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Received September 28, 1962. P.S.E.B.M., 1962, v111.

Inactivation of Methicillin, Oxacillin and Ancillin by *Staphylococcus aureus.**† (27850)

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The effect of penicillin G (benzyl penicillin) on penicillinase-producing staphylococci (1-3) and on some gram-negative penicillinase-producing organisms(3) has been shown to depend in part on the size of the inoculum. The individual cell, or small concentrations of cells of different strains of *Staphylococcus aureus*–both penicillinase-producers and non-penicillinase-producers–have been found to be about equally susceptible to small concentrations of penicillin G(1). Moderate concentrations of some alpha phenoxyalkyl penicillins (*e.g.,* phenethicillin and propicillin) appear to be active *in vitro* against somewhat larger inocula of penicillinase-producing staphylococci than the same concentrations of penicillin G but are equally ineffective against larger inocula of the same strains(4, 5). Only a few of the semisynthetic penicillins, such as methicillin(4,5), oxacillin(6) and ancillin(7) have *in vitro* activity that is virtually independent of inoculum size. The latter penicillins, which are active against large inocula of penicillinase-producing staphylococci have been generally considered to be resistant to that enzyme(8).

In the course of attempts in this laboratory to develop (select) staphylococcal strains resistant to some of the semisynthetic penicillins, it was found that a drop of a fully grown culture added to 0.5 ml of broth containing 100 or 200 μg of ancillin per ml and incubated at 37°C resulted in growth of the staphylococci although the minimum inhibiting concentration (MIC) of this penicillin for the same culture in the conventional test was only 0.4-0.8 μg/ml. The inoculum in the latter test provides about 10^5 viable units per ml, whereas about 1000 times that number was provided in the former. The MIC of ancillin for the staphylococcus used was the same when the starting inoculum of 10^5 viable units was provided from a culture that had never been exposed to the antibiotic or when it was obtained from a culture previously grown in the presence of 100 μg/ml from the larger inoculum.

Because these findings suggested that the drug may have been inactivated by the larger inoculum of staphylococci, experiments were performed in which simultaneous determinations of residual antibiotic activity and of the

* Aided by a grant from Nat. Inst. of Health.

† Methicillin (2,6-dimethoxyphenyl penicillin, X1497, Staphcillin) and oxacillin (5-methyl-3-phenyl-4-isoxazolyl penicillin, P12, Prostaphlin) were provided by Bristol Laboratories; ancillin (2-biphenyl penicillin, SKF 12141) was supplied by Smith, Kline and French Laboratories; each of these 3 penicillins was furnished as the hydrated sodium salt.

number of viable cells (units) were made at appropriate intervals after inoculation in order to determine the relation between disappearance of the drug and changes in the bacterial population.

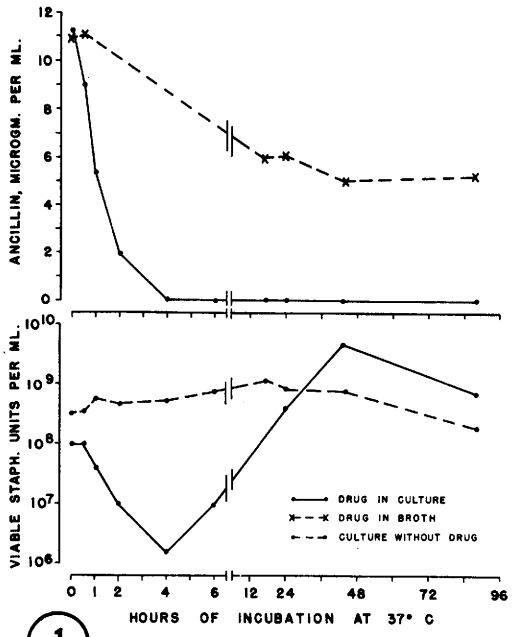
Materials and methods. The antibiotics tested were methicillin(4), oxacillin(6) and ancillin(7)–3 semisynthetic penicillins said to be highly resistant to staphylococcal penicillinase. The starting concentrations used were about 15 times the "usual MIC" as determined in the 2-fold dilution test in brain heart infusion broth with an inoculum of about 10^5 viable units per ml; actual concentrations used, in μg/ml, were: methicillin 50, oxacillin 6, and ancillin 12. The test organism used throughout was *Staph. 60/1,* a clinical isolate that was hemolytic, coagulase positive, phage type 80/81/KS6, resistant to >200 μg/ml of penicillin G(5) and shown to produce extracellular diffusible penicillinase (9). In each experiment an Erlenmeyer flask containing 60 or 120 ml of brain heart infusion broth together with antibiotic and organisms was incubated at 37°C. Control flasks containing broth with either drug or organism, but not both, were incubated at the same time to determine the effect of incubation on the number of viable organisms and on the antibiotic potency. Two additional controls were run in the experiment with oxacillin, namely, organisms plus antibiotic in broth at 4°C, and organisms and antibiotic in phosphate bufler, pH 6.4 at 37°C. The inoculum used in each experiment was obtained from a 5-6-hour growth of culture and was adjusted to provide a starting concentration of about 10^8 viable units per ml. At specified intervals, 10 ml of culture were removed; 0.5 ml were used for agar plate counts of viable organisms and the rest was passed through a Seitz filter. This permitted recovery of all of the antibiotic entirely free of staphylococci and of the staphylococcal penicillinase(9). The filtrates were stored in screw-capped tubes at –20°C until they were assayed for residual antibiotic by the *Sarcina lutea,* agar-diffusion (cylinder plate) method of Grove and Randall(10). Finally, the MIC of each of the organisms that grew in the presence of the antibiotics and of a subculture of the parent strain was determined simultaneously using a starting inoculum of 10^5 organisms per ml in order to detect any possible changes in susceptibility of the organisms resulting from exposure to the antibiotic.

