

# Correlation of *in vitro* Sulfonamide Resistance of the Gonococcus with Results of Sulfonamide Therapy\*†

CHARLES M. CARPENTER, M.D., F.A.P.H.A., AND  
HELEN ACKERMAN

*University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

AND

MILLARD E. WINCHESTER, M.D., F.A.P.H.A., AND  
JANE WHITTLE

*Glynn County Board of Health, Brunswick, Ga.*

PREVIOUS studies have demonstrated that *in vitro* the gonococcus acquires resistance to gradually increased concentrations of sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine.<sup>1-4</sup> It has been shown, furthermore, that induced resistance of an organism to one sulfonamide compound usually increases resistance to other derivatives of sulfanilamide.<sup>3, 5</sup> These observations, together with an apparent decrease in the therapeutic efficacy of sulfonamides in gonorrhea, suggested the investigation of a possible correlation between the laboratory and clinical findings. Information as to the prevalence of sulfonamide resistant strains of the gonococcus in a community following the widespread use of sulfonamides would in addition provide data of epidemiologic significance. Cohn and Seijo<sup>6</sup> have recently reported a direct relationship between the therapeutic

efficacy of sulfathiazole in patients with gonococcal infection in New York City, and the susceptibility of the patient's strain of gonococcus to the drug *in vitro*.

The present report records the results of treatment with sulfathiazole on 105 patients with gonococcal infection and the *in vitro* behavior to sulfanilamide and sulfathiazole of the strains of the gonococcus isolated from those patients. This study was carried out at the Glynn County Board of Health, Brunswick, Ga.

## MATERIALS AND METHODS

Patients with symptoms of uncomplicated urethritis of cervicitis reporting at the venereal disease clinic were examined for evidence of gonococcal infection. The film and cultural methods were used to verify the diagnosis of the disease. Those from whom the gonococcus was isolated were treated with a single course of therapy consisting of 20 gm. of sulfathiazole administered in one of the two following manners: 3 gm. daily for 6 days and 2 gm. on the 7th day or 4 gm. daily for 5 days. In a few instances sulfapyrazine was sub-

\* Presented before the Epidemiology Section of the American Public Health Association at the Seventy-second Annual Meeting in New York, N. Y., October 14, 1943.

† This study was carried out in cooperation with the Division of Venereal Diseases, U. S. Public Health Service, Washington, D. C.

stituted for sulfathiazole. The patients returned to the clinic for examination once a week for at least 3 weeks, and at each visit films and cultures were again taken. A series of three negative bacteriologic examinations together with the absence of clinical evidence of disease comprised the criteria of cure. Thus, patients responding to one course of therapy were considered "cured," and those requiring additional treatment were designated "failures." The 105 patients included in this study were classified according to race and sex as follows: Negro, 79; white, 26. The Negroes comprised 54 males and 25 females; the white patients, 13 males and 13 females. Included in this group were only those patients with no history of previous treatment with sulfonamide compounds.

The strain of gonococcus isolated from each patient before treatment was tested *in vitro* for growth in blood-broth media containing sulfanilamide and sulfathiazole. As a rule, the tests were carried out as soon as possible after isolation, the strains examined for the most part being between the 5th and 10th generations. In addition to the 105 strains of the gonococcus recovered from this group of patients, similar tests were made on 109 strains isolated in this same community. The observation period of the study extended from May 1, 1942, to August 1, 1943.

The medium was prepared in the following manner. Sulfanilamide to yield final concentrations of 5, 10, 20, and 40 mg. per cent was added to Douglas's broth containing 0.05 per cent each of sodium phosphate and sodium nitrate. After sterilization in the autoclave at 121° C. for 10 minutes, 5 ml. of defibrinated lapine blood were added to each 100 ml. of the medium. The sulfathiazole broth was prepared in a similar manner except that concentrations of 1, 2, 5, and 10 mg. per cent of the drug were employed.

Five ml. of medium in test tubes were inoculated with 0.1 ml. of a 24 hour Douglas's broth culture of the strain to be tested. A tube of blood broth without drug was also inoculated to serve as a control. After 48 hours of incubation at 37° C. subcultures were made to "chocolate" agar plates which were similarly incubated, and the presence or absence of growth was recorded. When growth occurred in any tube containing drug, the strain was designated "resistant." If no growth was detected, the strain was considered "nonresistant." The minimal concentrations of 5 mg. per cent and 1 mg. per cent of sulfanilamide and sulfathiazole, respectively, were selected because they were significantly higher than the concentrations which were bactericidal for strains of the gonococcus isolated from patients readily cured with a single course of sulfonamide therapy.

