the tubercle bacillus in a medium containing low concentrations of promin resulted in attenuation of virulence.[11] It is possible therefore that the beneficial effect of the combined action of the 2 chemotherapeutic agents consists in eliminating or attenuating strains which might acquire a resistance to the antibiotic.

Summary and Conclusions. The chemotherapeutic action of streptomycin used alone or in combination with one of two sulfones or sulfadiazine was studied in experimental tuberculosis in guinea pigs.

Evidence has been obtained to indicate that the therapeutic effect from combined treatment is greater than the sum of effects from the individual components.

The chemotherapeutic effectiveness (ratio of extent of tuberculous involvement in a group of untreated controls and treated groups) in a group of guinea pigs treated with 40 mg (40,000 units) streptomycin per kg of body weight daily was 14.4. Previously treatment with 10 to 15 mg per kg per day under similar conditions gave a chemotherapeutic effectiveness of 5.2.

The chemotherapeutic effectiveness of combined therapy with 20 mg streptomycin per kg per day plus 500 mg 4-amino-4′-propylaminodiphenylsulfone per kg per day was 28.6. The chemotherapeutic effectiveness of the sulfone alone was 3.7.

Similar though less marked potentiation was obtained in combined therapy with streptomycin and 4-amino-4′-succinimidodiphenylsulfone. By itself this latter sulfone was less effective than the *n*-propyl derivative.

Combined therapy with streptomycin and sulfadiazine, a substance of doubtful efficacy in experimental tuberculosis, gave inconclusive evidence of potentiation.

11 Emmart, E. W., and Smith, M. I., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 320.

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Further Studies on the Testing of Sterility of Concentrated Streptomycin Solutions.

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The inactivation of streptomycin with semicarbazide, as an aid in testing the sterility of concentrated solutions of streptomycin, was proposed in an earlier paper.[1] A similar method of sterility testing involving the use of hydroxylamine has been described in tentative minimum specifications for streptomycin issued by the Food and Drug Administration.[2] As is shown in Table I, semicarbazide, as compared to hydroxylamine mol for mol, has the advantage for such sterility test procedures in that the bacteriostatic action of the former is less than that of the latter for most organisms tested.* If instead of thioglycolate broth, which was used in the present tests, tryptone broth is used, the differences in the bacteriostatic action of the two compounds are even more marked in favor of semicarbazide.[1] The same is true of effects on bacteria in aqueous streptomycin solutions in which the antibacterial power of the carbonyl reagent would be exerted in the suggested sterility tests.

However, recent experiences lead to the

1 Rake, G., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 31.

2 *Tentative Minimum Specifications for Streptomycin,* Food and Drug Admin., June 28, 1946.

* Amongst the organisms tested were a number of strains kindly made available to us by Dr. W. A. Randall of the Food and Drug Administration and said to be less susceptible to hydroxylamine than to semicarbazide.

TABLE I.
Comparative Bacteriostatic Action of Semicarbazide-HCl and Hydroxylamine-HCl Against Various Test Organisms in Thioglycolate Broth.

Test organism	M.I.C.* Semicarbazide HCl mg/ml	Semicarbazide HCl millimols/L	Hydroxylamine HCl mg/ml	Hydroxylamine HCl millimols/L	Mol ratio SC/HA
E. coli SR†	0.60	5.4	0.30	4.3	1.25
K. pneumoniae (A.T.C.C. No. 9997)	0.21	1.9	0.17	2.4	0.79
No. 2411‡	0.15	1.3	0.044	0.63	2.06
No. 2435‡	0.14	1.3	0.039	0.56	2.32
B. brevis§	0.71	6.4	0.18	2.6	2.46
B. circularis§	0.79	7.1	0.20	2.9	2.44
B. subtilis (Peoria)§	0.76	6.8	0.23	3.3	2.06
Pseudomonas aeruginosa§	0.30	2.7	0.17	2.4	1.12
Aerobacter aerogenes§	0.37	3.3	0.25	3.6	0.92
B. subtilis (Merck)§	0.35	3.1	0.13	1.9	1.63
Staphylococcus aureus (Osgood)§	1.16	10.4	0.14	2.0	5.20

* Minimum inhibiting concentration.

† Strain of *E. coli* isolated from solution containing *ca.* 100,000 units streptomycin/ml.

‡ Psychrophilic organisms isolated from solution containing *ca.* 100,000 units streptomycin/ml.

§ These strains of bacteria were obtained from the Food and Drug Administration through the kindness of Dr. William A. Randall.

conclusion that the use of either carbonyl reagent can result in false negative results as to the presence of contaminants in concentrated streptomycin solutions. These findings were anticipated somewhat in the earlier paper[1] when it was pointed out that, since carbonyl reagents *per se* are bacteriostatic substances, the procedure suggested (for inactivation of and sterility testing of streptomycin) was valid only to the extent that viable organisms in streptomycin solutions were able to withstand the action of carbonyl reagents.

