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# Effect of Glucose, Peptone, and Salts on Streptomycin Activity.*†

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Among the factors which influence the antibacterial activity of streptomycin, the composition of the medium occupies a prominent place.[1,2] The influence of reaction and the effect of glucose, peptone, and of various salts have received special consideration.

Several explanations have been or can be suggested to account for the repressive action upon streptomycin potency by the lowering of the pH of the medium: 1. streptomycin is largely active in a free basic or dissociated state and to a lesser extent in the form of a salt; 2. the active part of the streptomycin molecule can largely be neutralized by an acid; 3. the streptomycin base and the hydrogen ions compete on the surface of the bacterial cell for the active centers; 4. dissociation of the streptomycin salt is repressed by a readily dissociated mineral salt.[3-8]

The effect of glucose in lowering the potency of streptomycin has been explained as due to the reducing properties of the sugar, to the production of an acid by the fermentation of the carbohydrates by the test bacteria, or to certain other factors such as the effect on the growth of the organism which would make it more resistant to the action of streptomycin.[4,6,7,9] The effect of certain peptones[2] and other organic compounds, such as sodium glycolate,[1] cysteine, and methionine, in lowering the activity of streptomycin has been explained by a reduction of the oxidation-reduction of the medium, by interaction with the active grouping of the antibiotic, or by some other mechanism.

Various theories have also been postulated to explain the effect of salts in reducing the potency of streptomycin. Loo *et al.*[5] noted that the addition of certain inorganic salts to streptomycin solutions caused a marked increase in activity of the antibiotic; in the presence of 0.1 *M* phosphate buffer, however, the effect of the salts was minimized, except at high concentrations. On the other hand, Klein and Kimmelman[10] reported that sodium chloride produced an antagonistic effect upon streptomycin activity. Berkman *et al.*[11] also observed a decrease in activity of streptomycin upon the addition of salts to nutrient broth.

In view of the importance of these constituents of the medium in determining the activity of streptomycin, a study was undertaken of the "pH effect," the "glucose effect," the "peptone effect," and the "salt effect."

*Methods.* Unless otherwise stated, *Escherichia coli* 9637 was used as a test organism.

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The inoculum was prepared by growing the organism on nutrient agar for 24 hours, removing with sterile tap water, centrifuging, and suspending in water. The turbidity of the suspension was standardized by use of a Cenco-Sheard-Sanford Photelometer; when 0.1 ml of the suspension was inoculated into 4.9-ml portions of medium, an approximate count of 40 million bacterial cells per milliliter was obtained.

The culture tubes immediately after inoculation showed no absorption of light, since the instrument is not sensitive to suspensions containing fewer than 100 million organisms per ml. As growth proceeded, the medium became turbid. The relationship between turbidity and numbers of cells proved to be a straight line function; suspensions having a turbidity of 13, 22, 38, 59 and 65 contained 200, 410, 1,220, 2,520, and 2,900 million cells per ml, respectively.

The glucose and nitrogen sources were sterilized separately. The initial pH of medium was 6.8. The nutrient broth used as the basic medium contained 0.3% meat extract and 0.5% peptone.

*Effect of glucose and peptone.* In preliminary experiments, the antibacterial potency of streptomycin was found to decrease in the presence of glucose in the medium. The presence of glucose (Table I) caused a decrease in the pH value of the culture after 24 hours of incubation, giving a final pH of 4.7, 5.6, and 6.5 with 0.5, 0.1, and 0.025% respectively. These sugar concentrations allowed a normal growth of *E. coli* even in the presence of 10 μg of streptomycin per ml. When concentrations of only 0.01 and 0.005% glucose were present, however, only a slight change in reaction occurred; these concentrations exerted no effect upon growth inhibition of *E. coli* by streptomycin.

