THE INFLUENCE OF CERTAIN SUBSTANCES ON THE
ACTIVITY OF STREPTOMYCIN

III. Differential Effects of Various Electrolytes on the Action
of Streptomycin

R. DONOVICK, A. P. BAYAN, P. CANALES, AND F. PANSY

Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick,
New Jersey

Received for publication January 19, 1948

The activities of both streptothricin and streptomycin are affected by substances and physical conditions that apparently do not affect these compounds per se. Foster and Woodruff (1943) noted that the presence of phosphates, some other salts, and sugars in test media decreased the activity of streptothricin. Similar observations with regard to streptomycin were presented by Waksman and Schatz (1945). Foster and Woodruff (1943) concluded that phosphates and sugars caused the observed interference through effects on pH, since it was also found that raising the pH of the medium increased the streptothricin activity and vice versa. They concluded that only the undissociated free base of this antibiotic was active, hence the increased activity at a higher pH. Since streptomycin responded similarly to pH changes (Waksman and Schatz, 1945), presumably one might apply the same deductions in this case.

Abraham and Duthie (1946), on the other hand, suggested the possibility of bacterial cell surface changes accompanying change in pH and posed the question of whether there might not be competition at the surface between hydrogen ions and the cations of dissociated streptomycin, hence the lower antibiotic activity at lower pH's.

In reporting the interfering action of sugars on streptomycin activity, Waksman and Schatz (1945) observed that such effects were probably caused by pH changes in the medium, but they also discussed the possibility of the reducing role played by sugars. Later, Geiger, Green, and Waksman (1946) concluded that the effects of sugars were after all caused by pH changes, since glucose was found to have no effect on streptomycin in the presence of a glycine buffer. This conclusion was questioned by Donovick and Rake (1946) because increased glucose concentrations, under the conditions of the test, did not lead to a lower pH but did decrease the activity of the antibiotic. In addition, the degree of glucose effect varied with the concentration of tryptone in the medium.

The explanation for the effects of salts other than phosphates on the action of streptomycin remains equally obscure. Berkman, Henry, and Housewright (1947) stated that the capacity of various salts to cause this interference was a function of the salt concentration. They reported little interference by salts at concentrations below 0.5 per cent. This antagonism was not due to stimulation of the growth of the test organism by the change of salt concentration.
They included several species of microorganisms and six different salts in their studies, and concluded that the results with all salts were alike, differences being only quantitative.

These authors proposed two possible explanations for the antagonism of salts to streptomycin action: interaction between salt and streptomycin, or the result of an interaction of salt and bacteria. They were unable to demonstrate any interaction between streptomycin and the salts studied. As to the possible nature of the interaction between salts and bacteria, no proposals were extended.

Another possible explanation for the action of salts, as well as one for a mode of action of streptomycin, was brought forward by Cohen (1946, 1947), who found that desoxyribonucleic acid (DNA) was precipitated by streptomycin and that this precipitate was caused to dissociate by the addition of salts. Since bacteria contain DNA, it was thought that perhaps streptomycin interfered with some normal cell functions through such precipitation. This action of streptomycin on DNA and its reversal by salts was confirmed by Berkman et al. (1947).

A different approach to the question of the interference by certain salts on the antibiotic activity of streptomycin was taken by Green (1947). This author reported that salts of pyruvic, fumaric, succinic, formic, maleic, and maleic acids all interfered with the action of this antibiotic, whereas lactate, acetate, propionate, glycerophosphate, as well as glycerol and lactose, had no antagonizing effect. This work was in line with the thought that the mode of action of streptomycin was in some way tied to the oxidative mechanisms of susceptible microorganisms. (Since this aspect of possible modes of action of streptomycin is outside the scope of the present paper, the literature dealing with it is not reviewed here. See Geiger, Green, and Waksman, 1946; Bondi, Dietz, and Spaulding, 1946; Donovick and Rake, 1946; etc.) Organic salts such as Green found to interfere with streptomycin action were thought to counteract the action of streptomycin by supplying some necessary intermediates in carbohydrate metabolism that are blocked by streptomycin.

