result from hydrolysis is negligible and would have little effect on the ratio (*4*), whereas the liberation of pantoate would have a considerable effect.

These observations do not detract from the possible usefulness of pantoyltaurine and other analogues of pantothenic acid against Group A streptococci and presumably other organisms which are incapable of utilizing, or cannot readily utilize, pantolactone or pantoic acid for pantothenic acid synthesis. As already indicated, reduction of analogue concentration (by hydrolysis) would be inconsequential. Furthermore, the inhibition of growth of Group A streptococci, for instance, with pantothenic acid analogues is an established fact.


### Summary

Like pantothenic acid, pantolactone, and the pantoate ion, pantoyltaurine and other pantothenic acid analogues of this type antagonize the inhibitory effect of salicylate on *E. coli*.

It is suggested that the active antagonist in a solution of such an analogue is pantolactone or pantoate. These (particularly the former) could conceivably be contaminants of the analogues. However, the liberation of pantoate by hydrolysis, appears to be a more likely explanation for the antisalicylate effect.

The presence of pantolactone or pantoate in a solution of pantoyltaurine and similar analogues of pantothenic acid provides a possible explanation for the ineffectiveness of these drugs in inhibiting the growth of microorganisms which can utilize pantolactone or pantoate for the synthesis of pantothenic acid.


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## Interference With the Antibacterial Action of Streptomycin by Reducing Agents[1]


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The authors have repeatedly observed that the antibacterial action of streptomycin is significantly reduced by incubation in an anaerobic jar. When filter-paper discs are saturated with streptomycin and placed on the surface of infusion blood agar plates inoculated with *E. coli*, the resulting zones of inhibition on plates incubated anaerobically are much smaller than those on the comparable aerobic plates. The reduced activity of streptomycin anaerobically is not due to better growth conditions, since *E. coli* produces its maximum growth when incubated aerobically. Similar results may be obtained with staphylococci and other species of bacteria whose growth is favored by aerobic incubation.

These observations seemed to us to be significant, and further studies were carried out, the results of which suggest that they may have some bearing on the antibacterial action of streptomycin.

*Method and results.* Duplicate sets of infusion agar plates containing 2-fold concentrations of streptomycin were inoculated with various species of bacteria. One set of plates was incubated aerobically, the other in the anaerobic jar by the palladium-hydrogen method as modified by Spaulding and Goode (*8*). Plates were read for evidence of growth inhibition at the end of 24 and 48 hours incubation at 37° C. Table 1 shows the results of these tests.

TABLE 1

Inhibition of Bacterial Growth by Streptomycin on Infusion Agar

| Organism | Incubation | Growth* in presence of streptomycin; units/ml. of agar | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | None | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 | 32.0 | 64.0 |
| *E. coli*-S | aerobic | 4 | 4 | 1 | – | – | – | – | – |
| | anaerobic | 3 | 3 | 3 | 3 | 3 | 3 | – | – |
| *E. coli*-M | aerobic | 4 | 4 | 2 | – | – | – | – | – |
| | anaerobic | 3 | 3 | 3 | 2 | – | – | – | – |
| *S. aureus*-SM | aerobic | 4 | 4 | 1 | 1 | 1 | – | – | – |
| | anaerobic | 4 | 4 | 3 | 3 | 2 | 2 | – | – |
| *S. aureus* #7739 | aerobic | 4 | 1 | – | – | – | – | – | – |
| | anaerobic | 3 | 3 | 3 | 3 | 2 | 1 | – | – |
| *S. paratyphi* B | aerobic | 4 | 4 | 4 | 3 | – | – | – | – |
| | anaerobic | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 |
| *S. typhi-murium* | aerobic | 4 | 4 | 4 | 3 | 2 | – | – | – |
| | anaerobic | 2 | 2 | 2 | 2 | 2 | 2 | 1 | – |
| Pneumo. Type I | aerobic | 4 | 4 | 4 | 4 | 4 | 1 | – | – |
| | anaerobic | 4 | 4 | 4 | 4 | 2 | 1 | – | – |
| *Str. Hemo-lyticus* C-203 | aerobic | 4 | 4 | 4 | 4 | 4 | 3 | – | – |
| | anaerobic | 4 | 4 | 4 | 4 | 3 | 1 | – | – |

* Readings made at end of 24 hours incubation; 48-hour readings were essentially the same.

Under anaerobic conditions the amount of streptomycin necessary to produce bacteriostasis was from 2 to 16 times greater than the comparable aerobic concentration. With one exception (*S. aureus*-SM), these results were observed only with organisms which produced their maximum growth on aerobic plates.

