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Bacteriostatic Effect of Sulfathiazol and Sulfamethylthiazol for Beta Hemolytic Streptococci in Tissue Culture Clots.*

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The bacteriostatic effect of 2(para-amino-benzene-sulfonamido) thiazol (sulfathiazol) and 2(para-amino-benzene-sulfonamido) 4-methylthiazol (sulfamethylthiazol) has been compared with that of sulfanilamide. Two strains of beta streptococci have been used, C 203 and No. 40. Strain C 203 has been used in many studies reported in the literature. Strain No. 40 is a Lancefield group C of human origin which has been studied extensively in this laboratory.

The Maximow culture technic was used. Details have been described previously.[1-4] A culture consists of one drop of heparinized rabbit plasma and 3 drops of rabbit serum extract of 7-day chick embryos.

The extract was inoculated with a culture of streptococci grown in the serum extract described above to which 5% of rabbit erythrocytes were added. Cultures are grown only until hemolysis occurs. For strain No. 40 this requires 3-5 hours and somewhat longer for C 203. Dilution of the bacterial culture was made rapidly through Tyrode into the extract to make a final dilution of 10^{-6}. The inoculated extract was then divided into 4 equal portions and equal volumes of the designated drug added to each of the experimental tubes and the same volume of Tyrode to the control.

A 400 mg % stock solution of each drug was sterilized by Berkefeld filtration through a filter used only for that drug. Solutions were kept in the ice box.

Due to the limited solubility of the thiazol derivatives these were used as the sodium salts.

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1 King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

2 King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

3 King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.

4 King, J. T., Henschel, A. F., and Green, B. S., *J. Am. Med. Assn.*, 1939, **113**, 1704.

pH determinations done with the glass electrode show that, in the amounts used, the change in pH caused by addition of the sodium salts is small.

Cultures were incubated at 37.5°C.

Readings were made at magnification of 60X with a standardized ocular micrometer using a mechanical stage (114 units = 1 mm). The average colony diameter was used as the index of bacteriostasis. Colony counts per culture are also given in the table.

The effect of 3 concentrations of each drug was studied for each strain. With one exception readings were made at or near the 24-hour period. The results are given in Table I.

It will be noted that even on the weight basis sulfathiazol compares favorably with sulfanilamide in inhibiting the growth of both strains studied. Sulfamethylthiazol is somewhat less effective.

When used in sufficient concentration to cause marked bacteriostasis, both drugs inhibit the development of the normal diffuse periphery usually seen around colonies growing in this medium. The periphery may be completely inhibited or it may be composed of a loose net of long, heavy chains of streptococci which are never seen in the controls. This influence on the periphery has been described previously for sulfanilamide in this medium by King, Henschel and Green.[4] Others have noted the formation of such chains in fluid media.

Sulfamethylthiazol is less effective than sulfathiazol in inhibiting the development of the normal diffuse periphery.

Strains which we have studied previously have usually not shown a significant decrease in the number of colonies when growing in tissue culture clots containing sulfanilamide. In the highest con-

TABLE I.

Action of Sulfanilamide (S), Sulfathiazol (ST), and Sulfamethylthiazol (SMT) on Two Strains of Beta Streptococci; 22-24 Hours Except as Noted.

Drug conc. mg%	50		10		1	
	No. Cols.	Diam.	No. Cols.	Diam.	No. Cols.	Diam.
			Strain C203.			
Control	44.2	60.8	40.7	38.7	23.1	45.1
S	5.2	5.6	20.2	20.9	18.6	42.3
ST	2.5	5.6	22.2	15.6	23.5	49.2
SMT	16.2	11.6	31.0	24.8	20.2	49.0
			Strain No. 40.			
Control	24.7	77.7	84.2	73.4*	53.6	85.4
S	14.5	9.4	85.2	22.8	52.3	57.2
ST	6.2	5.8	89.3	19.7	53.6	72.0
SMT	8.0	8.4	92.3	26.6	50.6	83.4

* 10 mg% conc. read at 48 hours.

centration of the drugs used in this study, strain No. 40 did show a decrease in colony count at 24 hours. By 48 hours, however, the counts had increased to 22.5 in cultures containing sulfanilamide, 20.5 in sulfathiazol and 21.7 in sulfamethylthiazol (controls 24.7).

Strain C 203, which we are using for the first time, shows a decrease in colony count at 24 hours not only in the 50 mg% concentration but also in 10 mg%. Furthermore, the count in the 10 mg% concentration had increased to only 32.7 in sulfanilamide, 31.0 in sulfathiazol and 38.7 in sulfamethylthiazol at 72 hours (control 40.7). In the 50 mg concentration there was no significant increase in average number of colonies per culture at 5 days. Some of the few colonies which were present continued to grow very slowly, however. This strain is definitely more susceptible in this respect than strains which we have studied previously.

Due to differences in method of assay and drug-organism combinations, it is difficult at this time to compare the results of *in vitro* tests. It is now generally realized that the medium used and the size of the inoculum markedly influence the results obtained.

Lawrence[5] found sulfathiazol and sulfamethylthiazol superior to sulfanilamide in their bacteriostatic effect against group A hemolytic streptococci. Long and Bliss[6] found sulfathiazol to be as effective as sulfanilamide against a number of organisms including group A streptococci.

Summary. Under the experimental conditions employed sulfathiazol is as effective a bacteriostatic agent against the strains of beta hemolytic streptococci tested as is sulfanilamide. Sulfamethylthiazol is somewhat less effective.

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5 Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 92.

6 Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.