THE STABILITY OF STREPTOMYCIN*

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The biological inactivation of streptomycin in acid and alkaline solution was first noted by Waksman and his coworkers (1). In a recent review, Waksman and Schatz (2) reported that solutions of crude streptomycin maintained their original potency during 15 to 17 days at 37°, but at 100° about 50 per cent of the activity was lost in 10 minutes. However, the pH of the solutions was not indicated. Other investigators concerned with degradation studies have described the products obtained by acid and alkaline hydrolysis of streptomycin. Folkers et al. (3–5) and Carter et al. (6) have shown that, on acid hydrolysis, streptomycin is cleaved into two basic fractions, streptidine, 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane (7, 8), and streptobiosamine. On acid hydrolysis the latter compound yields N-methyl-L-glucosamine (5), and an alkaline hydrolysis of streptidine yields streptamine, 1,3-diamino-2,4,5,6-tetrahydroxycyclohexane (7, 8).

The rapid inactivation of streptomycin in N sodium hydroxide was indicated by Carter et al. (9). When the hydrolysis of streptomycin hydrochloride was carried out in N sodium hydroxide at 100° or for a longer period at 40°, a substance was isolated and characterized as maltol (10).

The present investigations were carried out to determine the effect of pH and temperature on commercial and pure streptomycin sulfate. The ranges covered in these experiments are summarized in Table I. Although streptomycin in aqueous solution is sensitive to acids, bases, and heat, it has been found that its optimum stability lies between pH 3 and 7, at temperatures at or below 28°.

EXPERIMENTAL

Materials and Method

Preparation of Pure Streptomycin Sulfate—Commercial streptomycin hydrochloride, 500 γ per mg., was dissolved in methanol and treated with an excess of a methanol solution of calcium chloride. After evaporation in vacuo, the streptomycin hydrochloride-calcium chloride double salt was allowed to crystallize at room temperature. The crude double salt was

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filtered and recrystallized from methanol. After drying it for 48 hours at 25° and 30 μ, 9.9 per cent methoxyl and 1.8 per cent water were found by the Karl Fischer reagent method. However, the double salt was converted to streptomycin sulfate and the aqueous solution was concentrated in vacuo to a low volume; it was lyophilized and further dried at 26° and 50 μ over barium oxide for 2 days; activity 860 γ per mg. (*Bacillus subtilis* plate assay); [α]D^25 = −79° (c, 1 in water).

Analysis—(C₂₅H₃₀N₁₂O₁₄)₂·(H₂SO₄)₂
Calculated. C 34.61, H 5.81, N 13.46, SO₄ 19.78
Found. " " 34.34, " " 6.02, " 13.28, " 19.50

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tr>
<td>Half Life of Pure Streptomycin under Conditions of pH and Temperature (t in Hours)</td>
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<table>
<thead>
<tr>
<th>pH</th>
<th>7°</th>
<th>28°</th>
<th>50°</th>
<th>95°</th>
</tr>
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<tbody>
<tr>
<td>0.8</td>
<td>1200</td>
<td>110</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>Stable</td>
<td>1500</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td>&quot;</td>
<td>Stable</td>
<td>900</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4600</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>&quot;</td>
<td>&quot;</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>8.6</td>
<td>&quot;</td>
<td>1100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>9.5</td>
<td>3000</td>
<td>300</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>11.2</td>
<td></td>
<td>16</td>
<td></td>
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Streptomycin sulfate (1.144 gm.) was dissolved in 75 ml. of water and cooled to 5°. The solution was treated with the calculated amount of 0.420 N solution of barium hydroxide and immediately titrated with 0.1067 N sulfuric acid at 5°, with a glass electrode. All the readings were corrected for temperature. Approximate pK values were obtained from the mid-points of titration (Fig. 1) which occur at pH 4.5 and 9.5. 3 equivalents of sulfuric acid were required for complete neutralization of the streptomycin.

