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Development of Sulfathiazole-Resistant Gonococci *In vitro.*

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Gonococci cultivated in gradually increasing concentrations of sulfanilamide or sulfapyridine readily develop resistance, or fastness, to the bacteriostatic action of these sulfonamides.[1,2] With sulfathiazole, however, attempts to produce resistant strains of gonococci have not been successful.[3] The potential clinical significance of this observation has been pointed out in a recent editorial in the *Journal of the American Medical Association* as follows, "This failure of the gonococcus to develop resistance to sulfathiazole suggests that sulfathiazole-fast strains are not likely to be developed in the clinic or to be spread to the general population."[4]

Recent experiments in this laboratory have indicated that under proper conditions organisms susceptible to the bacteriostatic action of any of the sulfonamides should be capable of becoming resistant to all of the sulfonamides.[5] If this theory is correct, it should be possible to develop sulfathiazole-resistant gonococci, and the following *in vitro* experiments were undertaken to determine whether this could be accomplished.

Materials and Methods. A strain of *Neisseria gonorrhoeæ* isolated from a male with gonococcal urethritis was employed for the experiments. Using the quantitative technic previously described,[5] a constant inoculum of about one million organisms per cc was transferred every 5 to 7 days in 20% ascitic broth containing gradually increasing concentrations of sulfonamides, beginning with sulfanilamide 25 μg per cc and sulfathiazole 0.025 μg per cc. After 3 transfers the sulfonamide concentrations were doubled, and this process was repeated until the concentration of sulfanilamide was 500 μg per cc, and that of sulfathiazole was 10 μg per cc. Growth of the control and resistant organisms was measured by turbidity readings on the Coleman Universal Spectrophotometer. Using the same technic, measurements were made of the bacteriostatic effect of sulfapyridine and sulfadiazine on the control and resistant organisms.

Results. Growth curves of the control and resistant organisms are presented in Fig. 1. It is evident that transfers in gradually increasing concentrations of sulfonamides caused the gonococci to become markedly resistant to both sulfanilamide and sulfathiazole. These same sulfonamide concentrations completely inhibited the growth of the control organisms; indeed, they were completely inhibited by concentrations less than one-eighth as great as those shown in the figure, even after 30 days.

TABLE I.

The Bacteriostatic Action of Four Sulfonamides upon Sulfathiazole-Resistant Gonococci. Growth is expressed in terms of optical density (Turbidity). See text for explanation.

Drugs	μg/cc	Control organisms	Sulfathiazole-resistant gonococci
Sulfanilamide	500	0	.36
Sulfapyridine	50	0	.35
Sulfadiazine	10	0	.32
Sulfathiazole	10	0	.30

Table I shows the results when the sulfathiazole-resistant organisms were grown for 5 days in media containing sulfathiazole 10 μg per cc, sulfadiazine 10 μg per cc, sulfapyridine 50 μg per cc, and sulfanilamide 500 μg per cc. Good growth occurred with all the sulfon-

[1] Boak, R. A., Charles, R. L., and Carpenter, C. M., Pub. No. 11, American Society for the Advancement of Science, Lancaster, Pa. The Science Press, 1939, p. 118.

[2] Westphal, L., Charles, R. L., and Carpenter, C. M., *Ven. Dis. Inf.*, 1940, **21**, 183.

[3] Carpenter, C. M., Charles, R., and Allison, S. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 527.

[4] Editorial, Sulfanilamide Fast Gonococci, *J. A. M. A.*, 1942, **119**, 31.

[5] Kirby, W. M. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.