TABLE I.
Effect of Glucose upon Antibacterial Activity of Streptomycin.
*E. coli* used as test organism; cultures incubated 24 hours at 28°C.

| Glucose, % | Streptomycin, μg/ml | Turbidity | Final pH of culture |
|---|---|---|---|
| 0 | 0 | +++ | 6.9 |
| 0 | 10 | 0 | 6.8 |
| .50 | 0 | +++ | 4.7 |
| .50 | 10 | +++ | 4.8 |
| .10 | 0 | +++ | 5.6 |
| .10 | 10 | +++ | 5.5 |
| .025 | 0 | +++ | 6.5 |
| .025 | 10 | ++ | 6.3 |
| .010 | 0 | +++ | 6.7 |
| .010 | 10 | 0 | 6.4 |
| .005 | 0 | +++ | 6.7 |
| .005 | 10 | 0 | 6.6 |

To determine whether the nitrogen source in the medium influenced the effect of glucose on streptomycin activity, the peptone in the medium was replaced by tryptone, and varying concentrations of glucose were added. The acidity produced in the presence of tryptone was comparable to that obtained in the peptone broth, 0.5% glucose giving a pH of 4.9 after 24 hours of incubation (Table II). Tryptone did not, however, exert any repressive effect upon growth inhibition of *E. coli* by streptomycin even in the presence of 2% glucose. These results point definitely to the fact that it was not the glucose as such nor the acidity produced from bacterial metabolism that was responsible for the reduction in effectiveness of streptomycin upon *E. coli* in the peptone broth.

A comparison was, therefore, made of a number of commercial preparations of peptones and other hydrolyzed proteins (Table III). Comparable acidities were obtained after 21 hours of incubation in all the tubes containing the various nitrogen sources, the pH values ranging from 4.4 to 4.9. Only peptone 383619, peptonized milk, and neopeptone favored the growth of *E. coli* in the glucose broth containing 10 μg of streptomycin per ml; normally, this organism is sensitive to 1 μg per ml of streptomycin. Regular inhibition of *E. coli* by streptomycin was obtained in the media containing tryptone, tryptose, proteose peptone, peptone 23368, and protone as sources of nitrogen. These results tend to indicate that neither the glucose nor the acidity produced in the metabolism of the sugar by the test bacterium was responsible for the reduction in streptomycin activity in the peptone medium.

*Effect of salt.* The above experiments pointed to the presence in some of the protein hydrolysates of another substance that interfered with the antibacterial action of

TABLE II.
Influence of Different Peptones upon Glucose Effect on Streptomycin.

| Nitrogen source | Glucose, % | Streptomycin, μg/ml | Turbidity* 5 hr | Turbidity* 21 hr | Final pH |
|---|---|---|---|---|---|
| Tryptone | 2.0 | 0 | 23 | 39 | 4.8 |
| " | 2.0 | 10 | 3 | 3 | 6.6 |
| " | 1.0 | 0 | 22 | 37 | 4.9 |
| " | 1.0 | 10 | 0 | 0 | 6.7 |
| " | 0.5 | 0 | 21 | 37 | 4.9 |
| " | 0.5 | 10 | 0 | 0 | 6.7 |
| " | 0.25 | 0 | 22 | 35 | 4.9 |
| " | 0.25 | 10 | 0 | 0 | 6.7 |
| " | 0.10 | 0 | 27 | 36 | 5.0 |
| " | 0.10 | 10 | 0 | 0 | 6.6 |
| Peptone | 1.0 | 0 | 18 | 34 | 4.6 |
| " | 1.0 | 10 | 15 | 35 | 4.7 |

* Absorption of light in per cent.

TABLE III.
Influence of Peptones and Protein Hydrolysates upon Glucose Effect on Streptomycin.
All media contained 1% glucose and 0.3% meat extract; cultures were incubated for 21 hours at 28°C.

| Protein hydrolysate* | Streptomycin, μg/ml | Turbidity | Final pH |
|---|---|---|---|
| Peptone 383619 | 0 | 41 | 4.4 |
| " 383619 | 10 | 26 | 4.7 |
| " 23368 | 0 | 43 | 4.4 |
| " 23368 | 10 | 0 | 6.7 |
| Tryptone | 0 | 44 | 4.8 |
| " | 10 | 0 | 6.7 |
| Tryptose | 0 | 48 | 4.9 |
| " | 10 | 0 | 7.1 |
| Peptonized milk | 0 | 52 | 4.9 |
| " " | 10 | 40 | 5.0 |
| Peptinum siccum | 0 | 30 | 4.5 |
| " " | 10 | 0 | 6.8 |
| Proteose peptone | 0 | 38 | 4.5 |
| " " | 10 | 0 | 6.7 |
| Neopeptone | 0 | 40 | 4.6 |
| " | 10 | 11 | 4.9 |
| Protone | 0 | 45 | 4.9 |
| " | 10 | 0 | — |

