as a result of their earlier studies demonstrating restriction of genetic transcription in vivo to one of the complementary DNA strands of bacteriophage φX174. There is evidence which is consistent with this prediction. A leucine auxotroph, *leu 39*, appears to result from inversion of a large portion of the leucine operon containing at least one complete cistron (leu III) which by complementation tests is physiologically nonfunctional (*4*). This suggests that whatever serves in the genetic code as a punctuation mark to designate the end of one cistron and the beginning of the next cannot correct for a reversed polarity.

Either of the foregoing hypotheses concerning the mechanism by which polarity of information transfer is determined can explain the conclusion (*23*) that in *Bacillus subtilis* the genetic information for the enzymes concerned with histidine synthesis is carried on the strand of the DNA molecule opposite to that carrying the information for the tryptophan-synthesizing enzymes. Technical difficulties make this conclusion somewhat uncertain. However, if correct, the work (*23*) indicates that (i) the histidine and tryptophan genes are on two different units of DNA each having opposite polarities within the *B. subtilis* chromosome (second hypothesis) or (ii) the histidine and tryptophan genes are merely located within operons having opposite polarities (first hypothesis).

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28 December 1964

Staphylolytic Substance from a Species of Pseudomonas

Abstract. *A substance that rapidly lyses living cells of* Staphylococcus aureus, S. roseus, Gaffkya tetragena, *and* Sarcina lutea *has been partially purified. The substance is produced extracellularly by a species of* Pseudomonas *which was isolated from soil by an enrichment procedure.*

Certain investigations into the physiology and genetics of *Staphylococcus aureus* have been restricted by the lack of a lytic method for isolating intracellular macromolecular components. Recently, enzymes which lyse *S. aureus* and several other Gram-positive bacteria have been isolated (*1*). We have now isolated another staphylolytic substance which differs from the enzymes described earlier in origin, spectrum of activity, and optimum *p*H.

A microorganism which produces a staphylolytic substance was isolated by means of the following procedure. A sample of soil was added to a sterile enrichment medium consisting of basal salts and lyophilized cells of *S. aureus* strain U9 (*2*) in distilled water. After the mixture had been incubated at room temperature for 7 days, a sample was inoculated by means of a wire loop onto plates of nutrient agar rendered turbid by the addition of lyophilized cells of *S. aureus* strain U9 at the time the plates were poured. These plates were incubated for 24 hours, and after this incubation clear zones were present around several colonies of a bacterium that produced a green pigment. When one of these colonies was subcultured into brain-heart infusion broth, a highly active staphylolytic substance was produced. The bacterium has been identified as a member of the genus *Pseudomonas* because it is oxidative, it has polar flagella, it is a Gram-negative rod, and it produces a green, water-soluble pigment.

The substance produced by the *Pseudomonas* sp. shows, toward cells of *S. aureus*, lytic activity which decreases as the salt concentration increases; in a phosphate buffer at concentrations above 0.07*M*, no lytic activity could be detected. When the salts were removed by dialysis, the activity was fully restored. A similar relation between salt concentration and lytic activity was observed by Hash in his study of the *Chalaropsis* B enzyme (*1*). The maximum lytic activity of the substance produced by the *Pseudomonas* sp. was obtained at a *p*H of 8.5 in phosphate and acetate buffers of low ionic strength.

All assays of the staphylolytic substance were performed at 25°C in 0.01*M* ammonium acetate adjusted to *p*H 8.5 with NH_4OH. A sufficient number of lyophilized cells of *S. aureus* was suspended in the buffer to obtain an optical density reading of 0.50 at a wavelength of 535 mμ. After addition of a sample of the staphylolytic substance to this suspension, changes in optical density were recorded at 1-minute intervals. Low concentrations of the substance produced nearly linear results over 5-minute periods (Fig. 1). At these low concentrations and at time periods of 5 minutes or less, one unit of the staphylolytic substance was defined as the amount of the substance required to reduce the optical density of a suspension of lyophilized cells of *S. aureus* strain U9 by 0.001 optical-density units per minute. Different cultures varied slightly in sensitivity when viable cells of *S. aureus* were tested as substrate in lysis experiments. Reproducible results were obtained with lyophilized cells of *S. aureus* strain U9, and therefore these were used in the standard assay of the substance.

The staphylolytic substance is colorless and precipitable with either acetone or ammonium sulfate. The activity of the substance is completely destroyed by incubation for 30 minutes at 56°C, but dialysis for 24 hours against 0.01*M* ammonium acetate results in no loss of activity. These re-

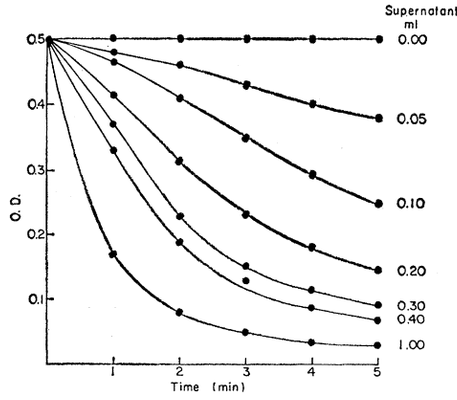

Fig. 1. Reduction of turbidity of lyophilized cells of *Staphylococcus aureus* strain U9 in 0.01*M* ammonium acetate, *p*H 8.5, by the addition of supernatant from a broth culture of the *Pseudomonas* sp. O.D., optical density.

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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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.