EFFECT OF COMBINATIONS OF ANTIBIOTICS ON LYSIS OF STAPHYLOCOCCUS AUREUS BY PENICILLIN

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In the presence of penicillin, suspensions of staphylococci tend to increase greatly in density for a few hours and then undergo rapid clearing. The increase in density has been shown to be due chiefly to swelling rather than multiplication, and the rapid clearing is caused by lysis of the organisms (Chain and Duthie, 1945). This phenomenon has been observed with visual methods of comparing the turbidity of bacterial suspensions (Todd, 1945; Spicer and Blitz, 1948) and has been measured more accurately with the aid of a spectrophotometer (Chain and Duthie, 1945; Kirby, 1945). It is not known whether penicillin exerts a specific lytic action upon staphylococci or whether they are destroyed by autolytic enzymes once they become nonviable. Bronfenbrenner and Muckenfuss (1927) found some years ago that staphylococcal autolysin has a powerful lytic action upon dead, but not living, staphylococci. This same is true of pneumococci (Avery and Cullen, 1923) which also are lysed by penicillin. On the other hand, hemolytic streptococci, which do not contain an autolysin, are said to undergo lysis in the presence of penicillin (Todd, 1945; Spicer and Blitz, 1948).

It has been shown recently that aureomycin, chloramphenicol, and oxytetracycline interfere with the bactericidal action of penicillin against hemolytic streptococci and enterococci (Jawetz, 1952). The present experiments were undertaken to study the effects of combinations of antibiotics upon killing and lysis of staphylococci. Striking alterations were observed in the lysis curves, and evidence was obtained which further clarifies the nature of lysis in the presence of penicillin.

MATERIALS AND METHODS

Overnight cultures of penicillin sensitive *Staphylococcus aureus* were diluted in tryptose phosphate broth to an optical density of 0.15 to 0.20 on the Coleman spectrophotometer at a wavelength of 550 mμ, and equal amounts (9 ml) of the diluted suspensions were pipetted into a series of sterile tubes. Penicillin and other antibiotics were diluted in broth and added to the suspensions in appropriate concentrations so that each tube contained a final volume of 10 ml. Optical density (turbidity) was recorded at intervals during 2 or 3 days' incubation at 37 C. Plate counts were performed to correlate the numbers of viable organisms with alterations in turbidity. In all, 6 different strains of *S. aureus* were studied, and each experiment was repeated on at least 10 occasions.

RESULTS

Aureomycin, oxytetracycline, and chloramphenicol all caused a marked modification of the lytic action of penicillin upon the staphylococcus. A typical experiment utilizing chloramphenicol is illustrated in figure 1. With penicillin alone, in a concentration of 0.1 unit per ml, the characteristic increase in turbidity during the first few hours is clearly shown, followed by rapid lysis which was maximal at ten hours. The addition of increasing concentrations of chloramphenicol from 10 through 80 μg per ml caused a progressive delay in lysis of the organisms. With concentrations above 80 μg per ml, i.e., 200 and 500 μg, the lysis curve was identical with that caused by 80 μg. Associated with the delay in lysis there was a progressive increase in turbidity so that with the higher concentrations of chloramphenicol (80 μg per ml or more) the maximal turbidity was greater than with penicillin alone. The results with aureomycin and oxytetracycline were similar to those produced by chloramphenicol. One to 10 μg per ml of these antibiotics altered the lysis curves to approximately the same extent as 10 to 80 μg per ml of chloramphenicol. Higher concentrations of aureomycin and oxytetracycline could not be studied because color changes caused by these antibiotics invalidated the turbidity readings. Changes in color when aureomycin and oxytetracycline are incubated in broth also have been noted by Jackson and Finland (1951).

In order to correlate these observations with viability studies, plate counts were performed at

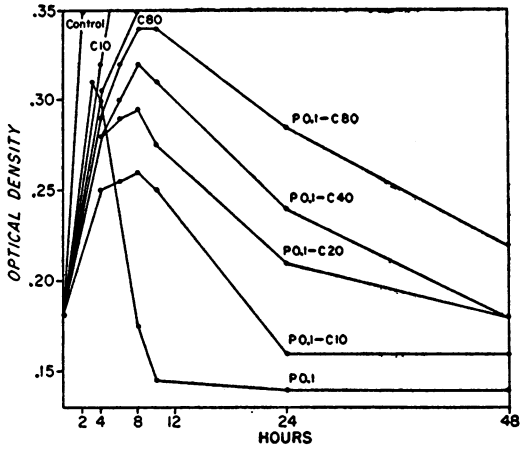

Figure 1. The effect of various concentrations of chloramphenicol upon the lysis of *Staphylococcus aureus* by penicillin.

