relative ineffectiveness of promin alone.[1] It was previously suggested that the slight retardation of the tuberculous process in guinea pigs by promin is probably due to an attenuating action on the tubercle bacillus.[10] The present experiments appear to indicate a slight bacteriostatic action by streptomycin against the tubercle bacillus *in vivo*, and a tuberculocidal action by the simultaneous application of streptomycin and promin. The mechanism of the combined action of the 2 drugs still remains to be determined.

It may be of interest to point out the relative value of the 3 tests employed to determine the presence of tubercle bacilli in the tissues. By direct smear examinations 19 or 39.7% of the 48 infected rats in the 4 groups gave positive results, by the subculture technic 29 or 60.4% were positive, and by the guinea pig subinoculation tests 36 or 75% were positive.

Summary and Conclusions. Rats inoculated with a human strain of tubercle bacilli were treated with streptomycin and promin, individually and in combination, and the effect of treatment determined by (a) direct tissue smears, (b) subculturing of lung suspensions, (c) subinoculation of lung suspensions in guinea pigs. Treatment with promin alone showed no beneficial effects, treatment with streptomycin alone resulted in an average lower colony count than in the controls, while treatment with both drugs appeared to indicate sterilization of 41.6% of the animals and marked decrease and attenuation of persisting viable tubercle bacilli in the remainder.

[10] Emmart, E. W., and Smith, M. L., Proc. Soc. Exp. Biol. and Med., 1942, **50**, 320.

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Streptomycin and Penicillin Resistant Staphylococci; Influence of pH, Body Fluids on Streptomycin Action.

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Interest in streptomycin has grown rapidly since its discovery by Schatz, Bugie, and Waksman.[1] The purpose of this report is to present experimental observations upon the development of a streptomycin- and penicillin-resistant strain of *Staphylococcus aureus*.

Materials and Methods. The *Staphylococcus aureus* used was the Smith strain.* Its viability was maintained by daily transfer in F.D.A.† broth, and it was found to be sensitive to 1.0 to 2.0 units of streptomycin per cc of medium. The same broth of pH 7.5 was employed for all sensitivity and culture studies. Streptomycin hydrochloride‡ of one lot number was used throughout the study. Streptomycin sensitivity tests were done by the serial broth dilution method, and assays by the agar cup plate method of Stebbins and Robinson.[2]

Organisms Naturally Resistant to Streptomycin. In making routine streptomycin assays by the cup plate method, it was noted that a few isolated colonies of the test organism, the Smith *Staphylococcus aureus*, grew within the clear zone of inhibition produced by the drug. These colonies, when picked and subcultured, produced organisms that were about 10 times more resistant to

[1] Schatz, A., Bugie, E., and Waksman, S. A., Proc. Soc. Exp. Biol. and Med., 1944, **55**, 66.

* Received from Merck and Co., Rahway, N. J.

† Formula: Peptone (Siccum, Armour) 10.0; Bacto Beef Extract (Difco) 5.0; NaCl 2.5; Distilled H_2O ad 1000 cc

‡ Streptomycin hydrochloride, lot No. 165, kindly supplied by Merck and Co., through the courtesy of Drs. Carlisle and Robertson.

[2] Stebbins, R. B., and Robinson, H. J., Proc. Soc. Exp. Biol. and Med., 1945, **59**, 255.

TABLE I.
Effect of Treatment with Streptomycin and Penicillin on Mice Infected with Sensitive and Resistant Strains of *Staphylococcus aureus* (Smith).

Infecting organism	Treatment	No. of mice	No. of mice surviving			
			24	36	48	72 hr
Staphylococcus aureus (Smith) Stock culture	None	5	2	1	0	0
	Penicillin	5	5	5	5	5
	Streptomycin	5	5	5	5	5
Staphylococcus aureus (Smith) Streptomycin Resistant	None	5	4	2	1	1
	Penicillin	5	5	5	5	5
	Streptomycin	5	5	5	0	0

streptomycin than the original stock culture of the Smith strain microorganisms.

Production of Streptomycin-Resistant Organisms. Streptomycin-resistant organisms could be produced at will by growing the sensitive Smith strain in serial transfers in broth containing increasing amounts of the drug. After 17 such passages, over a period of 30 days, such organisms grew well in broth containing 2500 units of streptomycin per cc.

Penicillin Sensitivity Tests. Titrations in broth were then made to note the sensitivity of the stock Smith organisms to penicillin. They were found to be sensitive to 0.1 to 0.2 units of penicillin per cc. The streptomycin-resistant organisms were equally sensitive to penicillin.