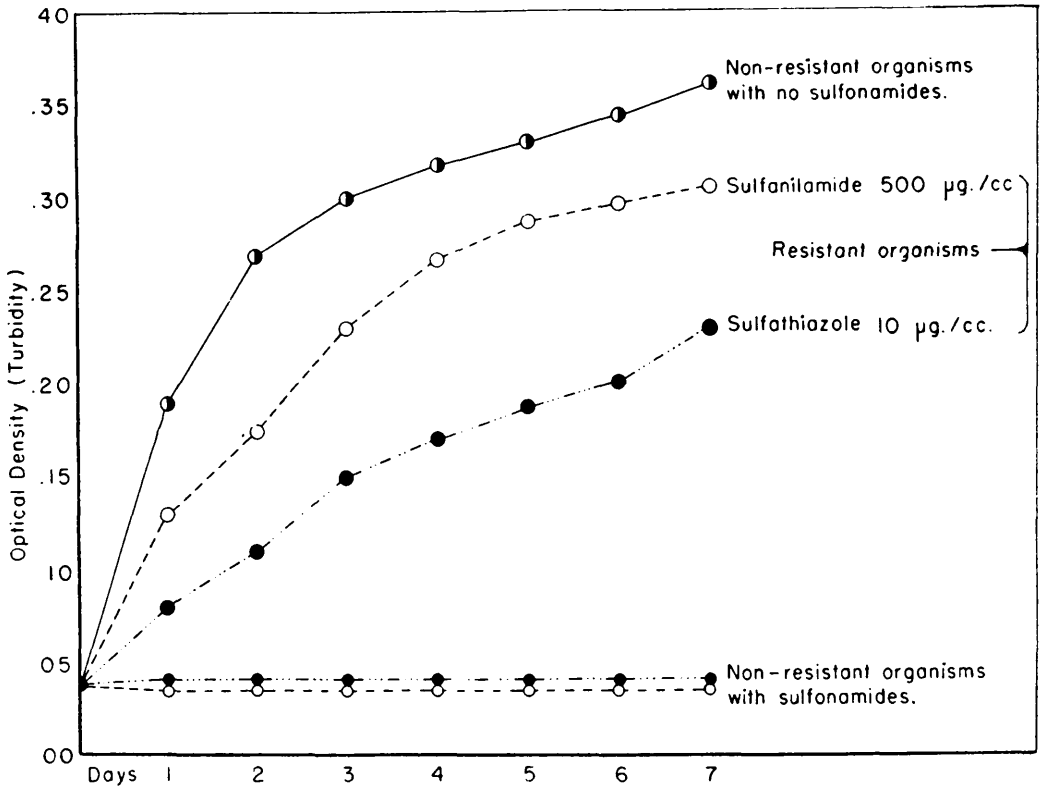

Figure I. Growth Curves of Resistant and Non-resistant Gonococci.

amides, while the controls were completely inhibited. The results when the sulfanilamide-fast gonococci were grown in media containing the other three sulfonamides were essentially the same, and therefore are not included in the table.

Comment. In these experiments sulfathiazole-resistant gonococci have been developed with comparative ease. The failure of previous workers to accomplish this may have been due to beginning with too high a concentration of sulfathiazole, for it must be realized that in these *in vitro* experiments the bacteriostatic potency of sulfathiazole is more than 50 times as great as that of sulfanilamide.

The sulfathiazole-resistant gonococci were also resistant to sulfanilamide, sulfapyridine, and sulfadiazine, and the sulfanilamide-resistant organisms were also resistant to sulfapyridine, sulfadiazine, and sulfathiazole. These findings, which are being extended to other strains of the gonococcus, are in accord with the previously advanced thesis[5] that the development of sulfonamide resistance represents an interaction between the organisms and the *p*-amino nucleus of the sulfonamides, and that organisms susceptible to the bacteriostatic action of any of the sulfonamides are capable of becoming resistant to all of the sulfonamides.

Summary and Conclusions. Using a simple *in vitro* technic, it has been possible to develop both sulfanilamide-resistant and sulfathiazole-resistant gonococci. The sulfathiazole-resistant organisms were resistant to sulfanilamide, sulfapyridine, and sulfadiazine, and the sulfanilamide-resistant organisms were also resistant to sulfapyridine, sulfadiazine, and sulfathiazole. The significance of these observations is discussed.