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Effect of Temperature on Bacteriostatic Action of Sulfanilamide upon Members of the Enterococcus Group.

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Until recently, the members of the enterococcus group were considered to be rather resistant to the bacteriostatic and bactericidal action of sulfanilamide, both *in vivo* and *vitro*. Thus, Helmholz and Osterberg[1] found that sulfanilamide when given by mouth produces a urine strongly bactericidal for microörganisms usually found in urinary infections with the exception of the *Streptococcus fecalis*. Bliss and Long[2] reported that hemolytic enterococci (Lancefield group D) were not affected by sulfanilamide in a concentration of 1:10,000 in beef infusion, neopeptone broth, even when the broths were inoculated with relatively small numbers of microörganisms. In contradistinction to fibrinolytic hemolytic streptococci, several strains of hemolytic enterococci were not or only slightly inhibited in 1% dextrose broth containing 0.8% sulfanilamide, when one loopful of a 16 to 18 hours broth culture was used for inoculation (Neter[3, 4]). Long and Bliss[5] reported that hemolytic enterococci, Lancefield Group D, are resistant to the bacteriostatic action of sulfanilamide in concentrations up to 800 mg %; above this concentration a slight retarding of growth was noted with a few strains.

Recent experiments, however, revealed that under certain conditions it may be possible to demonstrate a definite bacteriostatic action of sulfanilamide upon members of the enterococcus group. Bliss and Long[6] found that the growth of enterococci may be definitely delayed, provided that high concentrations of sulfanilamide and small inocula are used. A marked bacteriostatic action of sulfanilamide in concentrations from 800 mg % to 1000 mg % upon hemolytic enterococci could be demonstrated,[7] employing as culture medium, 1%

1 Helmholz, H. F., and Osterberg, A. S., *Proc. Staff Meetings of the Mayo Clinic*, 1937, **12**, 377.

2 Bliss, E. A., and Long, P. H., *New England J. Med.*, 1937, **217**, 18.

3 Neter, E., *J. Bact.*, 1938, **36**.

4 Neter, E., *J. Lab. Clin. Med.*, 1939, **24**, 650.

5 Long, P. H., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds*, 1939, 102.

6 Bliss, E. A., and Long, P. H., *Abstracts, Third Internat. Congress for Microbiology*, New York, 1939, 251.

7 Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 668.

maltose phenol red broth containing from 6.5% to 7% sodium chloride. In this broth the growth of hemolytic enterococci is markedly delayed. This culture medium was chosen because of the possibility that the delay in growth of the microörganisms may overcome the lag-period in the action of sulfanilamide. Bacteriostasis was also obtained with broth containing only ¼% maltose. Further experiments[8] revealed that hemolytic enterococci in 1% sulfanilamide broth, that failed to show visible growth upon incubation in contradistinction to the control, lost their viability more rapidly than the microörganisms in the control broth. On the other hand, the death rate of very small inocula of hemolytic enterococci, that failed to show visible growth in both the sulfanilamide and control broths, was not materially affected by sulfanilamide. Similar results were obtained with non-hemolytic enterococci. White and Parker,[9] and White[10] recently reported that sulfanilamide is more effective toward hemolytic streptococci at 39°C and 40°C than at 37°C. Wengatz, Boak, and Carpenter[11] showed that 0.01% of sulfanilamide may shorten by 50% the thermal death time of gonococci at 41.5°C. In view of these observations it was decided to determine the influence of temperature on the bacteriostatic activity of sulfanilamide toward members of the enterococcus group.

The experiments were carried out as follows: 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. To one part of this culture medium, 1% of sulfanilamide was added, to the other ½% of sodium chloride. Sodium chloride was added to the control broth in order to compensate for increased osmotic pressure of the sulfanilamide broth. The culture media were autoclaved at 15 pounds pressure for 15 minutes. Several strains of hemolytic and non-hemolytic enterococci were used; 2 of these strains were obtained through the courtesy of Dr. J. M. Sherman, Ithaca, New York. Decreasing amounts (volume 0.1 cc) of the respective broth culture were used for inoculation of sulfanilamide and control broths (volume 5 cc). Parallel experiments were carried out at 37°C and 43°C, respectively. Visible growth was noted at various intervals.

