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Biochemical Properties of Sulfonamide-resistant Strains of *Streptococcus mitis*, and Their Similarity to Enterococci. (17609)

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In a previous investigation(1) of the properties of sulfathiazole-resistant green streptococci, it was found that 4 strains which were originally unable to grow on 40% bile agar, acquired this ability after a high level of sulfonamide resistance had been induced. Sherman, Niven, and Smiley(2), Evans and Chinn(3), and Swift(4) have indicated that the ability to grow on bile agar, in .1% methylene blue milk, in 6.5% NaCl broth, and under certain other conditions not usually considered as optimum for the streptococci, is useful in differentiating the enterococci from the viridans group. It seemed of interest to determine whether any other properties characteristic of the enterococci (in addition to the growth on 40% bile) were acquired by these strains of *Streptococcus mitis* in becoming sulfathiazole-resistant. Further, since enterococci are resistant to penicillin and streptomycin, it was of interest to determine the resistance of the "trained" *mitis* strains to these antibiotics, and to compare the resistance of these trained strains and of enterococci to bacitracin* and aureomycin. Finally, it seemed desirable to develop bile resistance and streptomycin resistance in the parent *Streptococcus mitis* cultures and to determine whether these

1. Clapper, William E., and Heatherman, Mary E., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 392.

2. Sherman, J. M., Niven, C. F., and Smiley, K. L., *J. Bact.*, 1943, v45, 249.

3. Evans, Alice C., and Chinn, Alice L., *J. Bact.*, 1947, v54, 495.

4. Swift, Homer F., *The Streptococci: Bacterial and Mycotic Infection in Man*, J. B. Lippincott Co., Phila., Pa., p. 253, 1948.

* We are indebted to the Lederle Laboratories for kindly supplying the aureomycin and to the Upjohn Company for the bacitracin.

TABLE I.
Biochemical Properties of Sulfathiazole-resistant Streptococci.

	Parent strains				Resistant strains			
	1	4	6	17	1-S	4-S	6-S	17-S
Mg sulfa/ml*	.1	.1	.1	.1	>30	>30	>30	20
Growth in								
40% bile	—	—	—	—	+	+	+	+
6.5% NaCl	—	—	—	—	+	+	+	+
10°C	—	—	—	—	+	+	+	—
45°C	—	+	—	—	+	+	+	+
.1% M. B. Milk	—	—	—	—	+	—	+	+
Reduction, before clotting, of Litmus Milk	—	—	—	—	+	—	+	—

* Concentration inhibiting growth in broth.

agents would select organisms with the same properties, or bring about the same changes, as those found after exposure to sulfathiazole.

Methods and Materials. Two of the strains of *Streptococcus mitis* from our previous study(1) which had been isolated from the mouth, and two additional strains, one isolated from the mouth and one from the urine, were streaked on blood agar and re-isolated from single colonies 8 times. They were then made resistant by growing in gradually increasing concentrations of sodium sulfathiazole in tryptose phosphate broth. The parent strains were also trained in the same manner to grow on 40% bile agar and in high concentrations of streptomycin. Several unsuccessful attempts were made to induce penicillin resistance. The sensitivity tests to sulfathiazole and the antibiotics were performed by making dilutions (usually 5-fold) of the various agents in tryptose phosphate broth, inoculating with the organism, and reading the lowest concentration completely preventing growth after 24 hours at 37°C, to determine the sensitivity of the parent strain and reading the highest concentration allowing good growth to determine the resistance of the resistant strain. Inoculum size was equalized by adjusting the 24-hour broth cultures of the parent and resistant strains to the same turbidity, by visual inspection, before diluting 1 to 1,000. One drop of this dilution was then added to each tube.

Results and discussion. The results shown in Table I indicate that after a high degree of resistance to sulfathiazole had been attained, organisms that originally had the properties of *Streptococcus mitis* now had certain properties of enterococci. Table II compares the sensitivity to antibiotics of the sulfathiazole-resistant strains to that of the susceptible ones. Although strains of bacteria trained to sulfonamide resistance are usually as sensitive to the antibiotics as the parent strains, the ones in this study were more resistant. A known strain of *Streptococcus fecalis* (A.T.C.C. No. 9790) showed very much the same sensitivity to the 4 antibiotics as did the sulfonamide-resistant organisms. All the trained organisms and the *S. fecalis* were sensitive to less than 1 μg/ml of aureomycin or bacitracin.

Table III shows the properties acquired by the parent strains when they were trained to grow on 40% bile agar. Strain 4B acquired all the properties of the enterococci that were shown by the sulfonamide-resistant cells including increase in sulfathiazole resistance. Strain 17B did not become sulfathiazole-resistant, nor acquire the other properties of an enterococcus, but did become more resistant to the 4 antibiotics. The remaining 2 strains showed no changes. When the parent strains were made 1,000 times or more resistant to streptomycin they did not change in resistance to any of the other agents, and showed no change in other biochemical properties, with the exception that 3 strains acquired the ability to grow on 40% bile agar. These results emphasize the fact that development of resistance to one bacteriostatic agent does not necessarily bring about the same metabolic

TABLE II.
Resistance of Parent and Sulfathiazole-resistant Strains of Streptococci to Antibiotics.

