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Comparative Study on Bacteriostatic Action of Sulfanilamide, Sulfapyridine, Sulfanilyl-Sulfanilamide and Sulfathiazol upon Enterococci.

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At the present time it is not as yet possible to predict from the chemical composition of various sulfanilamide derivatives their chemotherapeutic activity toward different microörganisms that are more or less susceptible to the action of sulfanilamide. Thus, it is necessary to test the efficacy of new compounds both *in vitro* and *in vivo*. It is generally agreed that sulfanilamide in therapeutic doses has little or no effect on enterococci. Recently Helmholz[1] reported that sulfathiazol and sulfamethylthiazol in urine are bactericidal toward enterococci. Hill[2] found that sulfanilamide, sulfapyridine and sulfathiazol in a concentration of 100 mg % have a marked bacteriostatic or germicidal action upon relatively small numbers of *alpha-Streptococcus fecalis* in urine. This author made the important observation that sulfathiazol as present in urine after administration of the drug exerts greater bacteriostatic activity than the same amount added to normal voided urine. Long and Bliss[3] observed that sulfanilamide, sulfapyridine and sulfathiazol in concentrations of 10 mg % only slightly inhibited the growth of hemolytic streptococcus zymogenes (Group D) in beef infusion, 2% Neopeptone, 0.075% dextrose broth. Clinically, Pool and Cook[4] reported sterilization of urine of patients harboring *Streptococcus fecalis* following the administration of sulfathiazol and sulfamethylthiazol, respectively. The concentration of the drug in the urine of these cases exceeded 150 mg %. The following *in vitro* experiments were carried out in order to determine the relative bacteriostatic activity of various sulfanilamide derivatives toward hemolytic and non-hemolytic enterococci.

As culture medium ¼% maltose phenol red broth base (Difco), containing tryptose (1%), sodium chloride (0.5%), dipotassium phosphate (0.1%), and phenol red, was used. Sulfanilamide, sulfapyridine, sulfanilyl-sulfanilamide (Disulon), sulfathiazol (2-sul-

1 Helmholz, H. F., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 65.

2 Hill, J. H., *J. Urol.*, 1940, **43**, 491.

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

4 Pool, T. L., and Cook, E. N., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 113.

fanilamidothiazol) and sulfamethylthiazol (2-sulfanilamidomethylthiazol) were added to this culture medium. The drugs were dissolved by heating the broths in a water bath. Then, the broths were tubed and autoclaved at 15 lb pressure for 12 minutes. It may be mentioned that a change of the pH to the acid side occurred when sulfanilyl-sulfanilamide, sulfthiazol and sulfamethylthiazol were dissolved in the broth.

The strains of hemolytic and non-hemolytic enterococci were the same as those previously used in a study on the bacteriostatic action of sulfanilamide upon members of the enterococcus group.[5] In addition to these, several strains were obtained through the courtesy of Dr. H. F. Helmholz, the Mayo Clinic, Rochester, Minnesota. The strains were cultured in brain-heart infusion broth for 18-24 hours; this culture medium was also used for diluting purposes.

Table I presents an experiment in which the bacteriostatic action of various sulfanilamide derivatives in a concentration of 100 mg % upon a strain of hemolytic enterococcus (dilution of 1:125,000) was tested both at 37°C and 43°C. It may be seen from this table, that: (1) at 37°C, sulfanilamide and sulfapyridine lacked growth inhibitory action, whereas sulfanilyl-sulfanilamide and sulfathiazol delayed the growth of the microörganisms for a short period only. (2) At 43°C, sulfanilamide failed to inhibit the growth of the enterococcus; sulfapyridine delayed its growth, and sulfanilyl-sulfanila-

TABLE I.

Bacteriostatic Action of 100 mg % of Sulfanilamide, Sulfapyridine, Sulfanilyl-sulfanilamide and Sulfathiazol upon a Strain of *Enterococcus hemolyticus* in ¼% Maltose Phenol Red Broth.

		1	2	3	4	5
	Hr. of Incubation	Control broth	Sulfanilamide broth	Sulfapyridine broth	Sulfanilyl-sulfanilamide broth	Sulfathiazol broth
			Incubation at 37°C.			
1.	10	++	+	++	—	—
2.	18	++++	++++	++++	+++	++++
3.	24	++++	++++	++++	++++	++++
4.	48	++++	++++	++++	++++	++++
5.	72	++++	++++	++++	++++	++++
6.	120	++++	++++	++++	++++	++++
			Incubation at 43°C.			
1.	10	—	—	—	—	—
2.	18	+++	+++	—	—	—
3.	24	+++	+++	+++	—	—
4.	48	+++	+++	+++	—	—
5.	72	+++	+++	+++	—	—
6.	120	+++	+++	+++	—	—

— = No visible growth.
\+ to ++++ =various degrees of growth.

[5] Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 52.

mide and sulfathiazol prevented visible growth for 5 days. (3) The growth of the enterococcus in the control broth was slightly retarded and inhibited at 43°C in comparison to that obtained at 37°C.

As in the case of sulfanilamide, the degree of bacteriostasis exerted by sulfanilyl-sulfanilamide and sulfathiazol depends, besides on other factors, upon the size of the inoculum; *e. g.*, in one particular experiment sulfathiazol in a concentration of 0.1% did not delay the growth of a hemolytic enterococcus when 0.1 cc of a 1:50 diluted broth culture was used for inoculation; with a 1:2500 dilution, it delayed the growth for 18 hours and with a 1:6,000,000 dilution it completely prevented visible growth for 8 days. Essentially the same results were obtained with hemolytic and non-hemolytic enterococci.

In preliminary experiments with sulfamethylthiazol it was found that this drug in concentrations of 0.1% or in saturated solution is bacteriostatic toward members of the enterococcus group and is more effective than sulfanilamide in equal concentrations (0.1% to 0.2%).

Previously, it was shown[5] that sulfanilamide in concentration of 1% may completely prevent visible growth of suitable numbers of enterococci in broth incubated at approximately 43°C. In order to further evaluate the relative efficacy of sulfanilamide and its derivatives, the bacteriostatic effect of 1% sulfanilamide was compared with that of sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in 0.2% concentration or saturated solution, respectively.

These experiments were carried out with hemolytic and non-hemolytic enterococci. It was found that 1% sulfanilamide is of greater effectiveness than are sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in the above mentioned concentrations.

In conclusion: (1) Sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in concentrations of 100 mg % or above exert definite bacteriostatic activity toward small numbers of both hemolytic and non-hemolytic enterococci in ¼% maltose broth at 43°C. (2) The growth inhibitory effect of these drugs is greater than that of equal concentrations (0.1% to 0.2%) of sulfanilamide and sulfapyridine. (3) The bacteriostatic effect of sulfanilamide and its derivatives upon the growth of enterococci is greater at 43°C than at 37°C.

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