

Bactericidal Action of Streptomycin-Penicillin Mixtures *In vitro*.

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Studies were carried out in order to determine whether streptomycin and penicillin in combination were synergistic in their bactericidal action against 4 common organisms. Synergism is defined for the purpose of this paper as a cooperative action greater than would be predicted were the action of the agents mathematically additive.

Methods. On the basis of preliminary experiments, all media were adjusted to a sodium chloride concentration of 0.25% and to a pH of 7.6. All tests were performed in beef infusion broth (Difco) containing 1% neopeptone. The bacteria were grown at 37°C and a 6-hour culture was diluted so as to contain approximately 500,000 bacteria per/ml of media. One ml of this dilution was used as the inoculum.

Test organisms were *Staphylococcus aureus* Oxford H, *Staphylococcus aureus* No. 209, *Staphylococcus aureus* SM, and *Streptococcus hemolyticus* C-203 MV. All organisms were tested separately for susceptibility to penicillin and streptomycin. Titration of penicillin was made in 2 ml of medium and 1 ml of standard inoculum was added. The cultures were inoculated at 37°C. Readings were made at 24 hours compared with a bacterial control and subcultures were made of each tube to blood agar to determine the presence of growth. Streptomycin titrations were made in the same manner. This method determined the minimum lethal concentration of penicillin and of streptomycin for each organism. In each instance it was also determined that growth did occur in concentrations of 75% or more of this minimum lethal concentration.

Using the minimum lethal concentration as a guide, titrations of the combined antibiotics were set up. A penicillin titration was made to which varying amounts of streptomycin were added. Penicillin, streptomycin, and

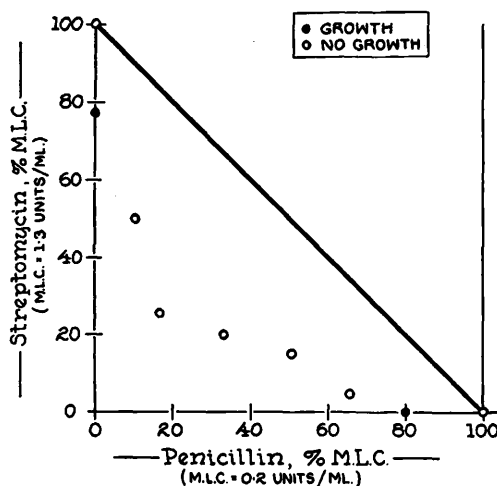


FIG. 1.

Staphylococcus aureus Oxford H.

bacterial controls were set up with each titration. Subcultures were made to blood agar and compared with subcultures of the antibiotic control.

Experimental Results. Fig. 1 is a typical graph for *Staphylococcus aureus* Oxford H. The minimum lethal concentration of penicillin was 0.2 unit per ml and for streptomycin was 1.3 units per ml. The diagonal line represents bactericidal combinations of penicillin and streptomycin which should be bactericidal if these two antibiotics were additive in their effect. For any given concentration of penicillin, the value representing the smallest amount of streptomycin which rendered the mixture bactericidal is plotted on the graph as a circle. For instance in this case, when the dose of penicillin was reduced to 16% M.L.C., the mixture was bactericidal when 25% M.L.C. of streptomycin was present.

In Table I are presented the results of similar studies on three strains of staphylococci and one strain of streptococci.

TABLE I.
Bactericidal Combinations of Penicillin and Streptomycin for Various Pure Cultures.

Organism	% M.L.C.* Streptomycin	% M.L.C.* Penicillin	Sum
Hemolytic streptococcus C-203 MV	5.6	50.7	56.3
	10.0	25.3	35.3
	14.3	23.0	37.3
	28.7	15.3	44.0
	43.4	5.0	48.4
	56.5	2.5	59.0
<i>Staphylococcus aureus</i> No. 209	11.0	76.0	87.0
	13.0	61.0	74.0
	14.0	50.0	64.0
	28.0	38.0	66.0
	43.0	25.0	68.0
	56.0	12.0	68.0
<i>Staphylococcus aureus</i> Oxford H	15.3	50.0	65.3
	20.0	33.0	53.0
	25.3	16.0	41.3
	50.7	10.0	60.7
<i>Staphylococcus aureus</i>	10.0	76.1	86.1
	15.3	50.7	66.0
	25.3	20.0	45.3
	76.1	10.0	86.1
<i>Staphylococcus aureus</i> SM	10.0	76.1	86.1
	15.3	25.3	40.6
	25.3	20.0	45.0
	50.7	15.3	66.0
<i>Staphylococcus aureus</i> SM	10.0	50.7	60.7
	15.3	25.3	40.6
	25.3	20.0	45.3
	50.7	15.3	66.0
	76.1	10.0	86.1

* M.L.C. Minimum Lethal Concentration of each antibiotic alone for the particular organism determined to within 25% (i.e., the organisms are known to grow at 75% M.L.C. or higher.)

Summary. Under the experimental conditions used, definite synergism was noted in the bactericidal action of penicillin and streptomycin upon staphylococci and streptococci *in vitro*.

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A Complement Fixation Test for Dengue.*

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Studies carried out by one of us (A. B. S.) since 1944 have established the fact that several immunologically distinct but related types of virus are responsible for the clinically typical and atypical forms of dengue.¹ Since

the adaptation of dengue virus to mice,² Sabin and Schlesinger found that the neutralizing antibodies which developed following infection were so highly type-specific, that by means of the neutralization test³ in mice one