THE EFFECT OF STREPTOMYCIN DERIVATIVES ON STREPTOMYCIN-DEPENDENT AND -RESISTANT STRAINS OF BACTERIA[1]

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Several authors (1, 2) have recently demonstrated the value of streptomycin-dependent strains of bacteria for the identification of streptomycin-producing organisms. The occurrence of streptomycin-dependent mutants of various bacteria was first demonstrated by Miller and Bohnhoff (3, 4). The streptomycin-dependent mutants were found in cultures which also contained streptomycin-resistant strains of the usual type.

In the case of the streptomycin-dependent strains, these organisms, according to Iverson and Waksman, apparently utilize streptomycin as a true growth factor. The authors stated that streptomycin is not used as a substrate or a nutrient since no destruction of streptomycin occurred in media in which the streptomycin-dependent strains were grown.

Accepting the suggestion that streptomycin represents a true growth factor for the mutant forms discussed above, it was of interest to determine whether any synthetic streptomycin derivatives could act either as anti-metabolites vis-à-vis the "growth factor" streptomycin or possibly as substitute growth factors for a streptomycin-dependent organism. It was also thought of interest to extend the study to include the effects of various streptomycin derivatives on streptomycin-resistant bacteria, as well.

Various streptomycin derivatives were therefore tested for antistreptomycin or streptomycin-like activity. The derivatives examined included dihydrostreptomycin (5) and certain N-substituted straight chain alkyl streptomycylamines first described by Winsten (6).

In testing different preparations of the streptomycylamine derivatives, it was necessary to insure that each derivative was tested for its effects free of any residual unchanged streptomycin or dihydrostreptomycin formed during the course of the synthesis of the streptomycylamine derivatives by catalytic hydrogenation of the corresponding Schiff bases. This could be most conveniently done by use of the paper chromatographic method of Winsten and Eigen (7).

METHODS

In carrying out the present study a solution of each preparation of a derivative (or derivatives) was first subjected to paper chromatographic separation. The resulting paper chromatograms were then laid on agar seeded with *E. coli* Strain E 158, a streptomycin-dependent strain obtained through the courtesy of Dr. R. J. Vanderlinde.

A desired amount of streptomycin was included in the Difco nutrient agar, in order to insure sufficient background growth of *E. coli* E 158 where this was desired. After incubation the substances being tested either caused enhanced zones of growth or zones of inhibition, seen along the loci of the strip chromatograms. The zones of growth were seen against the lighter background growth caused by the streptomycin in the medium. The zones of inhibition were seen as on usual plates against the background bacterial growth. Indeed it sometimes occurred that a locus of a chromatogram exhibited both a zone of growth and a zone of inhibition. This situation occurred whenever a preparation of a derivative which was an inhibitor also contained unchanged streptomycin or dihydrostreptomycin which caused zones of growth. A schematic drawing of such a chromatogram is shown in Figure 1 for the case of an N-n-hexylstreptomycylamine preparation.

RESULTS

The results of a series of experiments in which dihydrostreptomycin, streptomycin and different preparations of the N-substituted straight chain alkylstreptomycylamine derivatives were first subjected to paper chromatography and then tested against *E. coli* E 158 are given in Table I.

From the table it is apparent that streptomycin and dihydrostreptomycin cause zones of growth of *E. coli* E 158, when the agar contains 10 γ/ml of streptomycin. At a level of 100 γ/ml of streptomycin the background growth was quite heavy, thus obscuring zones of growth caused by these two substances.

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Rake (8) has also reported that organisms which require streptomycin for growth can also use dihydrostreptomycin for the same purpose.

It is also evident from Table I that in the presence of 10 γ of streptomycin per ml of agar, N-n-propylstreptomycylamine did not cause a zone of growth or of inhibition. The N-n-butyl derivative and higher homologues caused zones of inhibition. In the presence of 100 γ of streptomycin per ml of agar, the N-n-propyl and N-n-butyl derivatives caused no zones of inhibition whereas the higher homologues did so. The N-n-hexyl derivative appears to be a borderline case; in one experiment a zone of inhibition hazy in character was observed whereas in a second experiment no zone of inhibition was observed.