A similar experiment was carried out with extract agar. Although there was some evidence that the antibacterial action of streptomycin was greater on the aerobic plates, the difference was not large. It appeared that various reducing agents in the infusion medium played a role in inhibiting streptomycin anaerobically. The greater reducing power of infusion agar was evident from the observation that methylene blue in a concentration of 1:100,000 in this medium

[1] Streptomycin used in this study was generously supplied through the courtesy of Dr. D. F. Robertson, Merck and Company, Inc., Rahway, New Jersey.

was reduced during anaerobic incubation, while methylene blue extract agar plates similarly incubated were not reduced. The presence or absence of streptomycin had no apparent effect.

The anti-*E. coli* action of streptomycin was subsequently determined in tryptone and infusion broth containing cysteine or sodium thioglycollate (Table 2).

TABLE 2

THE EFFECT OF CYSTEINE AND THIOGLYCOLLATE ON THE ANTIBACTERIAL ACTION OF STREPTOMYCIN AGAINST *E. coli*

| Inhibiting concentration of streptomycin | |
|---|---|
| Medium | Units/ml. |
| Tryptone* | 1.0 |
| Tryptone plus 0.1 per cent thioglycollate | 2.0 |
| Tryptone plus 0.1 per cent cysteine | 64.0 |
| Infusion† | 16.0 |
| Infusion plus 0.1 per cent thioglycollate | 64.0 |
| Infusion plus 0.1 per cent cysteine | > 128.0 |

* Difco tryptone 1.0 per cent.
† Veal infusion, 1.0 per cent tryptose, 0.03 per cent glucose.

Aside from the added SH compounds, infusion broth itself contains interfering substances, since 16 times as much streptomycin was necessary for bacteriostasis in the infusion control as in the tryptone control. The presence of cysteine increased the concentration required for bacteriostasis 64-fold in tryptone and at least 8-fold in infusion broth; the increase with thioglycollate was only 2- to 4-fold.

TABLE 3

EFFECT OF INORGANIC REDUCING AGENTS ON THE ANTIBACTERIAL ACTION OF STREPTOMYCIN AGAINST *E. coli*

| Inhibiting concentration of streptomycin | |
|---|---|
| Reducing agent* | Units/ml. |
| Tryptone control | 2.0 |
| 0.5 per cent sodium formate | 32.0 |
| 0.05 per cent stannous chloride | 32.0 |
| 0.05 per cent sodium bisulfite | 32.0 |
| 0.05 per cent sodium hydrosulfite | 16.0 |
| 0.5 per cent sodium thiosulfate | 32.0 |

* Final concentration in tryptone broth.

The effect of cysteine on streptomycin could be due either to a reducing action or to some specific chemical reaction, especially with the sulfhydryl groups. The inactivation of penicillin (*2*) and other antibiotic agents (*1*) by cysteine is well known. The action of cysteine on streptomycin appears, however, to be different from that on penicillin, as was demonstrated in the recent report of Denkelwater, Cook, and Tishler (*4*), published while this manuscript was being prepared. These workers state that the inactivation of streptomycin by cysteine is reversible. Similar interference by sulfhydryl compounds with the antibacterial properties of mercurials (*6*) and quinones (*3*) has been reported.

If interference with the activity of streptomycin by sulfhydryl compounds is due to reducing powers, other reducing agents should likewise interfere with its action. Consequently, various reducing agents have been tested. Each of the substances studied definitely antagonized the antibacterial action of streptomycin against *E. coli,* as shown in Table 3. It is evident that the antibacterial action of streptomycin is greatly diminished by a variety of reducing agents other than sulfhydryl compounds.

*Discussion.* Reducing agents definitely antagonize the antibacterial activity of streptomycin. Further work will be required, however, to determine the true nature of this interference. Whether it is due to a lowering of oxygen tension or to some specific chemical reaction or both acting simultaneously, is still unknown. The SH compounds and the inorganic reducing agents could function in either capacity. The reduction in activity of streptomycin in the presence of glucose, as reported by Waksman and co-workers (*9*), could be attributed to the reducing action of this carbohydrate.

It is entirely possible that this phenomenon is related to the mode of action of streptomycin. This antibiotic displays its greatest activity against bacteria which grow better aerobically than anaerobically, e. g. *E. coli, S. aureus,* and *Myco. tuberculosis.* None of the obligate anaerobes is susceptible (*7*). The antibacterial action of streptomycin may be due to its ability to block some enzyme system, oxidative in nature, which is essential only to the growth of susceptible aerobic bacteria, an enzyme system which anaerobes do not possess. If this is true, it is possible that the antagonism by the reducing agent is a specific effect upon this streptomycin-enzyme relationship rather than a direct effect on the streptomycin itself. In fact, addition of the SH compounds and the inorganic reducing agents to a concentrated solution of streptomycin (1,000 units/ml.) does not result in material loss of activity, although Denkelwater and co-workers (*4*) recently reported that this antibiotic in a lower concentration was inactivated by cysteine.