Table I presents the results of an experiment in which the bacteriostatic action of 1% sulfanilamide toward a strain of non-hemolytic enterococcus was tested, both at 37°C and 43°C. It may

[8] Neter, E., *J. Bact.*, 1940, in press.

[9] White, H. J., and Parker, J. M., *J. Bact.*, 1938, **36**, 481.

[10] White, H. J., *J. Bact.*, 1939, **38**, 549.

[11] Wengatz, H. F., Boak, R. A., and Carpenter, C. M., *J. Bact.*, 1938, **35**, 36.

TABLE I.

Bacteriostatic Action of Sulfanilamide (1%) on *Streptococcus fecalis* in ¼% Maltose Phenol Red Broth at Different Temperatures. Inoculum from 48-hour Culture (0.1 cc).

		Dilutions.									
		I 1/50		II 1/2500		III 1/125,000		IV 1/6,250,000		V 1/312,500,000	
Hr of incubation		C	S	C	S	C	S	C	S	C	S
					Incubation at 37°C.						
1.	16	++++	+	++	—	—	—	—	—	—	—
2.	24	++++	++	++++	—	++++	—	++	—	—	—
3.	48	++++	+++	++++	++	++++	—	++++	—	—	—
4.	72	++++	+++	++++	+++	++++	+	++++	++	—	—
5.	96	++++	+++	++++	+++	++++	+++	++++	+++	—	—
6.	120	++++	+++	++++	+++	++++	+++	++++	+++	—	—
					Incubation at 43°C						
1.	16	++++	—	+++	—	—	—	—	—	—	—
2.	24	++++	—	+++	—	+++	—	+++	—	—	—
3.	48	++++	—	++++	—	++++	—	++++	—	—	—
4.	72	++++	—	++++	—	++++	—	++++	—	—	—
5.	96	++++	—	++++	—	++++	—	++++	—	—	—
6.	120	++++	—	++++	—	++++	—	++++	—	—	—

C = 1% NaCl ¼% Maltose Phenol Red Broth.
S = 1% Sulf. ½% NaCl ¼% Maltose Phenol Red Broth.
— = No visible growth.
+ to ++++ = Various degrees of visible growth.

be seen from this table that (1) sulfanilamide at 37°C delayed the growth, but did not completely prevent it, even when small inocula (1/6,250,000 dilution of a 48 hours culture) were used; (2) sulfanilamide at 43°C completely and continuously inhibited visible growth of the enterococcus; (3) sulfanilamide at 43°C was bacteriostatic toward much larger numbers (1:50 dilution of a 48 hours culture) of enterococci than at 37°C.

This increased bacteriostatic activity of sulfanilamide in concentration of 1% at 43°C could be demonstrated with 3 strains of hemolytic and 3 strains of non-hemolytic enterococci, even when relatively large numbers of microörganisms (1:50 dilution of a 18 to 48 hours culture) were used for inoculation. With one strain of non-hemolytic enterococcus, growth was not completely inhibited but definitely retarded by sulfanilamide at 43°C.

It is important to mention that the growth of the enterococci in the control broths was not markedly delayed or suppressed at 43°C in comparison to that obtained at 37°C. This observation supports the view of White,[10] namely, that the increase in activity of sulfanilamide toward hemolytic streptococci cannot be explained solely on the basis of a deceleration of the growth rate at higher temperature.

In conclusion, at 43°C sulfanilamide in concentration of 1% is markedly more bacteriostatic toward both hemolytic and non-hemolytic enterococci, than at 37°C.

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Tyrosinase in Feather Germs.

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The presumable enzyme system involved in melanin formation by growing feathers does not seem to have been investigated, although it might be supposed to be a tyrosinase or dopa-oxidase, by analogy with melanogenesis in mammalian skin and hair roots, amphibian skin, insect hypoderm, etc. That a tyrosinase *is* present, whatever its rôle, in feather germs of black and red chickens, and absent or somehow masked in the germs of certain white breeds, is shown by the following study.

Feather germs, in which the rhachis tip had not yet (or just re-