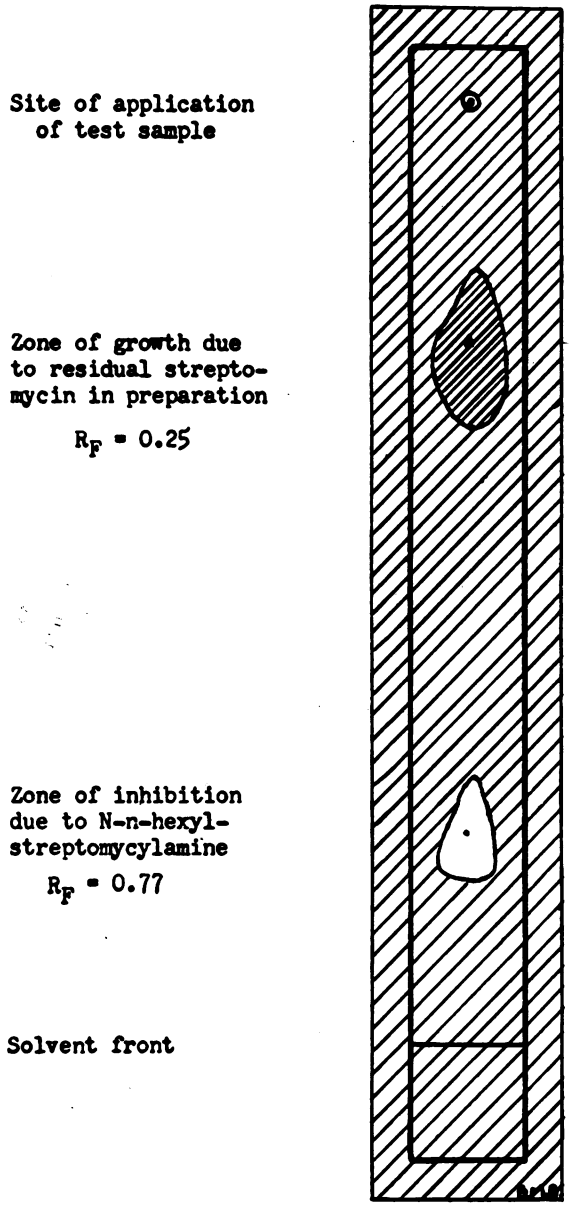

FIG. 1. SCHEMATIC DIAGRAM OF LOCUS OF A CHROMATOGRAM ON AN AGAR PLATE SEEDED WITH STREPTOMYCIN-DEPENDENT STRAIN *E. coli* E 158

The more widely spaced diagonal lines represent the lighter background bacterial growth caused by the presence of 10 γ of streptomycin per ml of agar. The chromatogram was developed for 18 hours on Whatman No. 1 paper using as a solvent wet butanol-2% piperidine-2% p-toluenesulfonic acid.

TABLE I

The effect of streptomycin and its derivatives on E. coli *E158, seeded into agar containing streptomycin*

G—indicates a zone of growth seen against the background bacterial growth. I—represents a zone of inhibition. N—represents neither a zone of growth nor of inhibition.

Substance studied*	Zones found	
	Amount of streptomycin in agar	
	10 γ per ml	*100 γ per ml*
Streptomycin	G	N
Dihydrostreptomycin	G	N
N-n-propylstreptomycylamine	N	N
N-n-butylstreptomycylamine	I	N
N-n-hexylstreptomycylamine	I	I, N†
N-n-octylstreptomycylamine	I	I
N-n-decylstreptomycylamine	I	I
N-n-dodecylstreptomycylamine	I	

* 10-microliter samples of 1–6% solutions of the various preparations were chromatographed on Whatman No. 1 or No. 4 paper for 18 hours using butanol-2% piperidine-2% p-toluenesulfonic acid as the developing solvent.

† The N-n-hexyl derivative in one experiment caused a hazy zone of inhibition. In a second experiment it caused no zone.

From these findings for the streptomycin-dependent *E. coli* E 158, there would appear to be an interesting metabolite-antimetabolite relationship between streptomycin and various N-substituted streptomycylamine derivatives which is some function of the length of the substituent side chain.