#### RESULTS

Of the 105 selected patients with gonococcal infection from whom *Neisseria gonorrhoeae* was isolated prior to sulfonamide therapy, 88, or 84 per cent, were "cured" with one course of 20 gm. of either sulfathiazole or sulfapyrazine. The remaining 17, or 16 per cent, failed to be cured with the same amount of drug.

Seventy-seven strains of the gonococcus, 87.5 per cent, isolated from the 88 patients designated as "cured" failed to grow *in vitro* in any of the concentrations, 5, 10, 20, and 40 mg. per cent sulfanilamide or in concentrations of 1, 2, 5, and 10 mg. per cent sulfathiazole. The remaining 11 strains, 12.5 per cent, on the other hand, resisted the action of one or both drugs *in vitro*.

Thirteen strains of the gonococcus, 76.5 per cent, recovered from 17 patients that failed to be cured likewise remained viable when exposed to the

TABLE 1

Correlation of Effect of Sulfonamides on *Neisseria gonorrhoeae* in Vitro with Sulfonamide Therapy in 105 Patients

Strain	Resistance to Sulfonamides of Strains of <i>Gonococcus</i> Isolated before Treatment		Clinical Results with Sulfonamide Therapy			
	No. of Strains	Per cent	Cured *		Uncured	
			No. of Patients	Per cent	No. of Patients	Per cent
"Nonresistant"	81	77	77	95	4	5
"Resistant"	24	23	11	46	13	54
Total	105	100	88	84	17	16

\* Criteria of cure consisted of three negative bacteriologic examinations at weekly intervals together with the absence of clinical evidence of gonorrhea.

above concentrations of sulfanilamide and sulfathiazole *in vitro*, while 4 strains, 23.5 per cent, did not survive the action of the drugs.

A correlation between the curative

ing the first 6 months of the study were "resistant," whereas observations made during the last 3 months showed that 59.3 per cent of all strains examined were "resistant" (Table 2).

TABLE 2

Trend of Prevalence of *in Vitro* Sulfonamide Resistance of the *Gonococcus* in Brunswick, Ga.

Time Intervals	Cultures Tested	Cultures "Nonresistant" to Sulfonamides <i>in Vitro</i>	Cultures "Resistant" to Sulfonamides <i>in Vitro</i>	"Resistant" Cultures
		No.	No.	Per cent
May, 1942–October, 1942	46	39	7	15.2
November, 1942–January, 1943	45	33	12	26.7
February, 1943–April, 1943	69	46	23	33.3
May, 1943–July, 1943	54	22	32	59.3
Total	214	140	74	34.6

effect of the drugs *in vivo* and the resistance of the specific organism *in vitro* showed that of 81 patients infected with "nonresistant" strains, 77, 95 per cent, were "cured," but that of 24 patients infected with "resistant" strains, only 11, 46 per cent, were "cured" with an equivalent amount of the compound (Table 1).

Of a total of 214 strains of the gonococcus isolated prior to treatment at Brunswick, Ga., during a 15 month period from May 1, 1942, to August 1, 1943, and tested for sulfonamide resistance, 74, or 34.6 per cent, were "resistant" to sulfanilamide and sulfathiazole *in vitro*. Of greater significance, however, is the finding that only 15.2 per cent of these strains tested dur-

## DISCUSSION

Cohn and Seijo<sup>6</sup> working with 107 strains of the gonococcus isolated from 101 patients in New York City have reported that 100 per cent of the patients with "nonresistant" strains were cured, but only 18 per cent of the patients with "resistant" strains recovered. In our study, 95 per cent of the patients with "nonresistant" strains responded favorably to sulfonamide therapy as well as 46 per cent of the patients with "resistant" strains. Thus in the group reported by Cohn and Seijo, only 3 of 17 patients with "resistant" strains of the gonococcus were cured, whereas in our group, 11 of 24 patients with "resistant" strains recovered. A valid comparison of the results obtained

in the two studies cannot be made because each patient of Cohn and Seijo received during the first course of therapy only a total of 14 gm. of drug. Our patients, on the other hand, were given a total of 20 gm. each.