Certain other practical implications are worthy of note. It is conceivable that in certain parts of the body where organic reducing agents are present or where a low oxygen tension exists, larger concentrations of streptomycin may be necessary to inhibit the growth of bacteria. Elias and Durso (*5*) recently reported that typhoid bacilli were isolated from stools in spite of the presence of large concentrations of streptomycin. These investigators suggested the presence in the body of a substance inhibitory to strepto-

mycin. It remains to be seen whether these two phenomena are related.

It is evident that the choice of a medium is important in assaying streptomycin and in determining the susceptibility of bacteria to its antibacterial activity. Because of its antagonism for streptomycin, an infusion medium would be an unwise choice for this purpose. Eventually one of these reducing agents may prove helpful in testing the sterility of streptomycin solutions or in the culturing of fluids from patients under treatment. Cysteine may prove to be such an agent.

*Summary.* The antibacterial activity of streptomycin in infusion agar plate cultures of *E. coli* and other bacteria is diminished by anaerobic incubation. The bacteriostatic activity of this antibiotic for *E. coli* is reduced in the presence of cysteine, sodium thioglycollate, stannous chloride, sodium bisulfite, sodium hydrosulfite, sodium formate, and sodium thiosulfate. Cysteine was the most active of the agents tested. Further investigation is necessary to determine the nature of this interference. It is possible that this phenomenon is related to the mode of action of streptomycin.

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## Water-level Regulator for a Series of High-temperature Water Baths[1]

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It was necessary in this laboratory to conduct a series of tests at 70° C., the nature of which required the use of water as a bath liquid. The tests were carried out in two water baths, and, as efficient covers for the baths were not feasible, the diagrammed system (Fig. 1), set up in less than an hour, served to maintain the levels in both baths to within ¼ in.

In operation, the reservoir bottle, located on a shelf above the water baths, is filled from a tap, J, by closing screw-clamps, I and I′, and opening clamp I″. After the reservoir bottle is filled, clamp I″ is closed and I and I′ are opened. Line D extends below the surface of bath B and serves as a delivery tube, while line E is the regulator and cuts off the supply of air to the reservoir, F, when it is reached by the water level in the bath. Siphon C keeps the level in bath A at the same level as that in B.

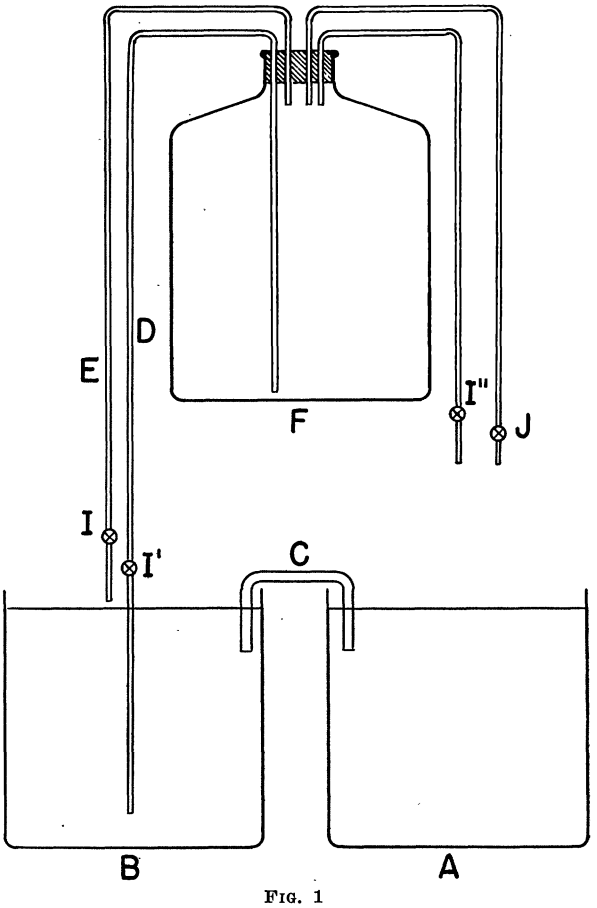


Fig. 1

The baths are of 6-gallon capacity, and the reservoir bottle holds 5 gallons. If the reservoir is filled in the late afternoon, this capacity is sufficient to make up for evaporation overnight. The tubes leading out of the reservoir are of glass, and the cork holding them is sealed in with DeKhotinsky cement. The siphon is of 15-mm. glass tubing to preclude stoppage by air bubbles.

With a sufficiently large reservoir, the system can be used to control a large number of baths. If the cost of the water is neglected, as it can be in most cases, this arrangement has a definite advantage over mineral oil baths in that cleaning of any glassware placed in the baths is much less troublesome.

[1] Contribution from the Industrial Fellowship on Optical Cements, Mellon Institute. Published by permission of the donor, Ordnance Department, U. S. Army.