with those incubated in their absence. There was no difference in the colony counts, suggesting that these agents did not produce proliferation of *S. sonnei*. Chemoresuscitation was not as pronounced as chemical protection. For example, the most effective agent, adenosine triphosphate, allowed us to recover 10^6 cells compared with a recovery of 10^2 cells in the absence of resuscitation. We were aware of the fact that impurities present in adenosine triphosphate may have been partially or totally responsible for the observed results, but had no way of eliminating their role completely. Adenosine triphosphate heated for 15 min at 75 C was completely without activity as a resuscitating agent. Chemical reactivation of irradiated cells was possible only if the organisms were incubated with the reactivating agent within 6 hr after ultraviolet treatment. Increased concentrations of the reactivating agent resulted in an increased number of viable cells recovered, until a maximum was reached at 1.5 mg/ml of the agent. We were aware that many of the chemicals studied were strong absorbers of ultraviolet light that may have screened out the lethal wavelengths from reaching the suspended cells.

Djordjević, Kostić, and Kanazir (Nature **195:** 614, 1962) postulated that deoxyribonucleic acid protected cells against ultraviolet irradiation because the irradiated cells had a lower rate of nucleic acid synthesis. Rene and Nardone (Exptl. Cell Res. **23:**549, 1961) were able to resuscitate ultraviolet-irradiated *Tetrahymena pyriformis* with adenosine triphosphate and nicotinamide adenine dinucleotide. They suggested that ultraviolet killing results from an inhibition of the synthesis of nicotinamide adenine dinucleotide and deoxyribonucleic acid. We are unable to state the mechanism of chemoresuscitation in our studies with *S. sonnei*. However, preliminary data indicate that more than one mechanism may be involved in restoring the viability of irradiated cells.

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ELIMINATION OF DRUG RESISTANCE OF *STAPHYLOCOCCUS AUREUS* BY TREATMENT WITH ACRIFLAVINE

SUSUMU MITSUHASHI, MASATO MORIMURA, KEI KONO, AND HIROSHI OSHIMA

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan

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In a previous paper (Mitsuhashi et al., Nature **189:**947, 1961), it was reported that the transmissible drug-resistance (R) factor of *Escherichia coli* and *Shigella flexneri* was eliminated spontaneously on standing, and the loss frequency was increased by treatment with acriflavine. It was also found that the R factor was eliminated by treatment with acridine orange (Watanabe and Fukasawa, J. Bacteriol. **81:**679, 1961).

From the epidemiological survey of *Staphylococcus aureus*, it was found that the multiple resistance of staphylococci of human origin has been increasing in Japan [Kosakai et al., Mod. Med. (Tokyo) **15:**2, 1960; Mitsuhashi et al., Rept. Min. Educ. (Japan), p. 165, 1961]. Hospital infections caused by multiply resistant staphylococci present another problem that has become increasingly serious (Barber et al., Lancet **2:**641, 1948; Ishiwara et al., Japan. J. Microbiol. **3:**427, 1959; Barber et al., Brit. Med. J. **2:**11, 1960).

Voureka (J. Gen. Microbiol. **6:**352, 1952) reported that the virulence and resistance to penicillin and to streptomycin of a strain of *Staphylococcus* decreased after exposure to injurious agents such as chloramphenicol, tetracycline, nitrogen mustard, etc., and that new characters remained unchanged through over 50 passages in normal laboratory media.

This paper deals with the elimination of multiple resistance to erythromycin (EM), leucomycin (LM), and oleandomycin (OM) of staphylococci by treatment with acriflavine and the joint transduction of EM-, LM-, and OM-resistance by International Typing Phage 81.

Two strains of *S. aureus* obtained by the present authors from patients with staphylococcal

infections were employed. They were coagulase-positive, multiply resistant (Table 1), and showed the phage-typing pattern 80/81. The recipient staphylococcal strains used in transduction were obtained from several different sources in the surgical wards. They were all sensitive to EM, LM, and OM, and of human origin.