The mode, or modes, of action of streptomycin and other antibiotic substances has attracted the attention of many laboratories, and numerous approaches to the problem have been chosen. The manner in which electrolytes affect the action of streptomycin was felt in this laboratory to offer an interesting point of attack on the problem. Relatively little is known about the effects of electrolytes on bacteria. The literature on this subject is tremendous, and no attempt can be made here to cover it. Porter (1946) provides a selected bibliography on this complex subject. Falk (1923) thoroughly reviewed the literature prior to 1923. A comment made by this author almost 25 years ago unfortunately still holds true today: "The basis of knowledge upon which rests the use of synthetic media is still essentially empirical. The physiological significance of this or that ion is not known and cation—or anions, for that matter—are thrown in or taken out of media according to whether growth is good, bad or indifferent."

Falk's paper covered both the physiologic and physicochemical aspects of the problem. An interesting study on the latter, insofar as adsorption of various cations by bacteria is concerned, was published by McCalla in 1940. Some of
his methods might well be applied to the investigation of the manner in which electrolytes compete with streptomycin. Do such electrolytes prevent streptomycin from reaching the vital site of reaction, or do they interfere with the final reactions in which streptomycin enters in causing inhibition? To attempt to answer some of these questions is the over-all purpose of the present work.

It appeared to the authors that it was necessary first to gather more exact data on the effects of electrolytes on the growth-inhibiting action of streptomycin on bacteria, and then, later, using this information as a baseline, to attempt to correlate it with data gathered from experiments specifically designed to test various theories of the mode of action of this antibiotic. *Klebsiella pneumoniae* (ATCC 9997) was chosen as the test organism because considerable information had previously been obtained on its response to streptomycin, and on the assumption that in general the mode of action of streptomycin was the same regardless of the test organism. Whether or not such an assumption is valid remains to be seen.

**PROCEDURE**

The basic medium used throughout this portion of the work consisted of 0.75 per cent tryptone in water, which upon autoclaving is found to have a pH very close to 7. Electrolytes that could withstand autoclaving at 15 pounds pressure for 15 minutes were incorporated into the medium before sterilization. Otherwise, the electrolyte solutions in 1-molar or 2-molar solution were sterilized by filtration through U.F. fritted Corning pyrex glass filters, and appropriate amounts were added to sterile tryptone broth to give the desired final concentrations of salt.

The trihydrochloride of highly purified streptomycin A1 (Fried and Titus, 1947) was used throughout the work described. To establish the minimal inhibiting concentration of streptomycin in the various media employed, the procedure was that described previously (Donovick and Rake, 1946). All M.I.C. figures reported are based on no less than eight assays, giving a standard error of approximately ±6 per cent.

**Differentiation between the Effects of Phosphate Ion and Hydrogen Ion on the Activity of Streptomycin**

Buffer solutions consisting of NaH₂PO₄·Na₂HPO₄ were prepared so as to give final concentrations of phosphate in tryptone broth ranging from 0.004 M to 0.067 M and at each concentration from pH ca. 6.0 to pH 7.5. In addition, as controls, tryptone broth was adjusted with NaOH or HCl over a range of pH 6 to 9. The M.I.C. of streptomycin A trihydrochloride was then determined in each of these media. The results, shown in figure 1, indicate that the effect of phosphate ion can be readily distinguished from that of hydrogen ion. At each concentration of phosphate, an increase in pH from 6 to 7.5 caused a 3- to 4-fold decrease in M.I.C. At any fixed pH an increase in phosphate concentration caused a relatively consistent increase in M.I.C.

1 The authors are indebted to Drs. Fried and Wintersteiner of the Division of Organic Chemistry, The Squibb Institute for Medical Research, for the streptomycin used in these studies.
The Behavior of Streptomycin in Vitro in the Presence of Various Electrolytes at Constant pH

Since the effects of phosphate ions could be distinguished from those of hydrogen ions on the action of streptomycin A, it appeared reasonable to expect the effects of other ions to be distinguishable as well. Hence studies including 17 different salts were undertaken, each at concentrations ranging from 0.004 M through 0.067 M. For studying anions, sodium was generally the cation of the salt used. For studying cations, on the other hand, chloride was generally the

