streptomycin per ml after 6 hours incubation were more resistant to its action. This resistance persisted through 4 subcultures in the absence of streptomycin and could be increased 5-fold by serial transfers in broth containing streptomycin. Washed organisms surviving after 5 hours incubation with 7.5 units of streptomycin per ml were not more resistant.

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A Procedure for Testing Sterility of Concentrated Streptomycin Solutions.

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Since the use of streptomycin as a therapeutic agent is rapidly increasing it is important to be able to test the sterility of highly concentrated solutions of this antibiotic. In such sterility tests it is, of course, a prerequisite that the sample of streptomycin taken be of sufficient volume to be representative of the lot of streptomycin involved. As the bacteriostatic levels of streptomycin, at least in the case of many organisms, differ greatly from bactericidal concentrations[1] the addition of such an adequate sample of streptomycin to the sterility test broth may result in inhibition of growth of any viable cells which may be present. Hence there is need for a method of inactivating streptomycin without destroying any living organisms present.

In order to test the sterility of streptomycin solutions in this laboratory, a procedure has been devised which incorporates the inactivation of streptomycin with semicarbazide[2,4] and the use, as the culture medium, of thioglycolate broth which interferes with streptomycin activity.[3]

Of the carbonyl reagents which inactivate streptomycin, semicarbazide was chosen because it is one of the least bacteriostatic members of this group (Table I) and is readily soluble in water. Thiosemicarbazide is also relatively low in bacteriostatic activity but is much less soluble.

TABLE I.
Comparative Sensitivity of Various Organisms to Streptomycin and to Certain Carbonyl Reagents.

Organism	Growth inhibiting concentration in 1% tryptone broth					
	Streptomycin mg/ml	HA-HCl mg/ml	HZ-H_2O mg/ml	SC-HCl mg/ml	TSC mg/ml	Ratio SC-HCl/streptomycin
K. pneumoniae (A.T.C.C. No. 9997)	0.000055*	0.0088	0.0068	0.073	0.116	1325
E. coli No. 33	0.00012*	0.016	0.010	0.064	0.088	533
Staph. aureus (Heatley)	0.000049*	0.018	0.017	>0.54	0.017	>11000
B. subtilis No. 558	0.000056*	0.012	0.012	0.48	0.23	8570
Bacillus sp. No. 290	0.000013*	0.010	0.0073	0.089	0.15	6840

* Based on pure streptomycin base as having 1000 units per mg.
HA-HCl = hydroxylamine hydrochloride.
HZ-H_2O = hydrazine hydrate.
SC-HCl = semicarbazide hydrochloride.
TSC = thiosemicarbazide.

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

[4] Donovick, R., Rake, G., and Fried, J., *J. Biol. Chem.*, in press.

Method. Materials required for inactivation of streptomycin solution containing 30,000 units per ml ± 10%:

1. Semicarbazide-hydrochloride solution: ca. 0.06 g per ml. Sterilization through U. F. fritted glass filter is the preferred method. The sterile solution is stored at 4°C.

2. Potassium acetate solution: ca. 0.05 g per ml. Solution is sterilized by autoclaving, 10 lb pressure for 10 minutes.

Procedure for inactivation of streptomycin. To each ml of streptomycin solution to be inactivated is added 1 ml of the potassium acetate solution and then 1 ml of semicarbazide-hydrochloride solution, observing aseptic precautions. The mixture is incubated at ca. 25°C (room temperature) for 24 hours.

After room temperature incubation, the inactivation mixture is tested for sterility in freshly prepared thioglycolate broth, using at least 30 ml of broth for each ml of inactivation mixture to be tested. The inoculated broth is incubated at 37°C for at least 24 hours.

Discussion. It has been shown that 2 γ of semicarbazide hydrochloride per γ (or unit) of streptomycin will cause 98% inactivation at pH 5.3.[1] Since the semicarbazide-hydrochloride solution is extremely low in pH, potassium acetate is first added to the streptomycin solution to bring the final mixture to the desired pH.

To justify the use of this method of inactivation of streptomycin as a step prior to sterility testing it must be shown that any organism resistant to 30,000 units of streptomycin per ml is also resistant to the concentration of semicarbazide present in the inactivation mixture. This question has been approached in 2 ways: Thus far we have found no vegetative cells resistant to such concentrations of streptomycin but have compared the sensitivities of a number of species to this antibiotic with their sensitivities to several carbonyl reagents at much lower concentrations of both substances. The results of such studies with 5 species are given in Table I. The second approach to this problem is based on the finding that spores of some species of bacteria are extremely re-

TABLE II. Recovery of *Bacillus sp.* No. 290 from Streptomycin Inactivated by Semicarbazide.