In Vivo Treatment with Penicillin and Streptomycin. A mouse experiment was done to determine the effect of treatment with both streptomycin and penicillin upon infections produced by the streptomycin-sensitive and streptomycin-resistant strains of the Smith *Staphylococcus aureus*. The mice were infected intraperitoneally with 1 cc of a 10-hour broth culture of each of these 2 strains, diluted 5 times in 5% gastric mucin.[3,4] One-third of each group of 15 were then treated with penicillin (250 units I.P. at the time of inoculation, repeated once in 4½ hours), another one-third with streptomycin (500 units I.P. at time of inoculation, repeated once in 4½ hours), and the remainder served as untreated controls.

From Table I it will be observed that an infection produced by a lethal dose of organisms made resistant to streptomycin can be favorably influenced by treatment with penicillin, while relatively large amounts of streptomycin remain quite ineffective.

Miller and Bohnhoff[5] recently published data for the gonococcus and meningococcus demonstrating the same relationships between streptomycin- and penicillin-resistant organisms of these species that we have just described for the staphylococcus.

Production of Penicillin-Resistant Organisms. It proved to be easier to adapt the *Staphylococcus aureus* to increasing concentrations of streptomycin than to penicillin. It took 39 passages over 45 days to make the organisms grow in broth containing 500 units of penicillin per cc and only 15 passages to have a similar effect with streptomycin. No difference was noted in the rate of adaptability to penicillin between the stock culture and the streptomycin-resistant strain.

The organisms made resistant to 2500 units of streptomycin retained their ability to grow well in at least 1000 units per cc of medium after 4 months in the refrigerator, after several rapid mouse passages, and after adapting the organisms to penicillin.

Using the agar plate method of Gots,[6] no substance capable of destroying streptomycin ("streptomycinase") could be demonstrated either in organisms made resistant to 2500 units of the drug, or in the naturally resistant organisms picked from a colony growing with-

[3] Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Infect. Dis.*, 1936, **59**, 11.

[4] Ercoli, N., Lewis, M. N., and Harker, E., Proc. Soc. Exp. Biol. and Med., 1945, **59**, 273.

[5] Miller, C. P., and Bohnhoff, M., *J. A. M. A.*, 1946, **130**, 485.

[6] Gots, J. S., *Science*, 1945, **102**, 309.

TABLE II.
Concentrations of Streptomycin Necessary for Complete and Partial Inhibition of Growth at Different pH Levels. Incubation 24 hr.

	pH	Concentration of streptomycin in units per cc for	
		Complete inhibition	Partial inhibition
Staphylococcus aureus (Smith)	7.7	1.6	0.8
	7.2	6.25	1.6
	6.6	6.25	3.2
	5.9	50.00	25.0
	5.2	100.00	40.0

Inoculum 0.1 cc 12-hr broth culture diluted 10 times.

in the clear zone of a streptomycin assay plate.

Effect of pH on the Action of Streptomycin. It has been demonstrated that the action of streptomycin varies markedly with change in pH.[7,8] Tubes of F.D.A. broth were adjusted to the following pH's: 5.2, 5.9, 6.6, 7.2 and 7.7, and to these was added enough streptomycin to give concentrations of 0.4 to 200 units per cc. Each tube was then inoculated with 0.1 cc of a 12-hour broth culture of staphylococcus, diluted 10 times.

Table II shows that the inhibitory efficiency of streptomycin in broth diminishes with increasing acidity.

To determine whether the drug is actually destroyed in acid solution, tubes of broth of a pH of 3.5, 6.4 and 7.2 were prepared to contain 10 units of streptomycin per cc. After standing for 2½ hours in the incubator, the tubes were quickly adjusted to pH 7.8 with N/1 NaOH, and streptomycin assays were made. All were found to contain about 10 units per cc, indicating that there had been no destruction of the drug.

Effect of Body Fluids, Pus, and Tissue Juice on Streptomycin. Four groups of broth tubes were set up. To the first group was added 6% by volume of purulent pleural fluid from an uncontaminated tuberculous empyema; to the second, the same volume of a sterile non-purulent tuberculous empyema fluid; to the third, the same volume of an opalescent sterile ascitic fluid; and the fourth group constituted the broth controls. Enough streptomycin solution was then added so that each group contained concentrations of the drug graded from 0.8 to 10 units per cc. The total volume in each tube was 5 cc. All of them were then inoculated with 0.1 cc of a 12-hour broth culture of staphylococcus, diluted 10 times. After 24 and 48 hours incubation at 37°C, each tube was examined for evidence of growth by visual turbidity, and one loopful of broth from each was streaked on an agar plate. The reaction in each group of tubes was checked after incubation, and the pH found to be between 7.2 and 7.5 in all. The results after 48 hours incubation are given in Table III.