Figure 1. Effect of phosphate on streptomycin activity at various pH's.
anion employed. Since the pH of the test medium exerts an effect on streptomycin action separate from that of other ions, it was desirable in this study to work within as narrow a pH range as possible. To this extent the pH of the 0.067 M sodium pyruvate was too high, and those of 0.067 M sodium lactate, barium chloride, magnesium chloride, and calcium chloride in 0.75 per cent tryptone broth were too low to be entirely satisfactory. Nevertheless, the results of these studies, presented in figure 2, indicate that sodium ion, per se, has little effect on the action of streptomycin, as exemplified by the results with sodium acetate and pyruvate, nor do lithium and potassium ions, since their chlorides are equal in activity to that of sodium chloride. Ammonium presents a puzzling problem because as an acetate it causes no interference, whereas as a chloride its action is greater than that of sodium chloride. Magnesium and calcium have very marked effects, and magnesium sulfate represents an interesting example of the additive action of two active ions (viz., Mg$^{++}$ and SO$^{--}$) causing greater interference than either sodium sulfate or magnesium chloride.

Barium chloride and calcium chloride presented special problems in concentrations of 0.067 and 0.033 M since they caused precipitates in the medium. The

---

**Figure 2.** Effect of various salts on streptomycin A action in vitro.
presence of such precipitates did not interfere seriously with the tests since they settled out and could be distinguished from the bacterial growth. Removal of the barium precipitates, by filtration, prior to use caused very little change in the results. However, when the precipitates caused by calcium chloride were filtered off, the pH of the broth dropped from pH 6.4 (unfiltered) to 6.2 and the M.I.C. rose accordingly. Hence the results shown in figure 2 are those obtained with the precipitate present.

Among the anions, acetate and pyruvate show little or no effect, whereas, in increasing order, the following interfere with streptomycin action: nitrate, chloride, lactate, phosphate, tartrate, citrate, and sulfate.

The explanation for the order of activities of these ions is not at once evident. At first glance one is tempted to associate ionic strength with the ability to cause interference. There are sufficient contradictions, however, to cause one to abandon this principle. Comparison of CaCl₂ and MgCl₂, the ionic strengths of which would be very similar in equimolar concentrations, exemplifies such a contradiction. It is the hope that future work will cast more light on the observed phenomena.

Effect of Salts on the Precipitation of Desoxyribonucleic Acid (DNA) with Streptomycin

Cohen (1946, 1947) has described the precipitation of desoxyribonucleic acid by streptomycin. Further he has shown that streptomycin also precipitates bacteriophage Tr—F, which is rich in DNA. Other pertinent points brought out in these publications are that desoxyribonuclease (DNase) depolymerizes the DNA present in this bacteriophage with a consequent decrease in viscosity but without inactivation of the bacterial virus. Streptomycin, on the other hand, precipitates the desoxyribonucleic acid and also inactivates the bacteriophage. Hence the possibility was presented that streptomycin acts through such precipitation. The description of the dissociation of streptomycin-DNA precipitates by salts (Cohen, 1946, 1947; Berkman et al., 1947) led the present authors to attempt to correlate the ability of various ions to interfere with the growth-inhibitory action of streptomycin with the power of these ions to prevent the precipitation of DNA with streptomycin. For this study the authors are indebted to Dr. Samuel Graff, of the College of Physicians and Surgeons, Columbia University, for a highly purified, relatively salt-free preparation of DNA.

Precipitation of desoxyribonucleic acid by streptomycin. A simple method was employed for measuring the precipitability of DNA with streptomycin. The minimal concentration of streptomycin trihydrochloride required to give a visible precipitate of DNA was determined by carefully adding to 1.5 ml of 0.1 per cent aqueous solution of DNA 1.5 ml of various concentrations of streptomycin in such a fashion as to give a ring. Although precipitates formed at once when adequate amounts of streptomycin were present, final readings were not made until the tests had stood at 4 °C overnight in order to ensure full development of the ring. Similar tests were carried out to determine the minimal con-
centration of DNA required to give visible precipitates at a fixed streptomycin concentration. In this case a 0.5 per cent streptomycin solution was used. The findings were as follows: DNA added in a concentration of 0.1 per cent showed a visible precipitate with streptomycin A trihydrochloride added in a concentration as low as 0.032 per cent. The minimal concentration of DNA showing a precipitate when added to 0.5 per cent streptomycin A trihydrochloride was 0.0032 per cent. For further work described below, both of these reagents were used in large excess as measured by this test.

