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.

15361

Buffers in the Range of pH 6.5 to 9.6.*

GEORGE GOMORI.
From the Department of Medicine, The University of Chicago.

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-

* This work has been done under a grant from the Douglas Smith Foundation for Medical Research at the University of Chicago.

1 Michaelis, L., *J. Biol. Chem.*, 1930, **87**, 33.

2 Delory, G. E., and King, E. J., *Bioch. J.*, 1945, **39**, 245.

3 Quastel, J. H., and Wheatley, A. H. M., *Proc. Roy. Soc. B.*, 1932, **112**, 60; *Bioch. J.*, 1934, **28**, 1251.

TABLE I.
pH Values of Buffers at 23°C and 37°C.

0.1 N HCl cc	Collidine		Tris(hydroxymethyl)-aminomethane		2-amino-2-methyl-1,3-propanediol	
	23°C	37°C	23°C	37°C	23°C	37°C
*5.0	8.35	8.28	9.10	8.95	9.72	9.62
7.5	8.18	8.10	8.92	8.78	9.56	9.45
10.0	8.00	7.94	8.74	8.60	9.38	9.27
12.5	7.88	7.80	8.62	8.48	9.26	9.15
*15.0	7.77	7.70	8.50	8.37	9.15	9.03
17.5	7.67	7.60	8.40	8.27	9.05	8.94
20.0	7.57	7.50	8.32	8.18	8.96	8.85
22.5	7.50	7.40	8.23	8.10	8.87	8.76
*25.0	7.40	7.32	8.14	8.00	8.78	8.67
27.5	7.30	7.23	8.05	7.90	8.70	8.58
30.0	7.22	7.14	7.96	7.82	8.60	8.50
*32.5	7.13	7.05	7.87	7.73	8.50	8.40
35.0	7.03	6.95	7.77	7.63	8.40	8.30
*37.5	6.92	6.84	7.66	7.52	8.30	8.20
40.0	6.80	6.72	7.54	7.40	8.18	8.07
42.5	6.62	6.54	7.36	7.22	8.00	7.90
*45.0	6.45	6.37	7.20	7.05	7.83	7.72

patible with Ca; however, its high cost is almost prohibitive.

Three new buffers: 2,4,6-collidine, tris(hydroxymethyl)-aminomethane and 2-amino-2-methyl-1,3-propanediol, are suggested for the use in the pH range between 6.5 and 9.6. They are quite soluble, do not precipitate Ca salts, and are low in price. They were found to be stable at room temperature for a period of over 3 months. Collidine and Tris(hydroxymethyl)-aminomethane, to be used in the pH ranges between 6.5 and 8.3, and between 7.2 and 9.0, respectively, were tested by Dr. E. S. Guzmán Barrón for their effect on the O_2 uptake of rat kidney slices in the presence of 0.01 M pyruvate. The concentration of the buffers was 0.02 M, phosphate buffer being used as a control. The results with the different buffers were all well within the limits of experimental error, thus showing complete lack of inhibitory action. Tris(hydroxymethyl) - aminomethane and 2-amino-2-methyl-1,3-propanediol (range, pH 8.0 to 9.7) were tested for their effect on alkaline phosphatase at pH 9.1, 0.005 M glycerophosphate being used as a substrate. Barbital and Delory and King's[2] carbonate buffers served as controls. Again, no inhibitory effect was noted.

The pK_b values of the new buffer substances were determined by the electrometric determination of the pH of their half-neutralized 0.05 M solutions at 23°C and 37°C. The apparatus used was a Leeds and Northrup potentiometer with glass and calomel electrodes. Phthalate buffer served as a standard.

1. 2,4,6-collidine (s-collidine).† Colorless liquid; pH 7.4 at 23°C; 7.32 at 37°C, pK_b 6.6 and 6.68, respectively.

2. Tris(hydroxymethyl) - aminomethane.‡ Colorless crystals; pH 8.14 at 23°C; 8.00 at 37°C; pK_b 5.76 and 6.0, respectively.

3. 2-amino-2-methyl-1,3-propanediol.‡ Colorless, somewhat hygroscopic crystals; pH 8.78 at 23°C; 8.67 at 37°C; pK_b 5.22 and 5.33, respectively.

The pH values of 0.05 M buffer mixtures, obtained by mixing 25 cc of a 0.2 M solution of the bases (collidine, 2.64 cc in 100 cc; tris(hydroxymethyl)-aminomethane, 2.43 g in 100 cc; 2-amino-2-methyl-1,3-propanediol, 2.1 g in 100 cc) with varying volumes of 0.1 N HCl and diluting the mixtures to the final volume of 100 cc, are given in Table I. The values marked with an asterisk were determined by potentiometric measurement, all the other ones were interpolated by calculation. The effect of salts on these values was not determined.

[4] Mertz, E. T., and Owen, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 204.

† Obtainable from the Eastman Kodak Co., Rochester, N. Y.

‡ Obtainable from the Commercial Solvents Corporation, 17 East 42nd Street, New York.