

Effect of Gradually Increased Concentrations of Sulfathiazole on the *Gonococcus in vitro*.*

CHARLES M. CARPENTER,[†] RUTH CHARLES AND SAMUEL D. ALLISON.[‡]

From the Department of Bacteriology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

Recent studies^{1, 2} have shown that many strains of the gonococcus readily acquire fastness to sulfanilamide and to sulfapyridine when cultivated in a medium to which gradually increased amounts of the drugs are added. It was observed, furthermore, that the strains tested retained this drug-fastness for at least 4 months after subsequent growth on a medium free from the compound. In addition, Bang and Bang³ observed that 2 of these sulfanilamide-fast strains which they studied had retained their resistance for at least one year.

The present study employing sulfathiazole was undertaken after this drug was proved to be more effective for the treatment of gonococcal infection than other sulfonamide compounds.

Materials and Methods. Ten strains of the gonococcus, recovered from various types of gonococcal infection, were studied. Six strains had been isolated from 1 to 3 years prior to the experiment, and 4 others had been under artificial cultivation for only 3 months. Seven of the strains were isolated from the genito-urinary tract of males, 1 from the cervix, 1 from the vagina of a child, and 1 from the conjunctiva.

The technic used to determine sulfathiazole-fastness was similar to that followed in previous studies on the development of sulfanilamide- and sulfapyridine-fast strains of the gonococcus.^{1, 2} The basic culture medium was a pork infusion broth containing 0.5%

* Studies in coöperation with the Division of Venereal Diseases of the United States Public Health Service.

† On leave of absence from the School of Medicine and Dentistry of the University of Rochester as a Fellow of the Division of Medical Sciences of The Rockefeller Foundation.

‡ On leave of absence from the Oregon State Board of Health, Division of Venereal Disease Control.

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 Bang, F. B., and Bang, B., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 527.

of potassium nitrate, 0.5% of potassium acid phosphate, and 5% of defibrinated rabbit blood. Sulfathiazole was added to yield final concentrations of 0.0005%, 0.001%, 0.002%, 0.003%, 0.004%, and 0.005%.

Each strain of the gonococcus was cultivated at 35°C for 24 hours on a chocolate blood agar slant. The growth was suspended in 1 ml of the infusion broth without rabbit blood, and the suspension immediately transferred to 3 ml of the infusion-blood broth. After 48 hours of incubation, inocula of 0.1 ml were transferred to a tube of the same medium, which served as a control, and to a series of tubes containing 0.0005%, 0.001%, 0.002%, 0.003%, and 0.005% concentrations of sulfathiazole. Tests for viability were made 24 hours after inoculation by means of subcultures on chocolate-blood agar plates. When growth became established at a given concentration of the drug, the strain was introduced into the medium in which the concentration was 0.0005% to 0.003% higher. At the same time, in order to safeguard the maintenance of the culture, it was also inoculated into a tube of broth containing an equivalent amount of drug in which growth had been established. Thus, transfers to the same and to higher concentrations were made every 48 hours, while subcultures for viability were made at the same intervals, but on alternate days. Throughout the study, control cultures were maintained in the infusion-blood broth. The study was in progress for approximately 3 months.

To ascertain if the strains selected for the study would acquire tolerance for sulfanilamide, 8 of them were grown in increasing concentrations of that compound. After it was demonstrated that all had become sulfanilamide-fast, they were inoculated into a blood broth containing 0.005%, 0.01%, and 0.025% concentrations of sulfathiazole to determine if simultaneously they had acquired tolerance for sulfathiazole.

Results. Ten strains of the gonococcus cultivated in gradually increasing concentrations of sulfathiazole grew moderately well in 0.0005%, 0.001%, and 0.002%. At levels of 0.003% and 0.005%, growth did not become established, although on some attempts the strain remained viable for 2 or 4, or in one instance 10, days at these higher concentrations. The cultures were grown in a 0.002% concentration for from 30 to 74 days before being transferred to the higher concentrations. This is in marked contrast to the 8 strains that were grown simultaneously in gradually increasing concentrations of sulfanilamide. Within 14 days, 2 grew in a 0.05% concentration of the drug, 3 in a 0.035% concentration, 2 in a 0.03% con-

centration, and 1 in a 0.02% concentration. In every instance growth was luxuriant. When the 8 sulfanilamide-tolerant strains were introduced into broth containing sulfathiazole in concentrations of 0.005%, 0.01%, and 0.025%, no growth occurred.

Discussion. The results of this study are striking in that the strains of the gonococcus studied acquired only slight, if any, tolerance for sulfathiazole, although resistance to sulfanilamide was readily established. Even though attempts were made repeatedly over a period of 3 months to establish growth in a medium containing sulfathiazole, success was attained at only the lowest concentrations, *i. e.*, 0.0005 to 0.002%. It is apparent that sulfathiazole exerts a more marked bacteriostatic and bactericidal action on the gonococcus than either sulfapyridine or sulfanilamide. Lawrence⁴ has reported that a 0.001% concentration of the drug inhibits the development of the gonococcus in 5 hours.

These *in vitro* studies are in accord with clinical observations of the superior chemotherapeutic action of sulfathiazole in gonococcal infection. The failure of the gonococcus to develop resistance to sulfathiazole *in vitro* suggests that sulfathiazole-fast strains of the organism are less likely to become established in the population at large than are sulfanilamide- or sulfapyridine-fast strains.

Summary. Ten strains of *Neisseria gonorrhoeae* developed little, if any, tolerance for sulfathiazole when grown for approximately 3 months in a blood broth containing concentrations of the drug varying from 0.0005% to 0.005%.

13363 P

Changes in Blood Urea Following Hypothalamic Lesions.

H. R. MILLER, E. KRUEGER, J. M. ALPER AND E. A. SPIEGEL.

From the Medical Division, Montefiore Hospital, New York City, and the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.*

The following observations are reported as part of a study of the influence of diencephalic lesions upon the nitrogenous constituents of the blood.

Methods and Material. Eight cats received lesions in the pre-

⁴ Lawrence, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 92.

* Aid from the Dudley D. Sicher Fund is acknowledged with thanks.