Antigenic Similarity of Some Trained Resistant Strains of Viridans Streptococci to *Streptococcus fecalis.** (19115)

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It has been shown in this laboratory(1) that some strains of viridans streptococci which were trained to resistance to sulfathiazole, penicillin, and aureomycin acquired many biochemical properties characteristic of enterococci. Trained streptomycin-resistant strains varied in their acquisition of these properties. Since the enterococci possess a common antigen, it seemed of interest to determine whether these resistant strains were antigenically related to a known strain of *Streptococcus fecalis*. This was found to be the case.

Experimental. Seven strains of viridans streptococci were used as the parent organisms. Four were obtained from saliva, one from urine, one from blood, and one from the pericardium of 7 different individuals. The resistant strains were prepared by repeated transfers in broth containing the various agents(1). The penicillin and aureomycin-resistant strains were retested and found to be resistant to at least 100 units per ml. The streptomycin-resistant strains were transferred once in broth containing 100 units per ml of streptomycin to insure a high resistance, since previous experience had indicated that this type of resistance may be lost after successive transfers in the absence of the antibiotic. All the parent strains were susceptible to 1 unit or less of penicillin and aureomycin, and to 5 units or less of streptomycin. None of these strains were able to grow in 0.1% methylene blue milk and on 40% bile agar, while all of the resistant strains were able to grow.

Preparation of materials for precipitin tests. The immune serum was prepared by injecting rabbits with formalin treated *Streptococcus fecalis* (ATCC No. 9790) cells. Lancefield's (2) and Fuller's(3) methods of extracting the antigen from the cells were tried with negative results. However, when the extraction was carried out after treating the cells with the enzyme preparation of *Streptomyces albus*, according to the method of Maxted(4), positive results were obtained. The precipitin tests were carried out by drawing undiluted antiserum into capillary tubes, followed by the extract containing varying dilutions of the antigen. The tubes were incubated one-half hour at 37°C, then placed in the refrigerator overnight, and the presence or absence of precipitate observed with the aid of a widefield microscope having a magnification of 6.3X. The various strains were grown for 18-24 hours in 10 ml of Bacto-Difco brain heart infusion broth, and adjusted to a turbidity of 200 on the Klett-Summerson colorimeter before further treatment. Each organism was cultured, harvested, and the antigen extracted from 3 to 6 different times.

Results and discussion. It was not possible in every case to obtain a positive precipitin reaction with our antiserum and the enterococcus used as the immunizing antigen. However, of 37 such tests, 26 were positive, and all 3 tests performed with another known *Streptococcus fecalis* were positive. Readings were made only when controls were positive. Table I shows that the sulfathiazole susceptible viridans strains did not give a precipitin reaction with antiserum prepared against *S. fecalis* No. 9790. The 2 strains which gave biochemical tests of viridans streptococci but were more resistant to sulfathiazole did react. When the parent strains were trained to resistance to sulfathiazole, aureomycin, and penicillin they acquired the ability to react with *S. fecalis* antiserum as well as other properties of enterococci. One of the 5 streptomycin-resistant strains did not react with the

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4. Maxted, W. R., *Lancet*, 1948, v2, 255

TABLE I. Precipitin Reactions of Parent and Resistant Strains with *S. fecalis* Antiserum.

Culture number	Parent strains		Sulfathiazole resis. strains		Penicillin resis. strains		Aureomycin resis. strains		Streptomycin resis. strains	
	Inhib. con. sulfa	% tests pos.	Inhib. con. sulfa	% tests pos.	Inhib. con. sulfa	% tests pos.	Inhib. con. sulfa	% tests pos.	Inhib. con. sulfa	% tests pos.
1	.1*	0†	30	66	30	100	30	66	30	100
4	.1	0	30	50	30	100	30	66	>30	66
6	.1	0	30	40	30	100	>30	66	30	66
17	10	33	30	80	30	100	>30	66	10	0
27	30	66	>30	100			>30	66		
34	1	0	>30	60						
57	1	0	30	50	30	66	>30	66	>30	33

* mg/ml of sulfathiazole required to inhibit growth.
† 3 to 6 tests performed on each organism.

antiserum. It was somewhat less resistant to sulfathiazole than were the other strains. We have reported that streptomycin-resistant strains, unless trained to a very high resistance, may not become as highly resistant to sulfathiazole as do other antibiotic-resistant trained strains from the same parents(1). Other biochemical properties appear to change more slowly, also, in this group of trained organisms.

Yegian, Budd, and Middlebrook(5) could demonstrate no change in antigenicity in a sulfonamide-resistant strain of *Mycobacterium ranae*. Gezon and coworkers(6-9) found some changes to occur in B-hemolytic streptococci when they were made resistant to penicillin, but little or no change when made resistant to aureomycin, bacitracin, and streptomycin.

We have now shown that viridans streptococci may be trained to resistance to sulfathiazole or to resistance to several antibiotics, and if they become sulfathiazole resistant at the same time, these strains become, in all the properties tested, exactly like enterococci. These properties include the ability to reduce methylene blue milk, to grow in 40% bile agar, and in broth containing high concentrations of sulfathiazole or of penicillin(1), to produce CO_2 from arginine and tyrosine(10), and to produce a positive precipitin reaction with *S. fecalis* antiserum. Sulfathiazole-sensitive viridans strains do not have these properties. There can be little doubt that the various agents used to select the resistant strains induced a change in the original organisms, since the resistant strains grow much more rapidly than the parent strains. If a few had been present in the original cultures, these would have grown out by transferring in broth in the absence of the inhibitors. Such a change was not found in any of our strains upon successive transfer in broth. Furthermore, planting a few resistant organisms in a culture of the parent strain resulted in a resistant culture after one transfer. It would certainly seem that several strains of viridans streptococci have been induced to change to enterococci.

Summary. Antigens similar to those found in a strain of *S. fecalis* were found in all but one of the sulfathiazole, aureomycin, penicillin, and streptomycin-trained resistant strains of viridans streptococci. The antigens were not present in 5 of the parent strains with low sulfathiazole resistance but were present in 2 with higher resistance.

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