Tube No.	Mixture incubated at 25°C, 24hr					Mixture diluted in thioglycolate broth, incubated at 37°C, 48 hr					
						Growth after dilution in broth					
	Spore suspension* ml	Streptomycin† ml	Semicarbazide-hydrochloride‡ ml	Potassium acetate§ ml	H_2O	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1	0.3	0.3	0.3 (undil.)	0.3	—	—	+	+	+	+	—
2	0.3	0.3	0.3 (½)	0.3	—	—	+	+	+	+	—
3	0.3	0.3	0.3 (⅓)	0.3	—	—	+	+	+	+	—
4	—	0.3	0.3 (undil.)	0.3	0.3	—	—	—	—	—	—
5	0.3	—	0.3 "	0.3	0.3	—	+	+	+	+	—
6	0.3	0.3	—	—	0.6	—	+	—	—	—	—
7	0.3	—	—	0.3	0.6	—	—	—	—	—	—
8	—	0.3	—	—	0.9	—	—	—	—	—	—
9	—	—	0.3 "	—	0.9	—	—	—	—	—	—
10	—	—	—	0.3	0.9	—	+	—	—	—	—
11	0.3	—	—	—	0.9	+	—	—	—	—	—
12	—	—	—	—	1.2	—	—	—	—	—	—

* Spore suspension contained ca. 1,000,000 spores per ml.
† Streptomycin solution = 28,700 units per ml = 0.0287 g per ml.
‡ Semicarbazide-hydrochloride solution = 0.0862 g per ml in undiluted solution.
§ 5% potassium acetate solution.
+ = growth; — = no growth.

sistant to the germicidal action of streptomycin even though the growth of their vegetative forms is inhibited by very low concentrations of streptomycin in broth.[1] The spores of such an organism (*Bacillus sp.* No. 290) were added to a solution containing 28,700 units of streptomycin per ml and the streptomycin solution then was inactivated in the manner described. The results of a typical experiment are tabulated in Table II.

It will be seen in Table I that the growth-inhibiting concentration of semicarbazide ranges from 533 to >11,000 times greater than the inhibiting concentrations of streptomycin for the organisms tested.

Ability of spores of Bacillus sp. No. 290 to resist the action of semicarbazide during the inactivation of streptomycin. Various mixtures of spores, streptomycin, semicarbazide-hydrochloride and potassium acetate were prepared as shown in Table II and incubated at 25°C for 24 hours. Each mixture was then diluted by 10-fold steps in freshly prepared thioglycolate broth, the broth dilutions were incubated at 37°C, and read for growth after 24, 48 and 96 hours incubation. Little change occurred after 48 hours incubation; therefore only the 48-hour readings are recorded in the table.

It is shown in Table II that the streptomycin-spore suspension mixture gave no growth until diluted 10^{-4} in thioglycolate broth, whereas growth occurred at 10^{-2} in mixtures containing 3, 2 and 1 γ of semicarbazide hydrochloride per γ of streptomycin. When spores were added to semicarbazide-hydrochloride and diluted in thioglycolate broth, growth again occurred in the 10^{-2} dilution. Hence under the conditions of the experiment presented, streptomycin, containing viable cells, when treated with the specified carbonyl reagent yielded growth at 1/100th the dilution required to obtain growth from the original streptomycin-spore suspension mixture.

Summary. 1. Details are given for a method of inactivating streptomycin with semicarbazide-hydrochloride in order to test the sterility of concentrated streptomycin solutions. It has been shown that although several carbonyl reagents inhibit bacterial growth, it requires from 533 to >11,000 times more semicarbazide-hydrochloride (one of the least toxic of the group) to cause this inhibition than is required of streptomycin.

2. Spores of *Bacillus sp.* No. 290, when added to a streptomycin solution containing 28,700 units per ml, were able to grow out when diluted 10^{-4} in thioglycolate broth. When a similar spore suspension-streptomycin mixture was treated with semicarbazide-hydrochloride (3, 2 or 1 γ carbonyl reagent per unit (or γ) of streptomycin) and then diluted in thioglycolate broth, growth occurred at a 10^{-2} dilution.

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Buffers in the Range of pH 6.5 to 9.6.*

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In the pH range between 6.5 and 9.6, the buffers generally used have been phosphate, barbital,[1] ammonium salts and carbonate.[2] Among these, phosphate and carbonate are incompatible with Ca salts; ammonium salt buffers are not entirely stable; barbital, on account of its low solubility, can be prepared in low concentrations only and, in addition, inhibits certain enzyme systems.[3] Mertz and Owen[4] have suggested the use of imidazole as a buffer in the physiologic pH range, com-

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