The above studies have been extended to a streptomycin-dependent strain of *S. aureus*. Starting with *S. aureus* SM, there was isolated an organism which grew readily in broth containing 1000 γ/ml of streptomycin. The culture was found to contain organisms which required streptomycin for growth. It was possible by subculture in streptomycin-free media to obtain organisms which grew in the absence of and were resistant to streptomycin.

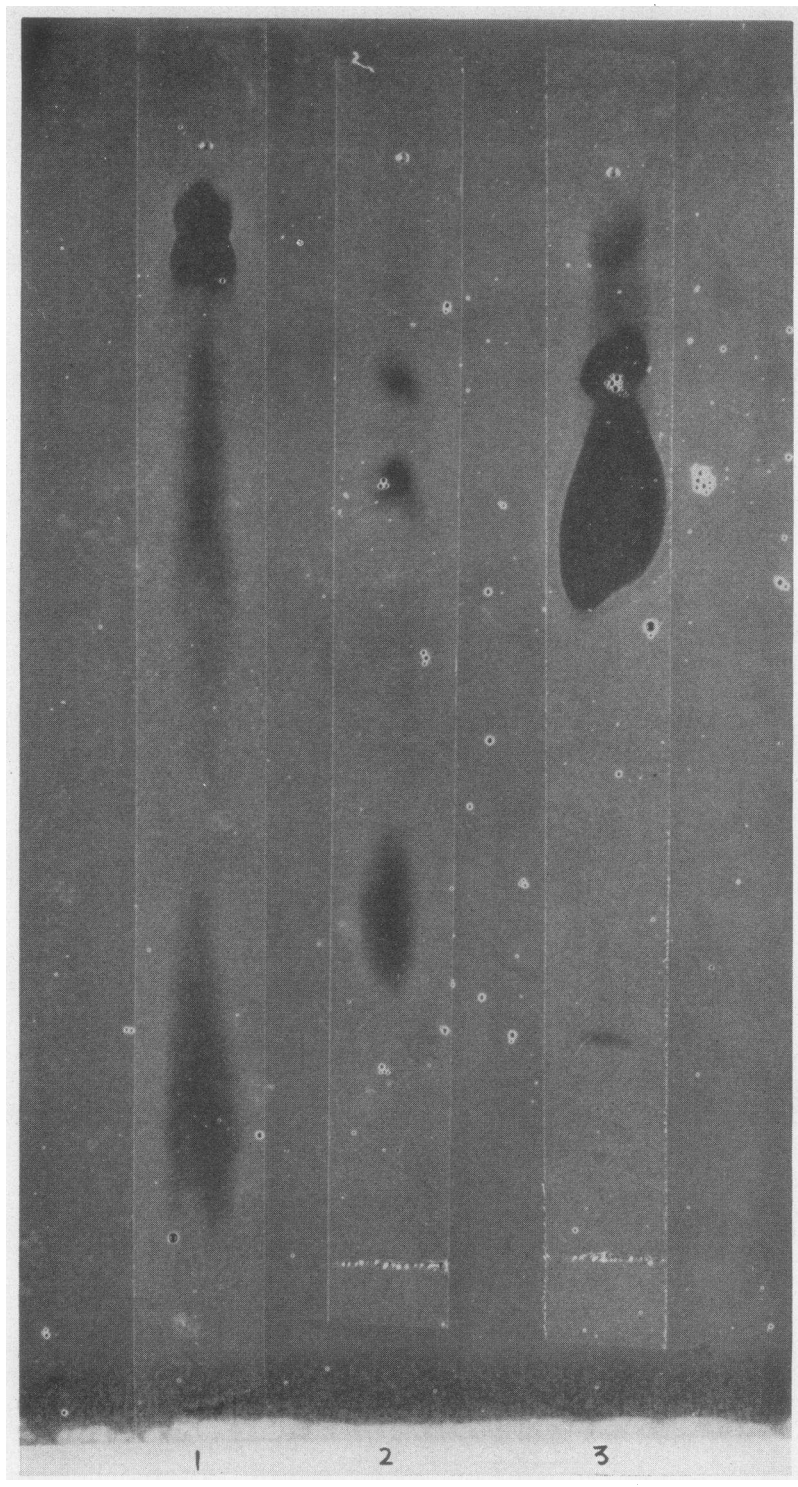

FIG. 2. LOCI OF CHROMATOGRAMS ON AGAR PLATE SEEDED WITH *S. aureus* H

A 10-microliter sample of a 3% solution of each preparation was chromatographed for 18 hours at room temperature using wet n-butanol-2% piperidine-2% p-toluenesulfonic acid as the developing solvent: Strip 1, N-n-propylstreptomycylamine preparation on Whatman No. 4 paper; Strip 2, N-n-butylstreptomycylamine preparation on Whatman No. 1 paper; Strip 3, N-n-hexylstreptomycylamine preparation on Whatman No. 1 paper (see text for discussion of results).