Two series of observations were made with use of (a) this pure streptomycin sulfate and (b) commercial clinical streptomycin sulfate (Pfizer) (400 γ per mg.). Weighed quantities of both purified and partly purified materials were dissolved in solutions of the desired pH to give concentrations of approximately 1000 γ per ml. Streptomycin solutions of both series were stored in 100 ml. ground glass-stoppered volumetric flasks. A small quantity of toluene was added to each flask to prevent the growth

*This assay was obtained by comparison with a standard related to the original Waksman standard.*
of microorganisms. The solutions were then raised to the desired temperature and a zero time sample (in duplicate) was withdrawn with a calibrated pipette. The samples for bioassay were transferred directly into phosphate buffers at pH 6.5 to retard inactivation of streptomycin.

When no buffer is used in solutions of sodium hydroxide at pH 8 to 10, the alakali is consumed and the streptomycin loses potency at a decreasing rate with a corresponding drop in pH. In this work, streptomycin sulfate was dissolved in phosphate buffers in regions at and above pH 8. All the solutions were checked periodically with a Cambridge pH meter.

The lowest temperature at which measurements were made was afforded by a refrigerator at 7° ± 0.3°. A constant temperature bath was used for the 28° and 50° observations and a well insulated electric oven was employed for the temperature at 95° ± 0.2°.

Assays—The plate assays were carried out essentially by the method of Schmidt and Moyer (11) which was used for penicillin, but with the following exceptions: (a) The medium used was Difco Bacto-streptomycin assay agar, originally employed by Skell (unpublished) for the assay.
of streptothricin; (b) *Bacillus subtilis*, American Type Culture Collection No. 9524, was used as the test organism; (c) a 1 per cent phosphate buffer at pH 7.9 to 8.0 was used.

Turbidimetric values were obtained by a modification of the method of McMahan (12) for penicillin assays. The test culture employed was a strain of *Escherichia coli* supplied by Dr. S. A. Waksman.

The *Bacillus subtilis* cylinder plate method gave more precise assays with less deviation than the *Escherichia coli* turbidimetric method throughout the whole course of these studies; consequently only *Bacillus subtilis* assay values were used to calculate the velocity constants of inactivation.

![Inactivation of commercial streptomycin with time at pH 6.6.](image)

**Fig. 2.** Inactivation of commercial streptomycin with time at pH 6.6. Curve 1 at 50°; Curve 2, at 95°. *Bacillus subtilis* plate assay. Ordinate, streptomycin in micrograms per ml.

**Results**

*Conditions of Stability*—There appears to be no inactivation of either pure streptomycin sulfate or of commercial streptomycin (400 γ per mg.) at concentrations of approximately 1000 γ per ml. during at least 60 days in the range of pH 3 to 7 at 7° and 28°.

*Inactivation at Elevated Temperatures*—Whereas streptomycin solutions are relatively stable in the range of pH 3 to 7 at or below room temperature, the rate of inactivation of commercial streptomycin becomes appreciable at more elevated temperatures, as is shown in Fig. 2. At 50° and pH 6.6 about 33 per cent of a sample of partially purified streptomycin was destroyed at the end of 15 days. More drastic decomposition took place at 95°, at which 50 per cent of the streptomycin was destroyed in 4.5 hours. Solutions of the pure normal salt of streptomycin sulfate (pH 5.5) at similar concentrations (1000 γ per ml.) are more stable at these temperatures, as is shown in Fig. 3.

*Inactivation by Acid*—The acid hydrolysis of streptomycin into the two basic fractions streptidine and streptobiosamine below pH 2 was found to
be a pseudounimolecular reaction by application of the first order equation,

$$k = \frac{1}{t} \ln \frac{c_0}{c_0 - c}$$  \hspace{1cm} (1)

where $c_0$ is the initial concentration of streptomycin and $c$ is the decrease

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**Fig. 3.** Inactivation of pure streptomycin sulfate (pH 5.5) with time at 50°, Curve 1, and at 95°, Curve 2. Bacillus subtilis plate assay. Ordinate, streptomycin in micrograms per ml.

