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Influence of Certain Substances on Activity of Streptomycin. I. Modifications in Test Medium.

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It was previously shown[1] that the amount of streptothricin required to inhibit the growth of the test organism, *Klebsiella pneumoniae*, in 1% tryptone broth varied with the lot of tryptone employed in preparing the broth. To this extent we had found that streptomycin behaves in a like fashion. Wallace *et al.*[2] have reported that suspensions of *Eberthella typhosa* showed marked drops in viable cell counts when exposed to streptomycin in the presence of "nutrient broth" and "nutrient broth diluted with an equal amount of water," whereas in brain heart infusion broth the same concentration of streptomycin had no apparent effect. *Staphylococcus aureus* suspensions under similar conditions showed marked drops in viable cell counts in all 3 media during the first 6 hours of exposure to streptomycin, but grew out heavily in brain heart infusion broth during the interval between the 6th and 9th hours of exposure.

The present paper deals with the growth-inhibiting activity of streptomycin in (a) a culture medium containing only tryptone and water or tryptone, dextrose and water, and (b) an enriched medium containing sodium thioglycollate.

A. The Activity of Streptomycin in Culture Media Containing Varying Amounts of Tryptone and Dextrose. In the paper already mentioned[1] it was shown that when streptothricin was added to 2 ml of 6-hour broth cultures of *K. pneumoniae* diluted 10^{-6} in broth consisting of 1% tryptone (or tryptose) and water, from 0.08 to >0.16 units of streptothricin per ml of broth were required to cause complete inhibition of growth, as observed after 17 hours incubation at 37°C. The minimum inhibiting concentration of streptothricin varied with the lot of tryptone or tryptose used. As indicated above, the action of streptomycin on *K. pneumoniae* was similarly affected by the various lots of tryptone. In the present paper it will be shown that even when a single lot of tryptone is used for preparing test media, the minimum inhibiting concentration of streptomycin will vary considerably with the concentration of tryptone used. Further, if dextrose is also added to the medium, the minimum inhibiting concentration will again be increased.

Procedure. Media were prepared containing from 0.5 to 1.0% tryptone (from a given lot) to which were added from 0 to 3% dextrose. A 6-hour culture of *K. pneumoniae* was diluted 10^{-6} in each of these media. The diluted cultures were dispensed into tubes in 2 ml volumes to which were then added increasing amounts of a standard streptomycin solution containing 2.0 units/ml according to the assay procedure referred to above.[1] The minimum inhibiting concentration (MIC) of streptomycin in each medium determined in this fashion is shown in Table I and Fig. 1.

Thus it is seen that as the concentration of tryptone rises in the test medium, the minimum inhibiting concentration of streptomycin increases. To a lesser extent addition of dextrose to the medium also raises the MIC, and interestingly has a more marked effect when added to 1.0% tryptone than to 0.50% tryptone. The addition of increasing amounts of dextrose to tryptone broth causes decreasing end pH values in the broth on autoclaving. Waksman and Schatz[5] have shown that lowering pH of agar lowers the

[1] Donovick, R., Hamre, D., Kavanagh, F., and Rake, G., *J. Bact.*, 1945, **50**, 623.

[2] Wallace, G. I., Rhymer, I., Gibson, O., and Shattuck, M., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 127.

[5] Waksman, S. A., and Schatz, A., *J. Am. Pharmaceut. Assoc.*, 1945, **34**, 273.