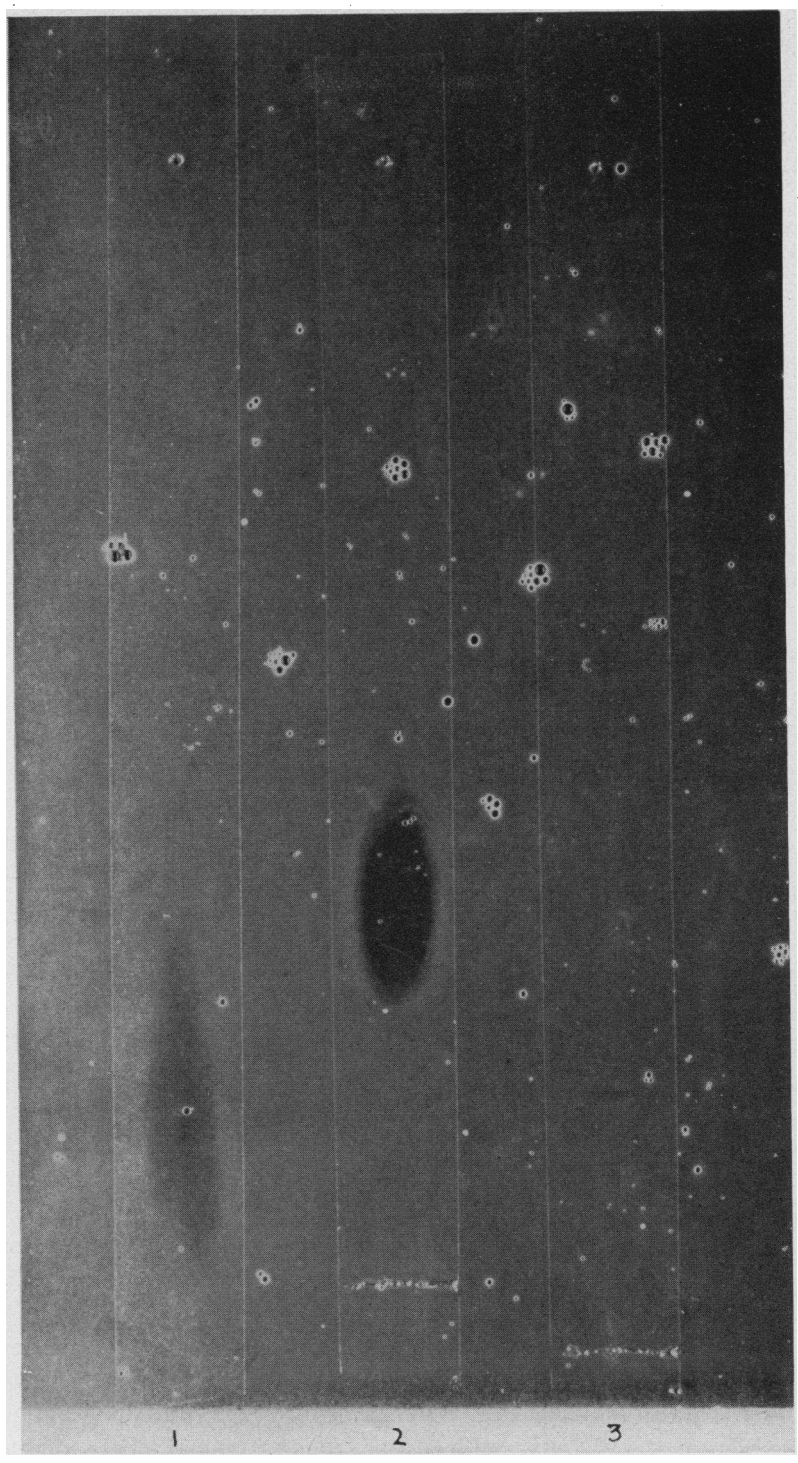

FIG. 3. LOCI OF CHROMATOGRAMS ON AGAR PLATE SEEDED WITH A HIGHLY STREPTOMYCIN-RESISTANT STRAIN OF *S. aureus*

A 10-microliter sample of a 3% solution of each preparation was chromatographed 18 hours at room temperature using wet n-butanol-2% piperidine-2% p-toluenesulfonic acid as the developing solvent: Strip 1, N-n-propylstreptomycylamine preparation on Whatman No. 4 paper; Strip 2, N-n-butylstreptomycylamine preparation on Whatman No. 1 paper; Strip 3, N-n-hexylstreptomycylamine preparation on Whatman No. 1 paper (see text for discussion of results).

TABLE II

The effect of streptomycin and its derivatives on a mixed culture of S. aureus *containing both streptomycin-dependent and -resistant strains of bacteria*

G—indicates a zone of growth seen against the background bacterial growth. I—represents a zone of inhibition. 30 γ per ml of streptomycin was included in the agar.

Substance studied*	Zones found
Streptomycin	G
Dihydrostreptomycin	G
N-n-propylstreptomycylamine	G
N-n-butylstreptomycylamine	G
N-n-hexylstreptomycylamine	G
N-n-octylstreptomycylamine	I
N-n-decylstreptomycylamine	I
N-n-dodecylstreptomycylamine	I

* 10-microliter samples of 1–6% solutions of the various preparations were chromatographed on Whatman No. 1 or No. 4 paper for 18 hours at room temperature using butanol-2% piperidine-2% p-toluenesulfonic acid as the developing solvent.

It is therefore likely that the culture containing the streptomycin-dependent organisms also contained a resistant strain as well. Using this mixed culture seeded into agar containing 30 γ/ml. of streptomycin to promote the background growth of the dependent strain, the results recorded in Table II were obtained in a manner similar to that described earlier for *E. coli* E 158. Inspection of the data shows that streptomycin, dihydrostreptomycin and the shorter chain streptomycylamine derivatives up to hexyl caused zones of growth. The longer chain length derivatives caused zones of inhibition.