The investigation of the resistance *in vitro* of recently isolated strains of the gonococcus to concentrations of sulfonamide compounds comparable to those obtained in the blood of patients after sulfonamide therapy has provided information of value in shedding light on the future course of sulfonamide therapy in gonorrhea. Evidence has been accumulated to indicate that the extensive use of sulfonamide compounds, chiefly sulfathiazole, for the treatment of gonococcal infection within a period of 15 months has markedly increased the prevalence of "resistant" strains in the community in which the study was carried out. This finding may possibly be attributed to the elimination of a high percentage of the "nonresistant" strains by chemotherapy, as well as to the development of "resistant" strains from exposure to the drugs *in vivo*. Inasmuch as the gonococcus acquires resistance to sulfonamides *in vitro*, undoubtedly the organism becomes resistant to the drugs *in vivo*.

The marked increase in the number of "resistant" strains isolated from untreated patients at the venereal disease clinic is striking. It is known that during the period of observation numerous patients in the community obtained sulfathiazole from local pharmacists presumably for the treatment of gonococcal infection. Therefore, self-treatment may have cured many patients infected with "nonresistant" strains of the gonococcus. Thus a proportion of patients attending the clinic may have comprised those who failed to cure themselves. It must be pointed out, however, that every effort was made to exclude previously treated patients from the study in order to eliminate strains

of the gonococcus known to have been exposed to sulfonamides.

Routine tests for the sulfonamide resistance of a strain of gonococcus *in vitro* are as yet impractical because of the time and expense involved. The present study points out, however, one of the difficulties encountered in carrying out with sulfonamide therapy an intensive program for the community control of gonorrhea.

It is interesting to speculate on whether "resistant" strains of the gonococcus were distributed in man previous to the era of sulfonamide therapy, or whether strains of the organism have acquired resistance as a result of contact with the drug. Further investigations including studies of strains isolated prior to the "sulfonamide era" may answer this question.

#### SUMMARY

The *in vitro* response to sulfathiazole and sulfanilamide of 105 strains of *Neisseria gonorrhoeae* has been correlated with the clinical responses of the patients from whom the organisms were isolated.

Of the group of patients from whom "nonresistant" strains were isolated, 95 per cent were "cured" in 5 days with 20 gm. of sulfathiazole. On the other hand, only 46 per cent of the group with "resistant" strains responded to an equivalent amount of sulfonamide therapy.

An increase in the prevalence of sulfonamide-resistant strains of *Neisseria gonorrhoeae* from 15 to 59 per cent in 15 months was observed in Glynn County, Ga., from May, 1942, to August, 1943, following the extensive use of sulfonamide drugs as a part of a special program for the control of gonorrhea.

#### REFERENCES

1. Boak, R. A., Charles, R. L., and Carpenter, C. M. Tolerance of the Gonococcus *In Vitro* for Increasing Concentrations of Sulfanilamide. *Am. A. Advancement Sc., Publ. No. 11*, pp. 118-119.

2. Westphal, L., Charles, R. L., and Carpenter, C. M. The Development of Sulfapyridine-Fast Strains of the Gonococcus. *Ven. Dis. Inform.*, 21: 183-186 (June), 1940.
3. Lankford, C. E., Scott, V., and Cooke, W. R. Studies of Sulfonamide Resistance of the Gonococcus. *J. Bact.*, 45:201 (Feb.), 1943.
4. Kirby, W. M. M. Development of Sulfathiazole-Resistant Gonococci *in Vitro*. *Proc. Soc. Exper. Biol. & Med.*, 52:175-176 (Mar.), 1943.
5. Lowell, F. C., Strauss, E., and Findland, M. Observations on Susceptibility of Pneumococci to Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole. *Ann. Int. Med.*, 14:1001-1031 (Dec.), 1940.
6. Cohn, A., and Seijo, I. Further Observations on the Correlation between Clinical and *in Vitro* Reactions of Gonococcus Strains to Sulfathiazole. *Am. J. Syph., Gonorr., & Ven. Dis.*, 27:301-308 (May), 1943.

NOTE: Sulfapyrazine was supplied by Mead Johnson and Company, Evansville, Ind.

---

## Health Workers Now Subject to W.M.C. Employment Stabilization Program

The War Manpower Commission, Washington, has announced that physicians, dentists, veterinarians, sanitary engineers, and nurses who are salaried employees in essential or locally needed activities are hereafter subject to the same provisions of any employment stabilization program as applies to other workers in such activities. Such professional employees may not change their jobs without securing statements of availability from the U. S. Employ-

ment Service or being referred to new jobs by this Service. It is understood that the U.S.E.S. will make referrals of such employees only after consulting the state chairman of the Procurement and Assignment Service. The W.M.C. state directors may delegate the duty of referring such employees to new jobs to the state and local offices of the Procurement and Assignment Service if this delegation is approved by the regional W.M.C. director.