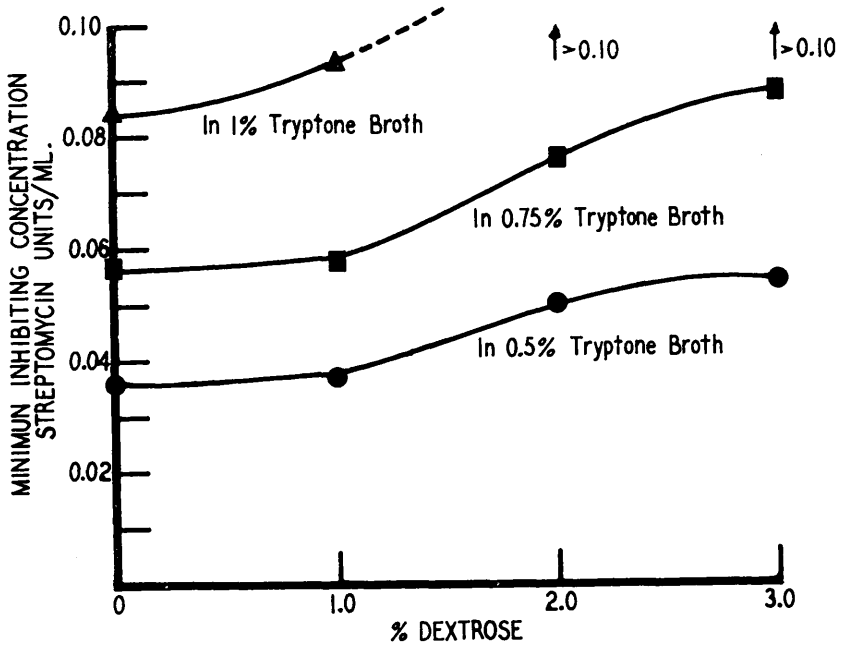

FIGURE I

Effect of Dextrose and Tryptone in Media on Minimun Inhibiting Concentration of Streptomycin for K. pneumoniae

activity of streptomycin in the cup-plate test. We have observed similar effects in broth. Thus the interference caused by dextrose may be due in part to the effect on pH. However this does not explain the finding that dextrose causes greater interference in broth containing 1.0% and 0.75% tryptone than in broth containing 0.5% tryptone since the pH drop with increasing dextrose was identical in all 3 concentrations of tryptone.

*B. The Activity of Streptomycin in an Enriched Medium Containing Sodium Thioglycollate.** Denkewalter, Cook and Tishler[3] have reported that streptomycin and streptothricin are "not inactivated to any significant extent by thioglycollic acid." Their data, however, shows that where a phosphate buffer control of streptomycin assayed at 49 units/ml, control solutions containing in addition 0.50, 2.50 and 3.0 mg respectively of thioglycollic acid per ml, assayed at 31, 39 and 26 units/ml respectively. Similar results were obtained with streptothricin.

In the present studies we have compared the activity of streptomycin in broth consisting of 0.75% tryptone with that in thioglycollate broth containing the following ingredients:†

Cystine	0.75 g
NaCl	2.5 "
Dextrose	5.0 "
Agar	0.75 "
Yeast Extract	5.0 "
Pancreatic digest of casein	15.0 "
Sodium thioglycollate	0.5 "
Resazurin (0.1%)	1 ml
Water to make 1000 ml	

* At the time that this paper was in proof Bondi, Dietz and Spaulding[4] published similar findings on the effects of sodium thioglycolate and other reducing substances on the action of streptomycin.

[3] Denkewalter, R., Cook, M. A., and Tishler, M., *Science*, 1945, **102**, 12.

[4] Bondi, A., Jr., Dietz, C. A., and Spaulding, E. H., *Science*, 1946, **103**, 399.

† Described in a circular from the National Institute of Health (January 15, 1945) revising the culture medium required for sterility testing of biologics.

TABLE I.
Effect of Tryptone and Dextrose on Minimum Inhibiting Concentration of Streptomycin.

% Tryptone	% Dextrose			
	0	1.0	2.0	3.0
		MIC* units/ml		
0.50	0.036	0.037	0.050	0.054
0.75	0.056	0.058	0.076	0.088
1.0	0.084	0.093	>0.10	>0.10

* Minimum inhibiting concentration of streptomycin.

TABLE II.
Comparison of Minimum Inhibiting Concentrations of Streptomycin in Tryptone and Thioglycollate Broths.

MIC in tryptone broth u/ml*	MIC in thioglycollate broth			
	Broth No. 1†		Broth No. 2‡	
	Age of broth§ days	MIC u/ml	Age of broth§ days	MIC u/ml
0.055	3	1.48	1	>2.98
0.053	6	1.37	2	2.41
0.051	7	1.25	3	2.81
0.057	15	1.04	7	1.52
0.059	16	0.99	8	1.35
0.051	23	1.06		
0.045	24	0.69		
0.055	42	0.63		
0.056	43	0.55		

* Units per ml.

† Prepared in this laboratory.

‡ Prepared from dehydrated broth obtained from Baltimore Biological Laboratory, Baltimore, Md.

§ Age of broth indicates the interval between the time of preparation of the broth and the time when the broth was used. During this interval the flasks of broth remained at room temperature.