Phage 81 was propagated on *S. aureus* MS 66 or MS 258 by the agar layer technique of Swanstrom and Adams (Proc. Soc. Exptl. Biol. Med. **78**:372, 1951). Phage was stored at 4 C after sterilization with a Chamberland L-2 filter, as described in the previous papers that reported the transduction of tetracycline resistance with phage 80 and EM resistance with phages 80 and 81 (Mitsuhashi et al., Gunma J. Med. Sci. **10**:298, 1961). The titers of the phage filtrate were 10^8 to 10^9 plaque-forming units per ml.

The recipient strains of *S. aureus* were grown in Heart Infusion (HI) Broth (Difco) to about 10^9 colony-forming units per ml, and were mixed with phage at multiplicities of infection of 0.1 to 1. The mixtures were kept at 30 C for 30 min. Free phages were removed by centrifugation, and the precipitated cells were plated on HI Agar containing EM (12.5 μg/ml). After 24 to 48 hr of incubation at 37 C, the colonies were picked, and two successive single-colony isolations were conducted on the same selective medium.

The drug resistance of all staphylococcal strains used was determined after growing them overnight at 37 C in HI Broth. One loopful of overnight culture of each strain in HI Broth was spread over the surface of each of the HI Agar plates containing serial twofold dilutions of the antibiotics. Mueller Hinton agar was used for the determination of sulfanilamide resistance.

TABLE 1. *Drug-resistance patterns of Staphylococcus aureus MS 66 and MS 582 before and after treatment with acriflavine*

Strain	Concn of acriflavine (μg/ml)	Drug-resistance patterns* (μg/ml)						
		TC	SM	SA	PC†	EM	LM	OM
MS 66	0	200	0.8	3,200	200	400	800	400
	12.5	200	0.8	3,200	200	0.2	0.2	0.2
MS 258	0	200	0.8	1,600	200	800	800	800
	12.5	200	0.8	1,600	200	0.2	0.2	0.2

* Abbreviations: TC, tetracycline; SM, streptomycin; SA, sulfanilamide; PC, penicillin; EM, erythromycin; LM, leucomycin; OM, oleandomycin.

† Units/ml.

TABLE 2. *Elimination of EM-, LM-, and OM-resistance of Staphylococcus aureus MS 66 and MS 258 by treatment with acriflavine*

Strain	Treatment for elimination	Elimination of resistance*
MS 66	With acriflavine treatment	15/300 (5)
		28/400 (7)
		24/300 (8)
		2/200 (2)
		21/300 (7)
	Without acriflavine treatment	0/400 (0)
		0/300 (0)
		0/500 (0)
MS 258	With acriflavine treatment	6/300 (2)
		9/300 (3)
	Without acriflavine treatment	0/400 (0)
		0/300 (0)

* Number of colonies from which EM-, LM-, and OM-resistance was eliminated/total number of colonies tested. Numbers in parentheses indicate the percentage of elimination of resistance.

Amounts (0.1 ml) of tenfold serial dilutions of an overnight culture in HI Broth of *S. aureus* MS 66 or MS 258 were inoculated in 10 ml of HI Broth containing acriflavine (12.5 μg/ml). After overnight incubation at 37 C, samples of suitable dilutions were plated on HI Agar. The colonies were transferred by the replica plating method onto HI Agar containing each drug, and their drug resistance was determined.

As shown in Tables 1 and 2, EM-, LM-, and OM-resistance of *S. aureus* MS 66 and MS 258 was jointly eliminated. For the transduction of EM resistance from *S. aureus* MS 66 and MS 258, about 10% of the strains among 60 strains used were competent recipients. The EM-resistant colonies in all transductant clones were all resistant to LM and OM. Even at lower multiplicities of infection (0.1), the EM-, LM-, and OM-resistance was jointly transmitted. The new characters, i.e., the EM-, LM-, and OM-sensitivity of *S. aureus* MS 66 and MS 258 after resistance was eliminated by treatment with acriflavine, and the EM-, LM-, and OM-resistance of the transductants, remained unchanged through more than 20 passages in HI

broth. The biological and biochemical properties of these strains will be described elsewhere.

From the results described above, it is suggested that the EM-, LM-, and OM-resistance of *S. aureus* MS 66 and MS 258 is controlled by a genetic element different from other resistance genes located on the staphylococcal chromosome, probably a cytoplasmic element.