Results. The data charted in Fig. 1, 2, and 3 depict the changes in number of viable staphylococcal units and in concentrations of active ancillin, methicillin and oxacillin, respectively, after various periods of incubating them together in broth at 37°C. In each instance the number of viable organisms declined until the level of antibiotic had fallen to about 1/15th of the starting concentration, *i.e.,* to below the conventional MIC, after which there was rapid multiplication of the organisms until their number approached or, in the case of ancillin, even exceeded the number in the control cultures grown in broth without antibiotic. Concentration of each antibiotic incubated at 37°C in broth (pH 7.4) without organisms also declined, but much less rapidly than when incubated with the viable staphylococci. Thus, there was an accelerated disappearance of antibiotic activity in each instance when *Staph. 60/1* was exposed to each of the 3 penicillins. The time required for the level of antibiotic activity in the presence of the staphylococci to fall below the MIC under the conditions of these tests varied; for ancillin this occurred between 2 and 4 hours, for oxacillin between 4 and 15 hours, and for methicillin between 2 and 5 days. In no instance was bacterial multiplication demonstrated before the concentration had fallen below that level. Changes in pH that occurred in the methicillin experiment are shown in the upper panel of Fig. 2.

For each of the 3 penicillins, the MIC of the organisms that had survived exposure to the high concentrations of antibiotic was the same as that of the organisms that had not been exposed to the antibiotic. With a starting inoculum of 10^5 organisms per ml, the minimum inhibiting and bactericidal concentrations were about the same.

The upper panel of Fig. 3 shows the data on decay in oxacillin activity during incubation under 4 different conditions:

1) In broth at 37°C with *Staph. 60/1* start-



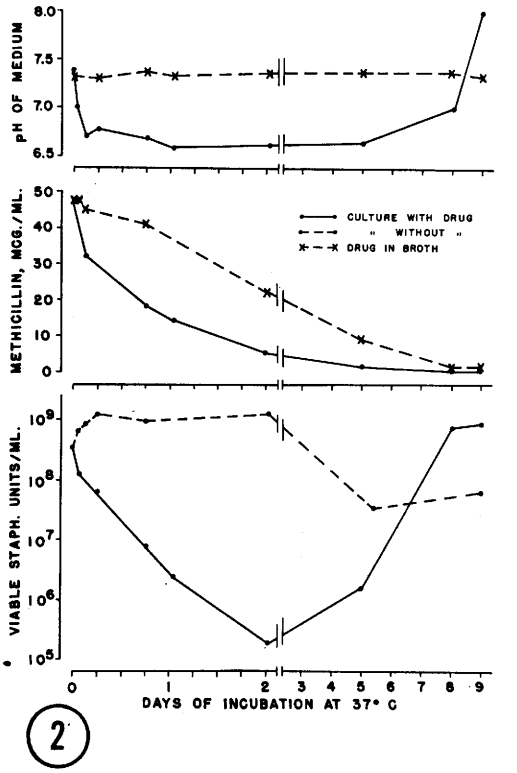


FIG. 1. Interaction of ancillin and *Staph. 60/1* at 37°C.

FIG. 2. Interaction of methicillin with *Staph. 60/1* at 37°C.

ing with 10^8 viable units per ml, 2) in broth at 4°C with *Staph. 60/1* starting with 10^8 viable units per ml, 3) in buffer pH 6.4 at 37°C with *Staph. 60/1* starting with 10^8 viable units per ml, and 4) in broth at 37°C without organisms. The oxacillin activity declined at an accelerated rate only when incubated in the nutrient medium with bacteria at 37°C. Exposure of oxacillin to the organisms at 4°C and incubation of the antibiotic in buffer at pH 6.4 failed to increase the rate of decay beyond that observed in broth, pH 7.4, at 37°C.