Effect of phosphate and hydrogen ions on the precipitation of DNA with streptomycin. A series of Na$_2$HPO$_4$-NaH$_2$PO$_4$ buffer solutions were prepared ranging from 0.268 m to 0.016 m (i.e., four times as concentrated as desired in the final concentration in the test in which a 4-fold dilution of the buffer solutions was involved), and at each concentration the pH ranged from 5.2 to 7.4. To 0.125 ml of buffer solution 0.25 ml of 0.25 per cent aqueous DNA solution were added and thoroughly mixed. To this 0.125 ml of 0.5 per cent aqueous solution of streptomycin A trihydrochloride were then carefully added to form a ring. All tests were permitted to stand overnight at 4 C to permit full formation of precipitate in border-line cases. Thus with streptomycin and DNA concentrations held constant, the minimal phosphate concentrations preventing precipitation at various pH's were measured. These results are given in table 1.

These findings at once reveal an interesting contrast between the manner in which phosphate and hydrogen ions affect the DNA-streptomycin precipitation system as compared to the streptomycin-growth-inhibiting system. Since in the former system the concentrations of DNA and streptomycin were held constant, it was necessary, for purposes of comparison, to pick from figure 1, shown earlier, similar data. In the bacterial system the microorganisms themselves were the only source of DNA, and as the inoculum was kept constant in all cases, this criterion had been met. In order to obtain data for the effect of phosphate and hydrogen ions on the bacterial system at constant streptomycin concentrations, however, extrapolation from the curves in figure 1 was necessary in some cases. For this comparison the point at which each pH-phosphate curve crosses the M.I.C. line of 0.4 micrograms per ml in figure 1 was determined, extrapolating where necessary. Although a certain amount of error is involved in this procedure, considering the nature of the curves involved, it was felt that the relative

### TABLE 1

<table>
<thead>
<tr>
<th>pH</th>
<th>Minimal Phosphate Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>0.062</td>
</tr>
<tr>
<td>5.9</td>
<td>0.062</td>
</tr>
<tr>
<td>6.4</td>
<td>0.047</td>
</tr>
<tr>
<td>6.8</td>
<td>0.031</td>
</tr>
<tr>
<td>7.4</td>
<td>0.023</td>
</tr>
</tbody>
</table>
values thus derived were valid. The data thus obtained and those shown in the foregoing table are plotted in figure 3.

It is evident that increasing the pH, which increases the antibiotic activity of streptomycin, causes an increase in the concentration of phosphate required to prevent the antibacterial action of streptomycin. On the other hand, a similar increase in pH in the DNA-streptomycin precipitation system causes a decrease in the amount of phosphate required to prevent precipitation. This point of difference having been established, it was of interest to compare how various other ions behave in the two systems described.

![Figure 3](http://jb.asm.org/)

**Figure 3.** Interrelationship between effects of phosphate and hydrogen ions at fixed streptomycin concentrations.

**Effects of various other ions on the DNA-streptomycin precipitation system.** A series of aqueous salt solutions were prepared ranging in concentrations from 0.268 M to 0.01 M. The minimal salt concentration preventing the formation of a DNA-streptomycin precipitate was determined in the manner described above. At the same time, from figure 2, the approximate point at which each salt-concentration-streptomycin-M.I.C. curve crossed the M.I.C. line of 1.5 micrograms per ml was determined by extrapolation. Since the curves for all salts less active than sodium tartrate in preventing the growth-inhibiting action of streptomycin approach straight lines, it was felt that the error involved here was not prohibitive for comparative purposes. This comparison is shown in table 2.

In the cases of some of the uni-univalent salts such as sodium chloride, sodium
nitrate, and potassium chloride, the relative action of the salts is similar in both systems. However, there are also cases of marked differences of behavior. In the DNA-streptomycin precipitation system, for example, no distinction is evident between the relative actions of sodium sulfate and calcium chloride, whereas in the prevention of the inhibition of bacterial growth the latter salt is twice as active as the former.

Of further interest are the differences in behavior of some of the salts of organic acids in the two systems, especially sodium citrate. One wonders whether the relatively low activity of such salts in their ability to interfere with the growth-

<table>
<thead>
<tr>
<th>SALT TESTED</th>
<th>MINIMAL SALT CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevention of growth inhibition</td>
</tr>
<tr>
<td></td>
<td>Absolute molarity</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.023</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.029</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>0.040</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>0.062</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.055</td>
</tr>
<tr>
<td>Sodium tartrate</td>
<td>0.076</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>0.098</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>0.10†</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>0.13</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>0.22</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.22</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.22</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>0.22</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>0</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>No effect</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>0</td>
</tr>
</tbody>
</table>

* As compared to sodium chloride taken as 1.0.
† Tested at pH 7.2.
‡ Tested at pH 6.8.

