Antagonism of Salicylate by Pantoyltaurine

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Ivánovics observed (*2*) that the inhibition of growth of *E. coli* and *Staph. aureus* in simple media by low concentrations (M/400–M/500) of salicylic acid was "specifically antagonized" by pantothenic acid and to a lesser extent by pantolactone. He concluded that under these conditions salicylic acid prevented the *synthesis* of pantothenate by inhibiting the formation of α, γ-dihydroxy-β, β-dimethylbutyric acid (pantoic acid). In a "complete" medium (*i.e.* one containing pantothenic acid or its precursor) *E. coli* is not affected by salicylic acid.

Pantoyltaurine, on the other hand, inhibits the growth only of certain bacteria (e.g. hemolytic streptococci) which require preformed pantothenate for growth. It has been considered that pantoyltaurine and other analogues of pantothenic acid act by interfering with the *utilization* of pantothenate (*3, 4*). It was suggested to us[1] that it might be possible to inhibit bacteria such as *E. coli* in a complete medium by some combination of salicylate (to prevent synthesis of pantothenic acid) and pantoyltaurine (to prevent utilization of preformed pantothenate).

Preliminary to experiments in a complete medium we wished to observe the effect of combinations of pantoyltaurine and salicylic acid on the growth of *E. coli* in a chemically defined medium, such as MacLeod's. It was found that whereas inhibition of growth was obtained with salicylate alone (confirming Ivánovics), normal growth occurred with the combination of salicylate and pantoyltaurine. It was thus evident that pantoyltaurine, like pantolactone, antagonized the inhibitory effect of salicylic acid. Table 1 illustrates the antisalicylate effect of this compound. Other analogues tested in the same way (unpublished data) also showed an antisalicylate effect.

These findings suggested that pantoyltaurine is hydrolyzed, yielding pantoic acid which at pH 6.8 would exist as the pantoate ion (*1*). Assuming that hydrolysis of pantoyltaurine is the process involved, it may be calculated from Table 1 that hydrolysis of about 3 per cent could account for the observed effect. This figure was arrived at by considering that the "d" isomer of pantoate is the only active antagonist of salicylate and that pantoate is about nine times more active than pantolactone in antagonizing salicylate inhibition of *E. coli* (*6*). This should be considered an approximation in view of the errors involved in the type of assay illustrated in Table 1.

[1] By Dr. R. O. Roblin, Jr., of these laboratories. McIlwain (*Brit. J. exp. Path.*, 1943, **24**, 203) considered the possibility of a synergistic action of salicylate and pantoyltaurine on *P. morganii* and reported finding synergism under certain conditions. According to Ivánovics (*2*), however, salicylate does not exert a specific antipantothenate action on *P. morganii*. He found that salicylate inhibited *P. morganii* only in high concentration and that the inhibition was not antagonized by pantothenic acid. He considered the inhibition in this case to be due to the protein-denaturing properties of salicylic acid.

TABLE 1

ANTAGONISM OF SALICYLIC ACID INHIBITION OF *E. coli* BY PANTOYLTAURINE

Conc. drugs $\times 10^{-3M}$	10 mg. per cent salicylic acid plus		
	d(−) panto-lactone	dl-sodium pantoyl-taurine	taurine
2	+	+	−
1	+	+	−
1/2	+	+	−
1/4	+	−	−
1/8	+	−	−
1/16	+	−	−
1/32	−	−	−
1/64	−	−	−

Drugs: All drugs used were made up in distilled water, sterilized by filtration and serial, twofold dilutions aseptically made in the medium; medium: MacLeod's (*5*), 5 ml. final pH about 6.8; inoculum: 0.2 ml. of a 10^{-6} dilution of an 18-hour broth culture (MacLeod) per 5 ml. medium; incubation: 37° C.; + = growth visibly equal to controls without drugs; − denotes absence of visible growth after 24 hours. In the absence of any salicylate antagonist, 10 mg. per cent salicylic acid prevented appearance of visible growth for at least three days. In the absence of salicylate, neither pantoyltaurine, pantolactone, nor taurine caused any inhibition of growth.

An alternative explanation for the antisalicylate effect of pantoyltaurine may be that the material was contaminated with the pantolactone used in its preparation. However, quantitative investigation of pantoyltaurine antagonism of salicylate, the details of which will appear in a later publication, does not support this view. For example, according to Table 1, about 25 per cent contamination of dl-pantoyltaurine with dl-pantolactone (which is extremely unlikely) would be required to give the observed effect. Furthermore, the fact that other crystalline and highly purified analogues of pantothenic acid also antagonize salicylate inhibition (unpublished data) suggests that contamination with pantolactone could not be the only explanation.

The data in Table 1, which indicate that a solution of pantoyltaurine contains pantolactone or pantoate, suggest that this analogue (and others which show an antisalicylate effect) would be at a disadvantage in inhibiting the growth of those organisms which can utilize pantolactone or pantoate for the synthesis of pantothenic acid. The amount thus synthesized might be of such an order that the resulting ratio of analogue to pantothenate would be less than that required to prevent growth. Whether this disadvantage is actually sufficient to account entirely for the inactivity of the analogues against such organisms is not known at this time. It should be pointed out that the reduction in analogue concentration which would

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

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