susceptibility which occurs among various strains of the same species, such as *Staphylococcus aureus,* the first logical approach is to tag the resistant members with a quality lacking in the sensitive strains. The results presented here show a marked correlation of penicillin resistance with the ability to produce a penicillin-inactivating substance, presumably penicillinase. This substance is soluble and diffuses readily into surrounding liquid and solid environment. Kirby's[6] failure to demonstrate the inactivator in Seitz filtrates of culture fluid may be due to the observation[5] that part or all of the enzyme's activity may be lost by Seitz filtration, presumably by adsorption.

In view of the available data, it cannot be denied that an organism's ability to synthesize penicillinase is not the sole factor in explaining its sensitivity or insensitivity to the action of penicillin. Sensitive organisms capable of producing penicillinase and insensitive organisms incapable of producing it have been described. Our own findings with the Shigella-Typhoid-Salmonella group of organisms confirm the findings of Bondi and Dietz.[3] This group is relatively insensitive to the action of penicillin, yet the Shigella organisms produce an inactivator and the Typhoid and Salmonella do not. Suffice it to say that the complete explanation for resistance must be awaited.

In certain bacteria at least, the resistance to penicillin can be definitely ascribed to their ability to produce penicillinase. Rather convincing are Woodruff and Foster's studies[5] on a bacillus capable of producing extracellular penicillinase at pH of 6.0 or above. At this pH the organism was uninhibited in growth by several hundred units of penicillin per ml of culture medium. When they suppressed the production of penicillinase by lowering the pH to 5.5, the organism became easily inhibited by 10 units per ml.

Those who have produced penicillin-resistant strains *in vitro* by culturing in increasing concentrations of penicillin have found that neither the resistant nor the sensitive progenitor was able to produce penicillinase. Inasmuch as we have yet to encounter a resistant strain, isolated directly from an infected process, which is incapable of producing an inactivator, it appears that there may exist a difference in the mechanism between *in vitro* acquired resistance and natural and *in vivo* acquired resistance.

Summary. A potent penicillin-inactivating substance is produced by penicillin-resistant *Staphylococcus aureus* but not by penicillin-sensitive strains. The substance is soluble and diffuses readily into both liquid and solid culture medium. The variation of susceptibility to penicillin among various strains of *Staphylococcus aureus* appears to be associated with a difference in this enzyme formation.

15129

Adaptability of Gonococcus to Four Bacteriostatic Agents, Sodium Sulfathiazole, Rivanol Lactate, Promin, and Penicillin.*†

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Chemotherapy of gonococcal infection has advanced markedly during the last decade. Sulfonamides appear to be less effective now, however, than when first introduced for the treatment of the disease. Two factors may be responsible for this evident decreased effi-

* This study was financed in part by a grant-in-aid from the Division of Venereal Diseases, U. S. Public Health Service, Washington, D.C.

† The following drugs used in this study were generously supplied by: (a) Rivanol lactate, Winthrop Chemical Company, New York, N.Y.; (b)

cacy: one, that the most susceptible strains have been eliminated leaving only the more resistant types to be transmitted throughout the population; the other, that continued exposure *in vivo* from inadequate therapy has resulted in the development of drug-fast strains.[1] Previous studies have shown that the gonococcus can be made sulfonamide-fast readily *in vitro* and recently penicillin-fast strains have likewise been developed.[2-5]

The present investigation was undertaken to find a possible solution to one of the basic problems inherent in chemotherapy; namely, acquired drug-resistance by the parasite. Because a single chemotherapeutic agent is not completely effective against an entire bacterial population, the use of several agents of widely-divergent chemical structures was an obvious approach to the problem. Such a procedure should insure a greater degree of inhibition of metabolic activity of the bacterial cell. Thus drug-resistance becomes more remote. To test the validity of this hypothesis, observations were made on the anti-bacterial effect of the combination of 4 therapeutic agents on the gonococcus. For comparison, the effects of the same drugs used individually were also determined. Information on the concurrent use of several agents will aid in solving the problem of drug-resistance. Furthermore, the combined administration of several agents for a specific parasite offers clinical possibilities.

Technic. The bactericidal agents selected for the study were sodium sulfathiazole, rivanol lactate, promin, and penicillin, representing respectively a sulfonamide, an acridine derivative, a sulfone, and an antibiotic agent. The available information on the mode of action of each substance indicates a special action for each that is consonant with the individuality of its chemical configuration and which has formed the basis for their selection in this study. Five non-resistant strains of the gonococcus identified as No. 515, 3215, 5609, 5723, and 8071, were selected for the experiment.‡ Two of the strains were isolated from male patients with urethritis and 3 from patients with cervicitis. The basic culture medium in which the organisms were grown was Douglas's broth with 0.05% KNO_3, 0.04% KH_2PO_4, and 5% lapine blood.

The 5 strains of the organism were grown in the above medium with gradually increasing concentrations of each agent separately. Initial concentrations used were 0.12 mg% of sodium sulfathiazole and of rivanol lactate (*i.e.*, 0.12 mg per 100 ml of medium), 32 mg% of promin, and 1.0 units % of penicillin (*i.e.*, 1 Oxford unit per 100 ml of medium). The strains were likewise subjected to the combined action of the following 3 drugs: sodium sulfathiazole, rivanol lactate, and promin, as well as to the same 3 combined with penicillin. Preliminary tests had shown that when the drugs were used in combination, the inhibitory action of each was approximately additive. The concentrations of the combined agents in which the cultures were first introduced, therefore, were approximately one-fourth that of the initial concentrations of the individual drugs, namely, 0.03 mg% rivanol lactate and sodium sulfathiazole, 8 mg% promin, and 0.25 unit % penicillin. Similarly, when 3 drugs were used, the concentration of each was one-third that initially permitting growth.

