determined. This is the first nucleic acid for which the structure is known.*

Transfer RNA's are the smallest biologically active nucleic acids known. Their function is to carry activated amino acids to the site of protein synthesis. During protein synthesis, the amino acid sequence of the polypeptide chain is determined by the interaction of a messenger RNA with transfer RNA's specific for a given amino acid. The structures of transfer RNA's are crucial in this process.

Three transfer RNA's, obtained from yeast, and specific for alanine, tyrosine, and valine, respectively, have been purified in our laboratories (*1*). Studies of the compositions of pancreatic ribonuclease digests have established that the three RNA's have quite different structures, as indicated by oligonucleotide sequences (*2*). The identification of a number of oligonucleotides obtained from the three RNA's has been described (*3*). We now summarize the determination of the first complete nucleotide sequence, that of the alanine RNA.

The structure determination involved the identification of small fragments formed by complete digestion of the RNA with pancreatic ribonuclease and takadiastase ribonuclease T1, followed by the determination of the structures of successively larger fragments, until the complete sequence of the RNA was established.

Table 1. List of fragments obtained by complete digestion of the alanine RNA with pancreatic ribonuclease.

	COH†	MeG-G-C-
13	C-	A-G-C-
	ψ-	A-G-DiHU-
6	U-	G-A-U-
	A-C-	I-G-C-
	MeI-ψ-	G-G-T-
	DiMeG-C-	G-G-DiHU-
2	G-C-	G-G-A-C-
4	G-U-	pG-G-G-C-
	G-G-G-A-G-A-G-U*-	

† The presence of a free 3′-hydroxyl group on this fragment indicates that cytidine occupies the terminal position in the purified alanine RNA. This establishes that the terminal adenylic acid residue is missing, as it is from most transfer RNA's isolated from commercial baker's yeast. A terminal adenylic acid residue is replaced under assay conditions before the amino acid is attached.

Complete digestion of the alanine RNA with pancreatic ribonuclease, an enzyme that cleaves the RNA chain next to pyrimidine nucleotides, for example, C- and U- (*4*), gives the 19 products listed in Table 1 (*5*). Complete digestion of the RNA with takadiastase ribonuclease T1, a highly specific enzyme (*6*) that cleaves the RNA chain next to G- and I-, gives the 17 products listed in Table 2 (*5*). Proof of the structures of these products required the use of both classical and new methods of sequence determination, as well as the identification of certain new nucleotides (*5, 7*).

Combination of the results summarized in Tables 1 and 2 permits description of the structure of the alanine RNA in terms of 16 oligonucleotide sequences, shown in Table 3 (*5*). Except for the positions of the two end sequences, the arrangement of the 16 oligonucleotide sequences is not established by these data. The presence of a 5′-phosphate on the pG-G-G-C- sequence establishes that this is the left end of the RNA molecule as conventionally written, and the 3′-hydroxyl on the U-C-C-A-C-C-AOH sequence establishes that this is the right end of the molecule. The 16 oligonucleotide sequences account for a total of 77 nucleotide residues and give a calculated molecular weight for the RNA of 26,600 as the sodium salt.

Takadiastase ribonuclease T1 cleaves the alanine RNA selectively, under controlled conditions, and gives a number of large oligonucleotide fragments (*8, 9*). Analysis of these large fragments, in combination with the data in Tables 1 to 3, has furnished sufficient information to establish the complete nucleotide sequence of the RNA.

The structure of the alanine RNA is shown at the top of Fig. 1. Large fragments that were crucial in the proof of structure are shown in the lower part of Fig. 1.

Summary of proof of structure. To determine the structures of the large oligonucleotide fragments *a* to *k* (Fig. 1), a large fragment was digested with ribonuclease T1 giving certain of the previously identified fragments listed in Table 2, and additional information was then used to establish the arrangement of these complete digest fragments.

The isolation and proof of structure of fragments *a*, *b*, *c*, and *d* (Fig. 1) have been described (*8*). These frag-