It can be seen from the table that 7.5 units of streptomycin per cc killed enough organisms in all 4 groups of tubes to prevent growth on subculture of a loopful of broth, and that 2 to 3 units per cc was sufficient to inhibit growth in the presence of all these exudates. Pus and body fluids, therefore, do not interfere appreciably with the bacteriostatic or bactericidal action of streptomycin in broth.

In another experiment, a streptomycin solution of 100 units per cc was diluted 10 times in tubes of ascitic fluid, purulent pleural fluid, guinea pig serum, tissue juice from livers and spleens of normal guinea pigs, and a saline control. These mixtures were then incubated at 37°C for 24 to 72 hours and then tested for streptomycin activity. When compared with the saline control, there was no significant decrease in activity in any of the above material.

Summary and Conclusions. 1. Certain organisms of the Smith strain *Staphylococcus*

[7] Waksman, S. A., Bugie, E., and Schatz, A., *Proc. Staff Meet. Mayo Clin.*, 1944, **19**, 537.

[8] Loo, Y. H., Skell, P. S., Thornberry, H. H., Ehrlich, J., McGuire, J. M., Savage, G. M., and Sylvester, J. C., *J. Bact.*, 1945, **50**, 701.

TABLE III.
Effect of Body Fluids and Pus on the Action of Streptomycin *in Vitro*. Incubation 48 hr.

Medium	Growth by visual turbidity. Units of streptomycin per cc							Growth by subculture.‡ Units of streptomycin per cc					
	1	2	3	4	5	7.5	10	2	3	4	5	7.5	10
Broth (control)	G*	0	0	0	0	0	0	+†	+	+	—	0	0
Broth + purulent pleural fluid	G	0	0	0	0	0	0	+	—	+	—	0	0
Broth + non-purulent pleural fluid	G	G	0	0	0	0	0	—	+	+	—	0	0
Broth + ascitic fluid	G	G	0	0	0	0	0	+	—	+	—	0	0

* G indicates visual turbidity.
† + indicates growth on subculture; 0 indicates no growth; — indicates tube not subcultured.
‡ One double loopful streaked on surface of agar plate.
Inoculum: 0.1 cc 1:10 dilution of 12-hour broth culture of *Staphylococcus aureus* Smith.

aureus possess a natural increased resistance to streptomycin. This resistance is not based on the production of a "streptomycinase." 2. A strain of staphylococcus, highly resistant to streptomycin, can be produced *in vitro* from the streptomycin-sensitive organisms. 3. Streptomycin resistance is a relatively permanent characteristic, and is not affected by several passages through mice. The resistant organisms retain their virulence for this host. 4. Streptomycin resistance and penicillin resistance are independent of each other. Staphylococci originally penicillin-sensitive, retain their sensitivity to penicillin after being made resistant to streptomycin, and vice versa. Treatment with relatively large amounts of streptomycin is ineffective in mice infected with a streptomycin-resistant strain of *Staphylococcus aureus*. These mice can be cured, however, with penicillin. 5. The bacteriostatic effect of streptomycin diminishes considerably as the acidity of the culture medium increases from pH 7.7 to 5.2; the greatest diminution in effect occurs between pH 6.6 and 5.9. This must be taken into consideration in making *in vitro* assays and sensitivity tests, as well as in evaluating the action of the drug *in vivo*. 6. Streptomycin is not destroyed after contact with broth of pH 3.5 for 2½ hours. 7. Streptomycin is not destroyed, nor are its bacteriostatic and bactericidal powers appreciably influenced, by serous body fluids, pus, or normal tissue juices.

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Electrophoretic and Allergenic Analyses of Fractions of Larvae of *Trichinella spiralis*.

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Fractions of larvae of *Trichinella spiralis* have been employed as diagnostic aids in detecting *Trichinella* infection in man. Bachman[1] used an acid-hydrolyzed, saline-soluble extract of *Trichinella* larvae to study intradermal responses in sensitized rabbits. Bozicevich[2] prepared an antigen for precipitin tests from the supernatant fluid of a 5% saline extract heated at 58°C for one hour to precipitate inert material; the same material, diluted and sterilized by intermittent

[1] Bachman, G. W., *J. Prev. Med.*, 1923, **2**, 169.

[2] Bozicevich, J., *Pub. Health Rep.*, 1938, **53**, 2130.