All cultures were transferred to the same concentration of drug in which they were growing and to a greater concentration every 48 hours for 4 months. Subcultures for viability were made on "chocolate" agar at the same time but on alternate days.

Results. The degree of resistance to each

Promin, Parke, Davis and Company, Detroit, Mich.; (c) Penicillin, Committee on Medical Research of the Office of Scientific Research and Development, Washington, D.C.

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4 Kirby, W. M. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 175.

5 Bahn, J. M., Ackerman, H., and Carpenter, C. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 21.

‡ Hereinafter multiple concentrations of each drug are given in numerical order of the 5 strains of the gonococcus studied, *i.e.*, No. 515, No. 3215, No. 5609, No. 5723, and No. 8071.

TABLE I.

Resistance of 5 Strains of the Gonococcus to 4 Drugs, Individually and in Combination.

Strain No.	Sodium Sulfathiazole Concentration		Rivanol lactate Conc.		Promin Conc.		Penicillin Conc.		Sodium sulfathiazole	Rivanol lactate	Promin	Penicillin
	Initial*	Maximal†	Initial	Max.	Initial	Max.	Initial	Max.	Fold increase in conc.			
	mg%‡		mg%		mg%		units %§					
Drugs individually												
515	.05	64.00	.12	8.00	32	256	0.50	100	1280	67	8	200
3215	.12	128.00	.12	8.00	32	512	1.00	100	1067	67	16	100
5609	.12	128.00	.12	8.00	32	256	0.50	200	1067	67	8	400
5723	.12	16.00	.12	16.00	32	256	0.50	100	133	133	8	200
8071	.12	2.00	.12	8.00	32	128	1.00	8	17	67	4	8
Three drugs in combination												
515	.04	.32	.04	.32	10	80	—	—			8	
3215	.04	.64	.04	.64	10	160	—	—			16	
5609	.04	.32	.04	.32	10	80	—	—			8	
5723	.04	.48	.04	.48	10	120	—	—			12	
8071	.04	.32	.04	.32	10	80	—	—			8	
Four drugs in combination												
515	.03	.03	.03	.03	8	8	.25	.25			0	
3215	.03	.03	.03	.03	8	8	.25	.25			0	
5609	.03	.03	.03	.03	8	8	.25	.25			0	
5723	.03	.03	.03	.03	8	8	.25	.25			0	
8071	.03	.03	.03	.03	8	8	.25	.25			0	

* Concentration initially permitting growth.
† Maximal concentration to which growth was adapted.
‡ Milligrams of drug per 100 ml medium.
§ Oxford units of penicillin per 100 ml medium.

drug acquired after 4 months' exposure varied greatly with the individual strains of the gonococcus. The maximal concentration of each drug tolerated by the strains, No. 515, 3215, 5609, 5723, and 8071, at the end of that period was as follows: sodium sulfathiazole 1280, 1067, 1067, 133, and 17 times greater, respectively, than those which initially permitted growth, and in rivanol lactate 67, 67, 67, 133, and 67; promin 8, 16, 8, 8, and 4, and penicillin 200, 100, 400, 200, and 8 times greater than the original concentration.

The 5 strains of the gonococcus acquired only a slight degree of fastness after 4 months' exposure to sodium sulfathiazole, rivanol lactate, and promin when used concurrently. The maximal concentrations in which these strains grew were 8, 16, 8, 12, and 8 times greater than the amounts of the mixed compounds into which the 5 parent strains were first introduced. When penicillin was added to the mixture of the same 3 drugs, none of the strains acquired resistance to the combined effects (Table I).

Discussion. The mechanism by means of which a bacterial cell becomes resistant to gradually increasing concentrations of bactericidal agents is complex. Knowledge of the metabolism of the cell and especially an understanding of its enzymic systems are necessary. The present studies serve as an indirect approach to the problem. The magnitude of increase ("fold increase") of the initial concentration of each agent permitting growth of the strain, served as the basis for comparison of acquired resistance to the drugs and undoubtedly constituted a sufficiently sensitive index. Fastness to each drug developed at different rates and to various degrees. This suggests that the mechanism by which the gonococcus becomes fast to an agent varies with the nature of the compound to which the cells were exposed. For instance, the adaptation to growth in the presence of a sulfonamide requires an altered cell metabolism probably different from that permitting growth in the presence of a sulfone, an acridine derivative, or an antibiotic agent.

Our observations indicate that promin and penicillin were more toxic for the gonococcus than sodium sulfathiazole and rivanol lactate and that the organisms could not adapt their growth readily to the presence of the former two substances. Penicillin, however, appeared to play the most important role in inhibiting the acquisition of resistance because its addition to the other compounds prevented growth of each strain tested in concentrations greater than those initially permitting growth. Since the cells were unable to survive *in vitro* in the presence of all 4 agents to which resistance had been acquired individually, a possible clinical application is indicated.

Summary. Five strains of the gonococcus were adapted to grow in gradually increased concentrations of each of the following agents: sodium sulfathiazole, rivanol lactate, promin, and penicillin. Only slight adaptation to growth was attained, however, when the strains were exposed to gradually increased concentrations of sodium sulfathiazole, rivanol lactate, and promin in combination. When penicillin was added to the above 3 drugs, none of the 5 strains developed resistance to their combined effects. Interpretation of the results and the possible clinical application of the *in vitro* observations are presented.