It is interesting to compare these results for a streptomycin-dependent strain of *S. aureus* with those obtained for *E. coli* E 158. It will be recalled that in the case of *E. coli* the N-n-propylstreptomycylamine derivative caused neither a zone of growth or of inhibition. On the other hand the streptomycin-dependent strain of *S. aureus* can utilize not only the N-n-propyl derivative for growth but the N-n-butyl and N-n-hexyl derivatives as well, both of which two latter derivatives inhibit *E. coli* E 158.

It would therefore appear that there are differences of major importance between different streptomycin-dependent organisms with regard to their response to streptomycylamine derivatives.

In the light of the above findings it was of interest to extend these studies to a streptomycin-resistant organism. It is well known that an organism resistant to streptomycin is also resistant to dihydrostreptomycin. It was anticipated that some streptomycin-resistant bacteria might be sensitive to various members of the N-substituted streptomycylamine family of antibiotics. Such appears to be the case for at least one highly streptomycin-resistant strain of *S. aureus*.

Preparations of N-n-propyl-, N-n-butyl- and N-n-hexylstreptomycylamine were first tested, using the paper chromatographic technique, against the parent strain *S. aureus* H from which a highly streptomycin-resistant strain was derived. The results of this experiment appear in Figure 2.

Inspection of Figure 2 reveals that the N-n-propyl preparation contained the faster moving derivative itself, some dihydrostreptomycin, and two slow moving antibiotics of unknown structure which formed a curious doublet zone of inhibition. The zones of inhibition appear somewhat smeared; this was probably due to the use of Whatman No. 4 paper, a chromatographically very fast paper.