* All hydrolysates were used in 0.5% concentration, except peptonized milk of which 1.5% was added.

streptomycin. The salt concentration appeared to be a possible factor, since various salts were found to exert a depressive effect upon the potency of streptomycin.

A chloride analysis of the various peptone preparations revealed significant differences in salt content. Peptone 23368, which did not prove favorable to the glucose effect upon streptomycin activity, contained the least amount of chloride, namely 1.52%, and tryptone contained 1.58% chloride. Neopeptone, however, contained 8.12% chloride; the peptonized milk contained 1.65% chloride, but since 1.5% of it was used as compared to 0.5% for the other peptones, there was three times this salt concentration in the medium.

The influence of salt on the activity of streptomycin in media containing peptone and tryptone was, therefore, investigated. The addition of sodium chloride to these media, with and without glucose, produced good growth of *E. coli* even in the presence of 10 μg of streptomycin per ml (Table IV). In the absence of sodium chloride, the various peptones, with the exception of 383611, and tryptone did not inhibit the antibacterial action of streptomycin. The addition of 0.5% sodium chloride resulted in a neutralizing effect of the streptomycin. These results tend to substantiate the above conclusion that glucose is not the agent responsible for the reduction of streptomycin activity but that the effect is due to the salt content of the medium.

The cultures of *E. coli* in which growth took place in the presence of 10 μg/ml of streptomycin were analyzed for the possible development of resistant strains or for the possible destruction of some of the streptomycin. When these cultures were inoculated with nutrient broth containing streptomycin no growth occurred, indicating that the organism did not become resistant to this antibiotic. An analysis of the antibiotic potency of the tubes in which growth occurred in the presence of streptomycin (10 μg/ml), due to the glucose-salt effect, gave the theoretical concentration of the

TABLE IV.
Combined Effect of NaCl and Peptones on Streptomycin Activity in Glucose Media.
Cultures incubated for 22 hours at 28°C.

| Peptone source | NaCl, 0.5% | Meat extract, 0.3% | Turbidity: Streptomycin, 0 | Turbidity: 10 μg/ml, + |
|---|---|---|---|---|
| Peptone 327393 | 0 | 0 | 27 | 0 |
| " 327393 | + | 0 | 19 | 19 |
| " 327393 | 0 | + | 45 | 30 |
| " 327393 | + | + | 47 | 38 |
| " 23368 | 0 | 0 | 22 | 0 |
| " 23368 | + | 0 | 23 | 20 |
| " 23368 | 0 | + | 43 | 0 |
| " 23368 | + | + | 41 | 37 |
| " 383611 | 0 | 0 | 25 | 0 |
| " 383611 | + | 0 | 22 | 21 |
| " 383611 | 0 | + | 43 | 27 |
| " 383611 | + | + | 44 | 35 |
| Tryptone | 0 | 0 | 30 | 0 |
| " | + | + | 37 | 29 |
| " | 0 | + | 45 | 0 |
| " | + | + | 48 | 38 |

TABLE V.
Effect of Salt upon Streptomycin Activity in Agar Media.
Cup method used; zone of inhibition measured in mm.*

| Concentration of NaCl, % | Salt added to agar. Streptomycin, μg/ml: 5 | 10 | 50 | Salt added to streptomycin solution. Streptomycin, 10 μg/ml |
|---|---|---|---|---|
| 0 | 13.0 | 14.0 | 18.5 | 17.0 |
| 0.5 | 14.0 | 16.0 | 20.0 | 18.0 |
| 1.0 | 12.0 | 14.0 | 19.0 | 18.1 |
| 3.0 | 9.0 | 9.0 | 14.0 | 19.0 |

* Diameter of cup 9 mm.

antibiotic, thus proving that the streptomycin was not destroyed.