GLUCOSE DEHYDROGENASE ACTIVITY IN *PEDIOCOCCUS PENTOSACEUS*

WALTER J. DOBROGOSZ[1] AND RALPH D. DEMOSS

Department of Microbiology, University of Illinois, Urbana, Illinois

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The genus *Pediococcus* is a recent addition to the family *Lactobacillaceae* (Breed, Murray, and Smith, *Bergey's Manual of Determinative Bacteriology*, 1957) and includes gram-positive, tetrad-forming cocci which resemble, metabolically, the homofermentative lactic acid bacteria. Felton, Evans, and Niven (J. Bacteriol. **65**:481, 1953) and Jensen and Seeley (J. Bacteriol. **67**:484, 1954) reported that, under certain conditions, many strains of these organisms exhibited catalase activity, an unusual property for members of this family. Recent studies with cell-free extracts of a catalase-positive strain of *P. pentosaceus* (Dobrogosz and Stone, J. Bacteriol. **84**:716, 1962) resulted in the observation of still another unexpected enzymatic property of this genus: their ability to catalyze an adenosine triphosphate (ATP)-independent oxidation of glucose with nicotinamide adenine dinucleotide phosphate (NADP) as the hydrogen acceptor. To the best knowledge of the authors, a reaction of this type has not been reported for any members of this family of bacteria.

Cultures of *P. pentosaceus* Az-25-5 were grown aerobically for 15 hr at 37 C, by use of a previously described casein hydrolysate-yeast extract basal medium (Dobrogosz and Stone, J. Bacteriol. **84**:716, 1962). Washed cells, suspended in 0.05 M $NaHCO_3$ were passed three times through a French Pressure Cell (American Instrument Co.) to obtain crude, cell-free preparations. Unbroken cells and debris were removed by centrifugation at 15,000 × *g* for 20 min. The pyridine nucleotide reduction assays were conducted in the usual manner at room temperature, with a Zeiss

[1] Present address: Department of Botany and Bacteriology, North Carolina State College, Raleigh.

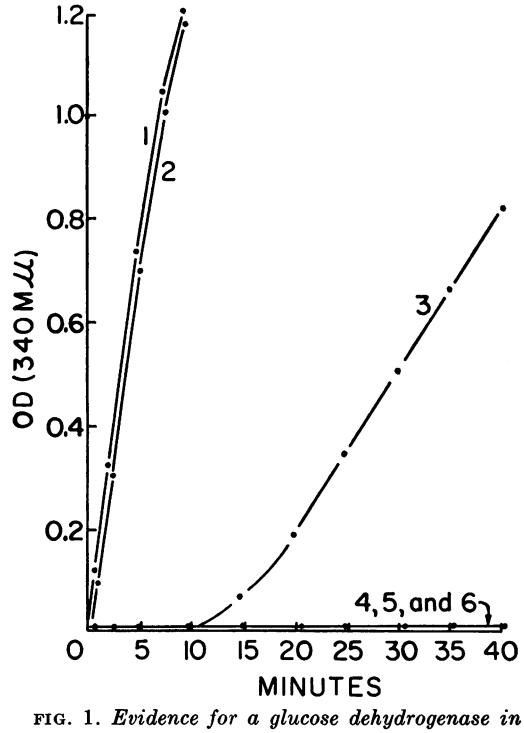

FIG. 1. *Evidence for a glucose dehydrogenase in cell-free extracts of Pediococcus pentosaceus grown on ribose. The reaction mixture contained the following (μmoles per 3 ml): tris buffer (pH 7.7), 75; sodium arsenate, 51; glutathione (red), 30; $MgCl_2$, 50; ATP, 20; thiamine pyrophosphate, 3; NAD or NADP (as indicated), 1.5; substrate (as indicated), 20; extract (from ribose-grown cells), 1.8 mg of protein; water to 3.0 ml. The reaction was initiated by the addition of substrate. Curve 1 = glucose + NADP + ATP; curve 2 = glucose + NADP; curve 3 = ribose + ATP + NAD; curves 4 and 5 = glucose + NAD with and without ATP, respectively; curve 6 = ribose + NAD.*