The inhibiting action of streptomycin may be due to the utilization of the anion by the bacteria. Preliminary tests on this point were conducted in a synthetic medium in which sodium lactate is ordinarily the sole source of carbon (Cohen, 1947). This medium contains 1 g of NH₄Cl, 0.7 g of K₂HPO₄, 0.3 g of KH₂PO₄, 0.1 g of Na₂SO₄, 0.01 g of MgSO₄, and 10 g of sodium lactate per liter of distilled water. The strain of K. pneumoniae used throughout these studies grows readily in this medium at least through 10 transfers.

To test the utilization of organic acid salts other than lactate, media were prepared in which 0.09 and 0.18 M sodium acetate, ammonium acetate, sodium pyruvate, sodium tartrate, and sodium citrate, respectively, were substituted
for sodium lactate. Ten ml of each of these media were inoculated with 0.1 ml of an overnight culture grown in the synthetic lactate medium. Each of these cultures was then incubated for 1 week at 37 C, observations for visible turbidity being made daily. Lactate controls were included with each day's tests. Usually no visible growth occurred in any of the media other than the controls, although sparse growth occurred once in the ammonium acetate, once in the sodium tartrate, and once in the sodium citrate media. There were still viable organisms present in all media at the end of 1 week, however, as shown by streak plates.

It is recognized, of course, that the inability of these salts to substitute for lactate in the synthetic medium used is no final measure of whether they are utilized by K. pneumoniae under other circumstances. This is shown in at least the case of sodium pyruvate, which when added in a concentration of 0.2 M to 0.75 per cent tryptone broth is fermented by K. pneumoniae with a consequent pH drop in 24 hours. Such acid formation with the other salts did not occur, and, hence, other types of tests for their utilization are necessary. These have not yet been performed.

On the basis of the behavior of streptomycin in the presence of phosphates and a number of other ions, it must be concluded that no good correlation is found between the precipitation of DNA and the prevention of bacterial growth. To test this even further still another approach was attempted.

Action of DNase on DNA-streptomycin precipitates. Aqueous solutions of DNA have a high viscosity, which is readily reduced by desoxyribonuclease (DNase) (McCarty, 1946). Cohen (1946, 1947) reported that Na DN (desoxyribonuclease) thus depolymerized by DNase "did not reduce the precipitability [with streptomycin] of the resulting Na DN after 1 hour of depolymerization." However, the present authors found that when DNA-streptomycin precipitates are treated with adequate amounts of DNase, dissolution of the precipitates occurred. (We wish to express our appreciation to Dr. M. Kunitz of the Rockefeller Institute, Princeton, New Jersey, for the crystalline DNase used in the following experiments.)

DNase solutions were prepared in 0.05 M MgCl₂ containing 0.25 per cent gelatin, under which conditions the enzyme is relatively stable when stored at 4 C (McCarty, 1946). With 0.025 M MgCl₂ containing 0.25 per cent gelatin as the diluent, the enzyme solution was diluted serially by 2-fold steps. To 0.125 ml of the various dilutions of enzyme were added, in turn, 0.125 ml of 0.5 per cent streptomycin A trihydrochloride and 0.25 ml of 0.025 per cent DNA. Readings were made for the presence or absence of precipitate immediately, and after incubating at 37 C over the time intervals shown in table 3. The experiment shown is one example of a number of repeat tests that were conducted.

It can be seen, thus, that DNA precipitated by streptomycin can still be depolymerized by DNase, with consequent disappearance of the precipitate. If the precipitation of DNA by streptomycin is a vital part of the manner in which this antibiotic inhibits bacterial growth, then one might expect DNase to interfere with the growth-inhibiting action of streptomycin. That DNase is capable
of entering at least into the dead bacterial cell and depolymerizing DNA has been demonstrated with *E. coli*, *Bacillus anthracis*, and *Corynebacterium diphtheriae* (Tulasne and Vendrely, 1947), as well as with the same strain of *K. pneumoniae* as has been used throughout these studies (Rake and Hamre, to be published). Hence, tests were set up to determine the M.I.C. of streptomycin in the presence of an excess of DNase. For this purpose 0.75 per cent tryptone broth containing 0.003 M MgCl₂ was used to ensure the presence of adequate amounts of Mg²⁺ to activate the enzyme. The DNase concentration of the broth was then brought to 0.5 µg per ml, the broth was inoculated with *K. pneumoniae*, and streptomycin titrations were run as usual. No significant differences were found in the M.I.C.'s of streptomycin A between broths containing DNase or lacking it, the former being 0.27 µg per ml and the latter 0.26 µg per ml. Not even at 10 times this concentration of DNase (i.e., at 5 µg per ml) were there significant differences.