Strain	Aureomycin	Bacitracin	Penicillin	Streptomycin
Parent 1	.05*	.01	1.0‡	5
Resistant 1	.5†	.05	10.	35
Parent 4	.05	.01	.5	5
Resistant 4	.5	.05	10.	25
Parent 6	.1	.01	1.0	5
Resistant 6	.5	.05	10.	25
Parent 17	.005	.001	.5	5
Resistant 17	.5	.01	10.	25

* Values represent concentration in μg/ml required to prevent growth for 24 hr.
† Values represent concentration in μg/ml through which good growth of the organism was obtained in 24 hr.
‡ Values for penicillin are given in units per ml.

TABLE III.
Biochemical Reactions and Resistance of Streptococci Trained to Grow on 40% Bile Agar.

	Strains trained on bile			
	1-B	4-B	6-B	17-B
Growth on				
40%Bile	+	+	+	+
10°C	—	—	—	—
45°C	—	+	—	—
.1% M. B. Milk	—	+	—	—
Reduction, before clotting, of Litmus Milk	—	+	—	+
Increase in sulfathiazole resistance	—	+	—	—
Increase in resistance to aureomycin, bacitracin, penicillin, and streptomycin	—	10-fold or more to all 4	—	10-fold or more to all 4

change as development of resistance to another. Each agent produces specific metabolic changes(5).

Enterococci are resistant to sulfathiazole, penicillin, streptomycin, and bile. If organisms having all these properties were originally present in small numbers in close association with the parent type, any of these agents would select organisms having the properties of enterococci. Furthermore, since our resistant organisms grow much faster than the parent strains, they would grow out in broth with no sulfathiazole in it, if they were already present. However, the same number of transfers were made in drug-free broth as were made in the presence of sulfathiazole, and no change in resistance was observed. To test further the possibility that a few variants possessing the properties of our resistant strains were present in the culture before exposure to sulfathiazole the following experiment was performed. Approximately 4 resistant cells of strain 6S, checked by plate counts of dilution, were mixed with 10^9 susceptible cells of strain 6 in tryptose phosphate broth. After 24 hours incubation, the sulfathiazole resistance of this mixed culture was determined. It was found to be the same as that of a pure culture of the resistant strain (6S). This would seem to be fairly good evidence that the parent culture did not have even a few highly resistant cells in it. Since the parent culture did not produce a resistant culture when transferred in drug-free broth, variants having the properties of enterococci must have been induced or selected stepwise by the sulfathiazole. Such a change might take place in a patient treated with sulfonamides, with the resulting organisms being resistant to penicillin and streptomycin and having other prop-

5. Sevag, M. G., Enzyme Problems in Relation to Chemotherapy. Adaptation, Mutations, Resistance, and Immunity, *Advances in Enzymology*, 1946, v6, 96, New York, Interscience.

erties of an enterococcus. Such an organism would apparently be inhibited by low concentrations of bacitracin or aureomycin, however. The results of our *in vitro* experiments suggest that resistance developed in *mitis* streptococci by contact with other agents does not necessarily bring about the overall increase in resistance shown to accompany sulfonamide resistance.

Summary. 1. At a certain level of sulfonamide resistance properties characteristic of enterococci were acquired by 4 strains of *S. mitis*. 2. Increased resistance to sulfonamides was accompanied by increased resistance to penicillin, streptomycin, bacitracin, and aureomycin in all 4 strains. The resistance of these trained strains to the antibiotics and sulfathiazole was similar to that of a strain of *S. fecalis*. 3. Acquisition of ability to grow on bile or in high concentrations of streptomycin did not generally produce the biochemical properties of enterococci, nor resistance to sulfathiazole or to the antibiotics.

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Influence of Certain Neurotropic Substances on Central and Synaptic Transmission in Callianassa. (17610)

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Although acetylcholine and cholinesterase have been shown to be present in the nervous system of crustaceans, evidence for the functional significance of these substances is generally lacking(1,2,3,4). A possible exception may occur in the anomuran *Petrolisthes armatus*, in which Welsh and Haskin(5) have found that injection of acetylcholine increases the tendency to autotomy of the walking legs. In this animal eserine in doses of 1.5 γ per gram elicited extreme activity and convulsions, while atropine exerted a depressive action. No attempt was made by Welsh and Haskin to localise the site of action of these substances. In an effort to obtain further information on the action of these and other neurotropic agents, we have carried out experiments on the macrurous anomuran *Callianassa californiensis* in which application of reagents was made at 3 different loci: 1. On the abdominal nerve cord and ganglia. 2. At the point of synapse between giant fibers of the nerve cord and the nerves to the flexors of the telson. 3. On the flexor muscles of the abdomen after severance of their connections with the central nervous system.

Methods. The abdominal nerve cord of *Callianassa* usually was exposed from the ventral side, freed entirely from adjoining tissues and left continuous with the thoracic cord. The abdomen of the animal was then cut away and the head and thorax, with the attached nerve cord, placed in a bath of sea water. Preparations kept well in running sea water for periods up to 24 hours. The drugs were made up in sea water and added in the proper concentrations directly to the (40 cc) bath. Electrical stimulation and recording were carried out by means of micromanipulated platinum electrodes. Responses were amplified through a Grass P3 amplifier and visualised on the screen of a DuMont 208 oscilloscope, the sweep of which was triggered from the stimulating circuit. The flexor muscles of the abdomen were denervated by ex-

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2. Welsh, J. H., and Schallek, W., *Physiol. Rev.*, 1946, v26, 447.

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4. Katz, B., *Biol. Rev.*, 1949, v24, 1.