Figure 2 also reveals that the N-n-butylstreptomycylamine preparation, chromatographed on the slower Whatman No. 1 paper, exhibited a zone due to the fast moving N-n-butyl derivative itself as well as two small zones of inhibition due to dihydrostreptomycin (the slowest antibiotic in the preparation) and streptomycin.

Chromatographic analyses of the N-n-hexylstreptomycylamine preparation used in this experiment reveal in Figure 2 that the particular preparation used contained only a trace of the fast moving N-n-hexyl derivative itself. The main zone of inhibition observed in Figure 2 for this preparation is due to streptomycin itself. The N-n-hexyl preparation therefore merely serves to provide streptomycin as a control substance in the plate chromatographic test of Figure 2.

In Figure 3 are shown the results of chromatographing identical amounts of the N-n-propyl, N-n-butyl, N-n-hexylstreptomycylamine preparations studied in Figure 2, using a highly streptomycin-resistant strain of *S. aureus* derived from *S. aureus* H as the test organism. From the figure it is evident that only the N-n-propyl- and N-n-butylstreptomycylamine derivatives caused readily visible zones of inhibition. If one examines the chromatogram of the N-n-hexyl preparation, the large residual streptomycin zone present in Figure 2 for the parent *S. aureus* H is now completely absent for the highly resistant daughter strain of

S. aureus derived from H. A trace zone due to the N-hexyl derivative itself can be seen on careful inspection of Figure 3 at about the same point as in Figure 2. In a separate experiment a second preparation of N-n-hexylstreptomycylamine containing a larger amount of the derivative as is evident in Figure 1 caused a larger zone of inhibition at a comparable point on the chromatogram. The zone was present when either *S. aureus* H or its streptomycin-resistant offspring was used as the test organism.

In still other experiments the N-n-octyl- and the N-n-decylstreptomycylamine derivatives caused zones of inhibition when using the resistant *S. aureus* as the test organism. These zones however were turbid or hazy, suggesting merely a retarded growth of the organism rather than complete inhibition.

The findings in Figure 3, wherein a highly streptomycin-resistant strain of *S. aureus* was inhibited by the N-n-propyl and the N-n-butyl derivatives, were confirmed in serial dilution tube assays. Whereas the resistant strain derived from *S. aureus* H was not inhibited by as much as 10,000 γ per ml of streptomycin, it was completely inhibited in a 24 hour test by 5 γ per ml of the N-n-propyl derivative or 4 γ per ml of the N-n-butyl derivative. 25 γ per ml of the N-n-decyl derivative were needed for inhibition while the N-n-octyl derivative failed to inhibit as 100 γ per ml (the highest amount tried). *S. aureus* H itself was inhibited by 1 γ per ml of streptomycin, 16 γ per ml (or less) of the N-n-propyl derivative and 63 γ per ml of the N-n-butyl derivative.

DISCUSSION

The above findings suggest that it may be possible to obtain N-substituted streptomycylamine derivatives which are active against streptomycin-resistant strains of bacteria. The results indicate however that a derivative active against one streptomycin-resistant organism may not be active against another. This is based on the findings reported earlier in this paper where the N-n-butyl derivative could be used for growth by one streptomycin-dependent organism (an *S. aureus*), but caused inhibition of another streptomycin-dependent strain (*E. coli* E 158) by antagonizing the growth promoting influence of streptomycin.

The results reported in this paper suggest that it may be possible to obtain a particular streptomycin derivative active against a particular streptomycin-resistant strain of bacteria. Further studies along this line will be reported at a later date.

SUMMARY

1. A streptomycin-dependent strain of *E. coli* was inhibited by various members of an homologous series of N-substituted alkyl streptomycylamines, in the presence of streptomycin.

2. A streptomycin-dependent strain of *S. aureus* was found capable of using N-n-propyl-, N-n-butyl-, and N-n-hexylstreptomycylamine for growth, replacing the need for streptomycin. Higher homologues inhibited the growth of the organism in the presence of streptomycin.

3. A streptomycin-resistant strain of *S. aureus* was inhibited markedly by N-n-propylstreptomycylamine, N-n-butylstreptomycylamine, and N-n-decylstreptomycylamine.

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