Thus, as in the case of the behavior of streptomycin in the presence of salts, its behavior in the presence of DNase does not confirm the theory that this antibiotic

---

**TABLE 3**

*The action of DNase on DNA-streptomycin A precipitates at 37° C*

<table>
<thead>
<tr>
<th>TUBE</th>
<th>DNase Conc.</th>
<th>STM. Conc.</th>
<th>DNA Conc.</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>4.5</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>µg/ml</td>
<td>%</td>
<td>%</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>4.5</td>
<td>24</td>
</tr>
<tr>
<td>b</td>
<td>28.7</td>
<td>0.125</td>
<td>0.0125</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>14.3</td>
<td>0.125</td>
<td>0.0125</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>0.90</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>0.45</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>0.22</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>0.11</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>0.056</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>0.028</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>0.014</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>0.0070</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = precipitate approximately as heavy as control tube a.  
± = precipitate present but lighter than controls.  
f. tr. = very faint trace of precipitate.  
- = no precipitate visible.

---

*First the effect of DNase itself on the growth of *K. pneumoniae* in this broth was tested. Tulasne and Vendrely (1947) had described the DNA present in several species of bacteria as being nuclei. It was anticipated, therefore, that perhaps the growth would be inhibited. Concentrations of crystalline DNase as high as 5 µg per ml, however, had no apparent effect on the rate of multiplication of our test organism.*
acts by precipitating the DNA in the living cell. Other observations also make this theory improbable. It must be pointed out that T2—F bacteriophage is not a good example from which to draw conclusions. DNA is not always present as a constituent of this bacteriophage, its presence or absence depending entirely upon cultural conditions; also it can be removed by DNase without interfering with phage activity; and yet streptomycin does inactivate the bacteriophage. Furthermore, as has been pointed out by Hamre and Rake (1947), streptomycin has no activity on one group of agents—the Chlamydozoaceae (lymphogranulomapsittacosis group)—members of which are known to be particularly rich in DNA.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Geoffrey Rake, Division of Microbiology, The Squibb Institute, for his many kind criticisms and suggestions in connection with this work.

SUMMARY

Quantitative studies of the effects of various ions on the action of streptomycin on bacterial growth as well as on its ability to precipitate desoxyribonucleic acid have been conducted.

Sodium, lithium, and potassium ions have little effect on the ability of streptomycin to inhibit bacterial growth.

Ammonium ion presents a puzzling problem since as an acetate it appears to have no effect on the growth-inhibiting action of streptomycin, whereas as a chloride its action is greater than can be accounted for by the action of the chloride ion alone.

Of the six cations studied, magnesium and calcium caused the greatest interference with streptomycin activity.

Among the anions studied, acetate and pyruvate caused little if any interference, whereas in increasing order the following were active in interfering with the prevention of growth by streptomycin: nitrate, chloride, lactate, phosphate, tartrate, citrate, and sulfate.

If both the cation and anion of a given salt show this interference, their effects may be additive, as is exemplified by magnesium sulfate.

Various salts also interfere with the precipitation of desoxyribonucleic acid by streptomycin, but the order of activity of the salts is different from that in the interference with the growth-inhibiting action of streptomycin. This and also studies with the enzyme desoxyribonuclease lead to the conclusion that the ability of streptomycin to precipitate desoxyribonucleic acid has little to do with the antibacterial action of this antibiotic.

REFERENCES


HAMRE, D., AND RAKE, G. 1947 Studies on lymphogranuloma venereum. V. The action of some antibiotic substances and sulfonamides in vitro and in vivo upon the agents of feline pneunomitis and lymphogranuloma venereum. J. Infectious Diseases, 81, 175-190.


RAKE, G., AND HAMRE, D. Effect of enzymes on microorganisms. To be published.