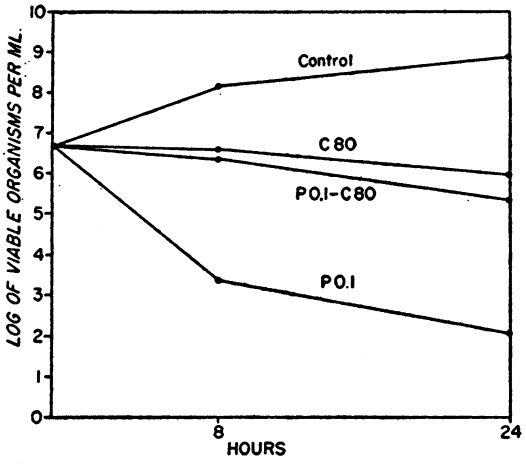

Figure 2. Results of plate counts performed to correlate the turbidity readings of figure 1 with the number of viable organisms at various intervals. The delay in lysis observed when both antibiotics were present was associated with a marked decrease in the rate of killing.

4, 8, and 24 hours. The results are shown in figure 2. With penicillin alone the marked swelling and lysis were associated with rapid killing of the bacteria. With chloramphenicol there was little change in the number of viable organisms, and lysis did not occur. With the combination, the delay in swelling and lysis were clearly correlated with a marked decrease in the rate of killing as compared with penicillin alone.

Time relationships were studied then. The effect on the lysis curves of adding chloramphenicol 80 μg per ml one-fourth, one, and two hours later than penicillin is shown in figure 3. Briefly, interference with the typical penicillin curve became progressively less marked as the time interval increased. At 2 hours the curve was similar to that produced by penicillin alone although a greater amount of lysis was produced by the combination. This observation was confirmed on many occasions, and its explanation is not entirely clear. When the situation was reversed, i.e., when penicillin was added one-fourth, one, and two hours after chloramphenicol, the curves became progressively more characteristic of those produced by chloramphenicol alone. The results of this last procedure are not shown in the figure.

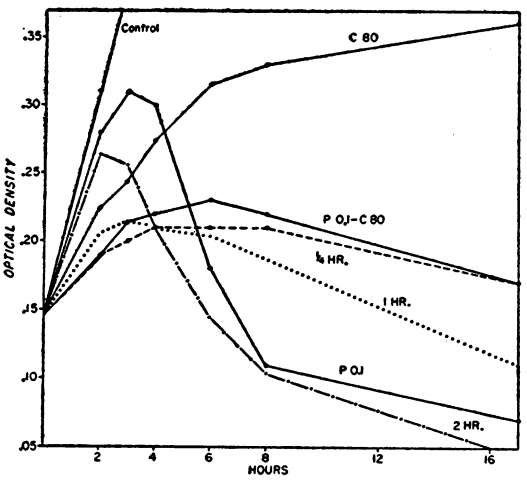

Figure 3. The effect on turbidity readings of adding chloramphenicol at various intervals after the action of penicillin has begun.

DISCUSSION

Remarkable alterations in the typical swelling and lysis of *S. aureus* in the presence of penicillin have been found to occur in the present studies when the bacteria are exposed to combinations of antibiotics. With the addition of aureomycin, oxytetracycline, or chloramphenicol, alterations in the pattern of lysis appear to be closely related to a slowing of the rate of killing as compared with that of penicillin alone. These observations are in keeping with the view that lysis results from the action of autolytic enzymes upon dead bacteria, rather than as a result of a specific lytic action of penicillin. Autolytic enzymes of this sort are known to occur in staphylococci (Bronfenbrenner

and Muckenfuss, 1927) and pneumococci (Avery and Cullen, 1923).

The present studies were carried out following the demonstration by Jawetz that aureomycin, oxytetracycline, and chloramphenicol interfere with the bactericidal action of penicillin against hemolytic streptococci and enterococci (Jawetz, 1952). These results with *S. aureus* confirm those of Jawetz in all respects, including the observations concerning the effects of adding the antibiotics at different time intervals. The chief differences are concerned with the unique swelling and lysis which characterize the response of *S. aureus*.

The nature of the interference of the broad spectrum antibiotics with the action of penicillin is unknown. It is of interest to note that the effect of adding chloramphenicol to a low concentration of penicillin appears to be identical with that of adding a much larger amount of penicillin. With 100 units per ml of penicillin, for example, swelling and lysis are delayed and staphylococci die at a much slower rate than with 0.1 unit per ml of penicillin (Kirby, 1945). This so-called "zone phenomenon" has been studied extensively by Eagle and Musselman (1948) and has been found to occur with a number of bacteria.

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SUMMARY

The marked swelling and lysis of *Staphylococcus aureus* which occur in the presence of penicillin are greatly altered by the addition of aureomycin, oxytetracycline, or chloramphenicol. Lysis is delayed, and with higher concentrations of chloramphenicol the amount of swelling is greater than with penicillin alone. These changes are correlated closely with a delay in the rate of killing in the presence of the combinations.

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