The salt phenomenon was shown to be effective not only for *E. coli* but also for *Bacillus subtilis* and *Proteus vulgaris*. Furthermore, other salts, such as magnesium chloride, sodium nitrate, and sodium sulfate, in *M*/3 concentrations, also inhibited the antibacterial activity of streptomycin. These results are in accord with those obtained by other investigators. The salt effect was not neutralized by the addition of phosphate buffer or of serum to the medium.

When sodium chloride was added to nutrient agar, and the activity of streptomycin tested by the agar diffusion or cup method, it was found (Table V) that 0.5% salt actually caused an increase in the width of the zone of inhibition due to streptomycin; the addition of 3% salt to the agar resulted, however, in a marked reduction in the size of the zone. When salt was added to the streptomycin solution before it was placed in the cup, there was no neutralizing action, but an actual stimulating effect upon the potency of streptomycin.

These results indicate that the effect of salt is due not to a modification of the streptomycin but to a change in the conditions in the medium which influence the growth of the test organisms.

*Summary.* 1. The potency of streptomycin is greatly influenced by the composition of the medium, the salt concentration being most significant.

2. The inhibiting effect of glucose upon streptomycin activity is due to the specific nature of the organic nitrogenous compounds

in the medium and to the salt concentration.

3. The effect of salt is exerted not upon the streptomycin itself but upon the medium, which in turn influences the growth of the organism.

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## Effect of Organic Acids on Streptomycin Activity.*†

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The antibacterial potency of streptomycin is still largely measured by its inhibiting effect upon the growth of certain bacteria in artificial culture media, under given conditions of cultivation. The activity of the antibiotic thus measured is influenced by a great many factors. Chief among these is the presence of certain organic and inorganic constituents in the medium,[1] the presence of certain reducing substances,[2,3] the nature of the test organism, its age and number of viable cells used.[4] In addition to these, the presence of certain organic acids that may be formed as intermediary metabolic products in the nutrition of various bacteria may also affect the activity of streptomycin. A study of the last phenomenon forms the subject of this report.

*Escherichia coli* ATTC 9637 was used as the test organism. Its growth in various media was determined by means of a Cenco-Sheard-Sanford Photelometer. Immediately after inoculation, the culture tubes gave a reading of zero turbidity. The final turbidity was expressed in terms of per cent of light absorption.

The organic acids used in these studies were neutralized to pH 7.0, sterilized by filtration through Mandler candles, and added to the medium to give a final concentration of 1%. The streptomycin solution was sterilized by heating at 60°C for 30 minutes. The basic nutrient broth used in these experiments contained 0.5% peptone and 0.3% meat extract.

The influence of different organic acids as compared to that of sugars and glycerol upon streptomycin activity was at first determined (Table I). The growth of *E. coli* in nutrient broth is usually inhibited by 1 μg of streptomycin per ml. When pyruvic or fumaric

TABLE I.
Effect of Various Carbon Sources in Medium upon Streptomycin Activity on *E. coli*.

| Carbon source 1% | Turbidity* 4 hr Streptomycin, 10 μg/ml 0 | + | 21 hr Streptomycin, 10 μg/ml 0 | + |
|---|---|---|---|---|
| Glucose | — | — | 41 | 0 |
| Lactose | 14 | 0 | 36 | 0 |
| Pyruvic acid | 23 | 13 | 67 | 31 |
| Fumaric acid | 14 | 12 | 53 | 30 |
| Formic acid | 0 | 0 | 15 | 12 |
| Succinic acid | 8 | 6 | 30 | 7 |
| Glycerol | 8 | 0 | 42 | 0 |
| Maleic acid | 10 | 6 | 40 | 12 |
| Malonic acid | 8 | 6 | 31 | 6 |
| Glycerophosphoric acid | 7 | 0 | 33 | 0 |
| Acetic acid | 6 | 0 | 34 | 0 |
| Propionic acid | 5 | 0 | 30 | 0 |
| Lactic acid | 9 | 0 | 52 | 0 |

* Absorption of light in per cent.

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