In recent months it has been noted that certain solutions of streptomycin, containing *ca.* 100,000 units per ml, on standing at room temperature or at 5°C, became turbid with what appeared to be bacterial growth. While sterility tests according to the procedure described[1] failed to show any growth, simple streaks of these original solutions on yeast beef agar plates yielded one, and in some cases 2, strains of bacteria. One of these was a strain of *Escherichia coli* and 2 others (No. 2411 and No. 2435, possibly identical, although isolated from separate sources) were Gram negative, psychrophilic rods which grew readily at 5° to 25°C but poorly at 37°C.

Pure cultures of these 3 organisms were inoculated into streptomycin solutions containing *ca.* 30,000 units per ml. The inoculated solutions were divided into several portions, some of which were treated with semicarbazide and potassium acetate as described earlier,[1] while the remainder served as controls. Although the organisms could be readily recovered from the untreated streptomycin solutions, they could not be reisolated from the solutions treated with semicarbazide. Similarly failure resulted from subsequent tests with equivalent amounts of hydroxylamine as the inactivating agent, and, as shown in Table I, all 3 organisms were more sensitive to hydroxylamine than to semicarbazide.

Thus, whereas the use of carbonyl reagents had aided in the demonstration of the presence in streptomycin solutions of certain spore formers,[1] they now played a detrimental role. There still remains a need for a non-germicidal agent which will inactivate streptomycin. Van Dolah and Christenson[3] have recently suggested the use of certain oxidizing and reducing agents, such as potassium permanganate and potassium periodate, for the inactivation of streptomycin. However, here again it is likely that, in higher concentrations required for a sterility test, some bacterial destruction would be caused by the reagent before the reaction had gone to completion and before the streptomycin-reagent mixture could be added to test culture media.

[3] Van Dolah, R. W., and Christenson, G. L., *Arch. Biochem.*, 1947, **12**, 7.

Further consideration apparently should also be given to the choice of culture medium and the temperature of incubation in sterility testing of streptomycin solutions. Broth containing sodium thioglycolate, because of the ability of the latter to interfere with the action of many antibacterial substances,[4] is widely used for sterility testing of biological materials. The extent to which this broth interferes with the action of streptomycin[5] led to its use in a proposed sterility test.[1] In attempts, however, to isolate the streptomycin-resistant *E. coli* strain, referred to above, from inactivated mixtures of streptomycin and carbonyl reagents, it was found that control cultures died out in one week at 37°C. On further examination it appeared that this organism could be carried in thioglycolate broth if daily subcultures were made, but if transfers were attempted after one week's incubation no growth ensued. It was found that this organism could be recovered from this broth through 6 days of incubation but not after the 7th day. Controls in yeast beef broth yielded positive subcultures throughout at least 8 days.

In the sterility testing of many biological materials it is not an uncommon practice to inoculate the material under question into thioglycolate broth, incubate for one week, and then subculture if there is any doubt whether growth has occurred. Should this be done with a material containing a contaminant such as the *E. coli* strain used here, the subcultures would yield no growth. In this connection we have found that the 2 psychrophils mentioned above do not grow well in thioglycolate broth, but multiply readily in yeast beef broth.

The occurrence of 2 psychrophilic organisms as contaminants in several highly concentrated streptomycin preparations which we have studied demonstrates also the need for incubating streptomycin sterility tests at more than one temperature. We have not yet found any thermophilic organisms occurring as natural contaminants in streptomycin solutions, but this possibility should not be overlooked.

Summary. 1. Further studies with semicarbazide and hydroxylamine confirm earlier findings that for most of the bacterial species tested the former substance mol for mol is less bacteriostatic than the latter. 2. The use of either carbonyl reagent in sterility tests of concentrated streptomycin solutions, however, may lead to false negative findings. 3. Three strains of microörganisms have been isolated from streptomycin solutions which failed to grow out from similar material inactivated with either carbonyl reagent. 4. These organisms isolated from the streptomycin solutions grow poorly in thioglycolate broth, and one species was found to die out in this broth on incubation prolonged beyond 6 days. 5. The demonstration of psychrophilic organisms in streptomycin solutions suggests the need for incubating such sterility tests at more than one temperature.

[4] a. Eagle, H. J., *Pharmacol.*, 1939, **66**, 436; b. Fildes, P., *Brit. J. Exp. Path.*, 1940, **21**, 67; c. Chow, B. F., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 175; d. Cavalito, C. J., Bailey, J. H., Haskell, T. H., McCormick, J. R., and Warner, W. F., *J. Bact.*, 1945, **50**, 61; e. Geiger, W. B., and Conn, J. E., *J. Am. Chem. Soc.*, 1946, **67**, 112.

[5] Donovick, R., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 224.