The media were sterilized by autoclaving at 15 pounds pressure for 20 minutes. After sterilization the pH of both types of media was 7.0-7.2.

A single lot of tryptone was used throughout for preparing the tryptone broth. Two thioglycollate broths, containing the given ingredients, were employed. One was prepared in this laboratory while a second was obtained in a dehydrated form from an outside source. The minimum inhibiting concentrations of streptomycin per ml of test broth are shown in Table II. For the tests a 6-hour culture of *K. pneumoniae* diluted 10^{-6} in the given broth was used.

As is shown in Table II, approximately 27 times more streptomycin was required to inhibit the growth of *K. pneumoniae* in thioglycollate broth No. 1, 3 days after the broth was prepared, than in 0.75% tryptone broth. Forty days later the ratio of MIC values in these 2 broths had dropped to 10:1. In thioglycollate broth No. 2, when used one day after preparation, the minimum inhibiting concentration was 54 times greater than that in tryptone broth. This ratio decreased to about 24.5:1 after the thioglycollate broth had stood for 8 days at room temperature. It was further found that in a broth containing the same ingredients as thioglycollate broth No. 1 in Table II, except that sodium glycollate was substituted for sodium thioglycollate, the minimum inhibiting concentration of streptomycin was about 10 times greater than that in 0.75% tryptone broth. This compares to thioglycollate broth No. 1 after it had stood at room temperature for 43 days.

Thus it is seen that the incorporation of sodium thioglycollate in a culture medium greatly interferes with the activity of streptomycin. Since sodium thioglycollate is readily

oxidized, this interference with streptomycin activity gradually decreases as the broth stands. What may be called the baseline of interference of the broth, which is reached after some 40 days of standing in one thioglycollate medium we have used, is similar to the interference caused by the same medium containing sodium glycollate instead of sodium thioglycollate.

It is interesting that the action of streptothricin is affected to a similar or perhaps even greater extent in thioglycollate broth. The minimum inhibiting concentration of a streptothricin preparation (320 units/mg) which was tested in thioglycollate broth No. 1 (7 days after preparation) was 2.65 units/ml as compared to 0.053 units/ml in 0.75% tryptone broth—a ratio of 50:1.

It perhaps should be pointed out that the concentration of sodium thioglycollate in the media tested was 0.5 mg per ml, while the concentrations said by Denkewalter, *et al.*[3] to cause no appreciable destruction of streptomycin ranged from 0.50 to 3.0 mg per ml. Hence remarkable interference with streptomycin activity may occur without destruction of the streptomycin. Since thioglycollates reduce the oxidation reduction potential of a culture medium, could this interference indicate that streptomycin interferes more with aerobic than anaerobic metabolism?

Summary. Under standard test conditions, raising the concentration of tryptone from 0.50% to 1.0%, in a medium containing only tryptone and water, raised the minimum inhibiting concentration of streptomycin from *0.036* units/ml to *0.084* units/ml. The further addition of glucose to the medium increased the minimum inhibiting concentration to a lesser, but nevertheless, definite degree.

The minimum inhibiting concentration of streptomycin in an enriched broth containing sodium glycollate was about 10 times greater than that in 0.75% tryptone. The substitution of sodium thioglycollate for sodium glycollate further greatly increased the MIC. This additional interference by sodium thioglycollate was observed to decrease as the broth aged (and hence as the thioglycollate was oxidized). Since thioglycollate *per se* is said to cause no significant destruction of streptomycin, it is proposed that its interfering action may be due to its role in reducing the oxidation-reduction potential of the medium and that perhaps streptomycin interferes more with the aerobic than anaerobic metabolism of the test organism.

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Metabolism of Glycine by the Completely Isolated Mammalian Heart Investigated with Carboxyl-Labeled Glycine.*

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When acetate containing heavy carbon, C^{13}, in the carboxyl position is administered to the completely isolated, working, mammalian heart, the isotope appears in the respiratory CO_2 rapidly and in large amounts, indicating that the 2-carbon chain is readily broken and converted to CO_2, in amounts approximating 20-30% of the total CO_2 output of the heart.[1] In the present experiments, glycine labeled with an excess of C^{13} in the carboxyl carbon, was injected into the blood perfusing the

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[1] Lorber, V., Lifson, N., Wood, H. G., and Barcroft, J., *Am. J. Physiol.*, 1946, **145**, 557.