Discussion. The data presented indicate that the activity of the 3 so-called penicillinase-resistant penicillins–ancillin, methicillin and oxacillin–decayed much more rapidly in a nutrient medium at 37°C when exposed to a moderately heavy concentration of the

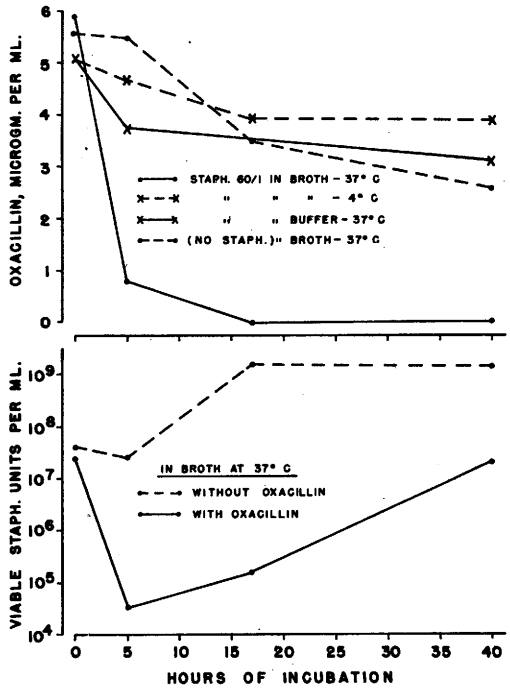


FIG. 3. Kinetics of interaction of *Staph. 60/1* with oxacillin.

penicillinase-producing *Staph. 60/1* than when incubated in the same medium without these organisms. Three possible explanations for this loss of activity might be considered: 1. the antibiotic was adsorbed on the bacteria; 2. the presence of the staphylococci had some antagonistic effect on the action of

the antibiotic, and 3. the antibiotic activity was destroyed by the bacteria or their product(s). The first 2 explanations seem unlikely because at least in the case of oxacillin, no such loss of activity, beyond that observed during incubation of the drug in broth without bacteria, occurred when the drug was exposed to the same concentration of bacteria in broth at 4°C or in the non-nutrient buffer, pH 6.4, at 37°C. Appreciable destruction of antibiotic apparently occurred when the drug was exposed to a concentration of 10^8 viable units of *Staph. 60/1,* but this destruction apparently continued even after the viable bacterial count had dropped below 10^5 units per ml. Thus, physical adsorption alone could not account for the more rapid rate and continuing decay in antibiotic activity.

It should be pointed out that, without the simultaneous measurements of both concentration of antibiotic activity and numbers of bacteria at the different intervals it would not have been possible to determine whether the organisms were multiplying or decreasing in number during their exposure to the high concentrations of the penicillin. With these measurements it was clearly shown that the number of bacteria actually declined until the level of the drug was low enough to permit multiplication of surviving organisms. It was also shown that these surviving organisms, after they had multiplied, were no less susceptible than the parent strain to the antibiotic to which they had been exposed.

The destruction of so-called penicillin-resistant penicillins by heavy concentrations of penicillinase-producing staphylococci may account for difficulties in some cases during methicillin treatment(11) in eradicating the staphylococci from infected loci, such as deep abscesses, where these organisms may be present in large numbers. This may also be true of foci in which there are moderate numbers of susceptible bacteria mixed with gram-negative bacilli which are also capable of inactivating these same types of penicillins (12).

‡ Tested only with oxacillin.

Summary. Each of the 3 semisynthetic penicillins–ancillin, methicillin and oxacillin–was studied for inactivation by a penicillinase-producing strain of *Staphylococcus aureus.* Starting with 10^8 viable staphylococcal units per ml and a concentration of each penicillin 15 times the minimum inhibiting (and bactericidal) concentration (MIC) for 10^5 organisms per ml in the same medium, these penicillins were inactivated at 37°C at a much more rapid rate than in the same medium at the same temperature without organism, or with the same number of organisms at 4°C,‡ or in a non-nutrient medium‡ (buffer, pH 6.4) at 37°C. The inactivation of each of these penicillins was accompanied first by a steady fall in the number of viable staphylococci until the concentration of drug fell below the "usual MIC," after which there was demonstrable multiplication of the organisms. The organisms that survived and multiplied were no less susceptible than the parent strain to the action of the penicillin to which they had been exposed.

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Received October 1, 1962. P.S.E.B.M., 1962, v111.