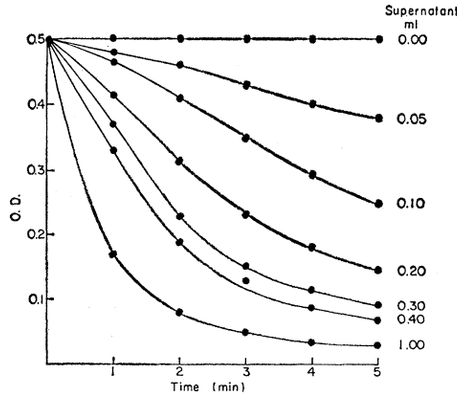

Fig. 1. Reduction of turbidity of lyophilized cells of *Staphylococcus aureus* strain U9 in 0.01*M* ammonium acetate, *p*H 8.5, by the addition of supernatant from a broth culture of the *Pseudomonas* sp. O.D., optical density.

sults indicate that the staphylolytic substance is a protein possessing enzyme activity. The substance was partially purified by the following procedure. The *Pseudomonas* sp. was grown in 17 liters of brain-heart infusion broth for 24 hours at 37°C, and the cells were removed by Sharples centrifugation. The supernatant fluid contained 4,200,000 units of the staphylolytic substance. Acetone was added drop by drop to the supernatant fluid at 0°C with continuous stirring until the acetone constituted 75 percent (by volume) of the mixture. The resulting precipitate was collected by centrifugation at 4°C. This precipitate, which was dissolved in 3 liters of 0.01*M* ammonium acetate, contained 3,900,000 units of the staphylolytic substance. The overall recovery of the substance was 93 percent.

Several bacterial species were examined for susceptibility to lysis by the staphylolytic substance. Washed suspensions of viable cells in 0.01*M* ammonium acetate, *p*H 8.5, were exposed to 100 units of the substance per milliliter. The suspensions of nine strains of *S. aureus*, differing in source, coagulase production, and sensitivity to bacteriophages and antibiotics, exhibited a decrease in optical density from 0.50 to 0.03 in 10 minutes or less. This rapid lysis was also observed with *Staphylococcus roseus* American Type Culture Collection (ATCC) 418, *Gaffkya tetragena* ATCC 10875, and *Sarcina lutea* ATCC 272.

Suspensions of *Micrococcus lysodeikticus* ATCC 4698 and several species of *Streptococcus* required 2 hours or slightly less to exhibit a decrease in optical density from 0.50 to 0.25 when exposed to the staphylolytic substance. None of the following Gram-negative organisms was sensitive: *Escherichia coli, Erwinia carotovora, Proteus vulgaris, Shigella boydii, Salmonella choleraesuis, Serratia kiliensis, Xanthomonas campestris, Achromobacter viscosus*, and *Aerobacter aerogenes*. Of the Gram-positive organisms examined, the following were not sensitive to lysis by the substance: *Bacillus subtilis, B. cereus, B. megaterium* strain KM, *Lactobacillus casei, L. acidophilus*, and *Micrococcus luteus*.

Broth cultures of *Pseudomonas fluorescens, P. aeruginosa*, and *P. saccharophila* were examined for the presence of staphylolytic activity, using lyophilized cells of *S. aureus* strain U9 as substrate. The cultures of *P. fluorescens* and *P. aeruginosa* exhibited staphylolytic activity, but the culture of *P. saccharophila* did not. Pyocyanine (*3*), the green pigment characteristic of the genus *Pseudomonas*, did not lyse cells of *S. aureus* strain U9, indicating that it is not similar in nature to the staphylolytic substance.

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4. Supported by NIH grant AI-04202.

22 January 1965

Cystinuria: Effect of D-Penicillamine on Plasma and Urinary Cystine Concentrations

Abstract. *Administration of penicillamine reduced the concentration of cystine in the plasma and urine of patients with cystinuria; renal clearance of cystine, lysine, and arginine remained unchanged. Cysteine–penicillamine disulfide and penicillamine disulfide were detected in the plasma.*

Crawhall *et al.* showed previously that oral administration of D-penicillamine reduces the amount of cystine excreted by patients with cystinuria (*1*). These authors originally postulated that penicillamine would react with cystine by thiol-disulfide exchange in the urinary tract. Further studies with an automatic amino acid analyzer show that at least some of this reaction must occur within the body before filtration takes place at the renal glomerulus.

Analysis of the urinary amino acids of five cystinuric patients treated with penicillamine (Table 1) revealed that the amount of cystine excreted was reduced in each case. Hartley and Walshe (*2*) found that administration of penicillamine to patients with normal renal function increased tenfold the urinary excretion of "total cysteine" (*3*) as a result of the formation of cysteine-penicillamine mixed disulfide. We have found that administration of penicillamine to patients with cystinuria also leads to the excretion of the mixed disulfide and of penicillamine disulfide. In four of five patients for whom full

Table 1. Daily urinary excretion of sulfur-containing amino acids before and during penicillamine therapy. Amino acids were analyzed with a Technicon automatic amino acid analyzer. The gradient elution buffer was modified from that recommended by Technicon by using a buffer at *p*H 3.7 in chamber 4 of the autograd. Figures in parentheses indicate the number of determinations.

Patient	Penicillamine administered*	Excretion (mg/24 hours)						B/A × 100	Recovery of penicillamine (%)
		Cystine		Mixed disulfide	Total cysteine (B)	Penicillamine disulfide	Total penicillamine		
		Before therapy (A)	During therapy						
J.C.	804	748(1)	372(3)	557	620	123	442	83	54
W.T.	1085	930(1)	414(2)	485	630	119	388	68	36
S.O.	482	719(2)	458(4)	244	566	39	175	79	36
	1085		241(2)	415	426	103	333	59	31
S.L.	602	470(1)	192(1)	169	261	48	148	56	25
J.O.	1085	383(2)	235(1)	412	413	119	347	120	32

* Milligrams of free base per 24 hours.

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

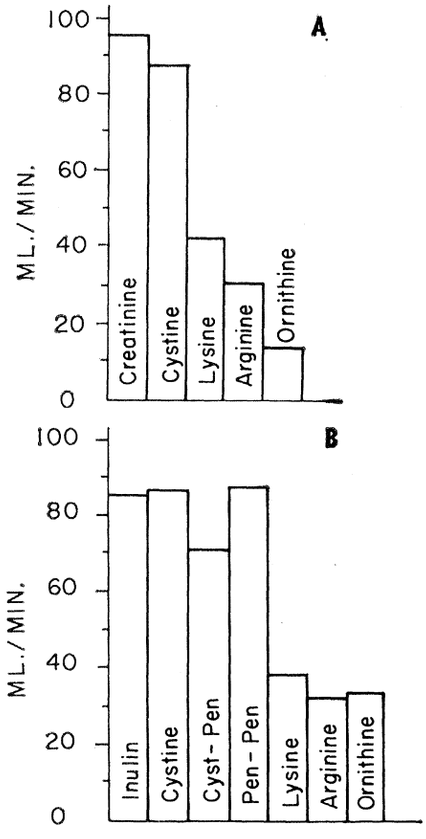

Fig. 1. Amino acids excreted in the urine of patient J.C. before (*A*) and during (*B*) the administration of penicillamine. Results (corrected to 1.73 m^2) of D-penicillamine hydrochloride administered orally at a rate of 450 mg once every 8 hours (*Cyst-pen*, cysteine–penicillamine disulfide; *pen-pen*,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