**Fig. 4.** Hydrolysis of streptomycin with time at 28°. Curve 1, partially purified streptomycin at pH 1; Curve 2, partially purified streptomycin at pH 2; Curve 3, pure streptomycin at pH 1.7; Curve 4, pure streptomycin at pH 0.95; Curve 5, pure streptomycin at pH 0.30. Ordinate, streptomycin in micrograms per ml.

After a lapse of time, $t$. When the concentration of streptomycin is plotted against time, exponential curves are obtained which when plotted on semilogarithmic paper produce straight lines (within experimental errors), as is shown in Figs. 4 and 5. The velocity constants ($k$ hour$^{-1}$) of inactiva-
tion of partially purified streptomycin sulfate (400 γ per mg.) at concentrations of about 1000 γ per ml. and at pH 1.0 were found to be 0.0025 at 28°, and 0.040 at 50°; at pH 2.0, 0.00043 at 28°, and 0.0043 at 50°. For the purified material at pH 0.8 k hour⁻¹ was found to be 0.00056 at 7°, 0.0065 at 28°, and 0.090 at 50°; at pH 1.7, 0.00045 at 28° and 0.0080 at 50°; at pH 2.7, 0.0007 at 50°. The rate of hydrolysis in acids is essentially the same for pure and partially purified streptomycin.

Inactivation by Alkali—The inactivation studies in the alkaline region were carried out in buffers in the range of pH 8.0 to 11.4 between 7° and 50°.

![Graph showing hydrolysis of streptomycin with time at 50°.](image)

The pH of these solutions remained constant during the course of the observations. The velocity constants obtained by alkali inactivation on different samples of commercial streptomycin sulfate were not reproducible; however, when the concentration of pure streptomycin is plotted against time, the curves in the alkaline range are similar to those obtained in acid medium. The data fall on a straight line when they are plotted on semi-logarithmic paper (Fig. 6). The reaction rate constants calculated from the first order equation (No. 1) in the alkaline range for purified material k hour⁻¹, was found to be, at pH 8.6, 0.00065 at 28°; at pH 9.5, 0.00023 at 7°, 0.0023 at 28°, and 0.024 at 50°; at pH 11.2, 0.044 at 28°.

A more complete summary of the data obtained in this work is given in Table I where the half life of purified streptomycin under conditions of pH and temperature is indicated.
Stability of Dry Streptomycin Salts—Salts of commercial streptomycin containing less than 1 per cent moisture have been shown to be stable at or below room temperature over long periods of time. No drop in potency has been noted in material which has been at laboratory temperature for 1 year. Some recent observations were made on both streptomycin hydro-

![Graph](http://www.jbc.org/)

Fig. 6. Inactivation of pure streptomycin with time. Curve 1, 7°, pH 9.5; Curve 2, 28°, pH 8.6; Curve 3, 28°, pH 9.5; Curve 4, 28°, pH 11.2; Curve 5, 50°, pH 8.6; Curve 6, 50°, pH 9.5. Ordinate, streptomycin in micrograms per ml.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Stability of Dry Streptomycin Salts; Bacillus subtilis Plate Assay</th>
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<tbody>
<tr>
<td>Streptomycin</td>
<td>Per cent moisture</td>
</tr>
<tr>
<td>Hydrochloride</td>
<td>0.80</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.68</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.89</td>
</tr>
</tbody>
</table>

chloride and sulfate at 50° for a period of 10 weeks. These stability data are given in Table II.

**SUMMARY**

The most favorable conditions for the stability of solutions of streptomycin are at temperatures at or below 28° and between pH 3 and 7. Inactivation takes place outside of this range; namely, below pH 3 and above pH 8. The velocity constants of inactivation have been determined in
both acid and alkaline regions and the reactions have been found to be irreversible and first order. In the acid region the velocity constants of the partially purified material are essentially the same as for pure streptomycin. Heat greatly increases the rate of decomposition over the whole pH scale.

Relatively dry neutral salts of streptomycin are stable at 50° over long periods.

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BIBLIOGRAPHY

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