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Inactivation of Penicillins by Thiol Broth

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Thiol broth with sodium polyanetholesulfonate inactivated penicillin G, carbenicillin, nafcillin, oxacillin, and gentamicin, but had no effect on cephalothin, cefoxitin, clindamycin, chloramphenicol, erythromycin, and tetracycline. Only Thiol broth was capable of this inactivation, which was not influenced by the presence of blood.

Because recovery of bacteria in blood cultures can be inhibited by the presence of antimicrobial agents in the blood specimen, a variety of approaches have been used to minimize this problem. Blood can be processed initially in a system containing resins which remove most antibiotics (5). However, this system (Antimicrobial Removal Device; Marion Laboratories, Kansas City, Mo.) was reported to inhibit the growth of some bacteria (7), and the additional manipulations with the Antimicrobial Removal Device system may pose technical problems for laboratories.

Another approach to neutralizing antibiotics in blood is to add supplements directly to the culture broths. Sodium polyanetholesulfonate is added to most blood culture media as an anticoagulant. It also is anticomplementary and antiphagocytic and inactivates clinically achievable concentrations of aminoglycosides (1, 4). However, sodium polyanetholesulfonate has no effect on other commonly used antimicrobial agents. Penicillinases can be added to the cultures when they are received in the laboratory, but this does not protect the bacteria during the period before the specimens are received in the laboratory. Additionally, pseudobacteremias can be caused by contaminated penicillinase solutions.

The manufacturers of Thiol broth (Difco Laboratories, Detroit, Mich.) report that a variety of antibiotics are neutralized in this medium, although we are not aware of conclusive data that support these claims (3). The study reported herein attempted to define which antibiotics were affected by Thiol broth.

(This work was presented in part at the 80th Annual Meeting of the American Society for Microbiology, 1980.)

The laboratory standard powders used in these studies were: penicillin G from Wyeth Laboratories, Philadelphia, Pa.; carbenicillin and tetracycline from Pfizer Inc., New York, N.Y.; cephalothin and erythromycin from Eli Lilly & Co., Indianapolis, Ind.; oxacillin and nafcillin from Bristol Laboratories, Syracuse, N.Y.; cefoxitin from Merck Sharp & Dohme, Rahway, N.J.; clindamycin from The Upjohn Co., Kalamazoo, Mich.; chloramphenicol from Parke, Davis & Co., Detroit, Mich.; and gentamicin from Schering Corp., Bloomfield, N.J.

To assess the neutralization of antibiotics, five concentrations (5, 10, 25, 50, and 100 μg/ml) of the 11 antibiotics were added to blood culture bottles containing tryptic soy broth or Thiol broth. Immediately after the antibiotics were added, the broths were inoculated with an indicator organism (*Staphylococcus aureus* ATCC 25923) at a final concentration of 10^3 colony-forming units per ml of broth. This indicator organism was selected because it was highly susceptible to all of the antibiotics tested (2). The ability of the broths to neutralize the antibiotics was determined by examination of the bottles for macroscopic growth of the indicator organism after incubation at 35°C for 5 days. This experiment was performed with both non-supplemented broths and broths with human blood (10%, vol/vol) added.

Of the 11 antibiotics that were examined, only the penicillins (penicillin G, carbenicillin, nafcillin, and oxacillin) were neutralized by Thiol broth. The *S. aureus* test organism was able to grow in all Thiol bottles supplemented with from 5 to 100 μg of penicillin per ml. However, growth of the indicator organism was delayed in the Thiol broths with the penicillins in comparison with antibiotic-free Thiol. This is consistent with the slow rather than the immediate inactivation of the antibiotics, the kinetics of which were examined in subsequent experiments. The presence or absence of blood did not influence inactivation of the penicillins by Thiol broth. In contrast with this, tryptic soy broth did not

TABLE 1. Effects of Thiol and tryptic soy broths on penicillin G activity

Time of exposure[a]	Penicillin G activity (μg/ml) in:	
	Thiol broth	Tryptic soy broth
0	100	100
5 min	95	100
15 min	90	100
30 min	82	100
1 h	66	100
2 h	58	100
4 h	37	100
8 h	18	100
12 h	9	100
24 h	2	100

[a] Time after the addition of penicillin G to broth.

neutralize the penicillins because the test organism was inhibited by even the lowest concentration of the antibiotic. Furthermore, the only other antibiotic that was neutralized by either broth was gentamicin, which was due to supplementation of both broths with sodium polyanetholesulfonate.

The ability of other blood culture broths to neutralize penicillin G was determined. The commercially prepared broth media used in these studies included brain heart infusion, Brucella, and Columbia from Difco; Schaedler and Trypticase soy from BBL Microbiology Systems, Cockeysville, Md.; and dextrose phosphate and thioglycolate from GIBCO Diagnostics, Madison, Wis. After penicillin G was added to broths incubated at 35°C (final concentration, 100 μg/ml), samples of broth were withdrawn at 0 and 24 h and immediately frozen at −70°C. Antimicrobial activity of the samples was measured by an agar diffusion bioassay with *Bacillus globigii* as the indicator organism (6). This assay can detect approximately 1 μg of penicillin per ml. The results of this experiment indicated that none of these broths reduced the activity of penicillin G.

The rate of penicillin G inactivation was determined. Penicillin G was added to the Thiol and tryptic soy broths incubated at 35°C (final concentrations, 100 μg/ml), and then samples from the bottles were removed with a syringe over a 24-h period. The samples were rapidly frozen by immersion in an alcohol-dry ice mixture and then stored at −70°C. Penicillin G activity in all samples was measured by the agar diffusion bioassay method after the samples were thawed in a water bath at 50°C. The rate of neutralization of penicillin G in Thiol and tryptic soy broths in summarized in Table 1. Approximately two-thirds of the penicillin G was inactivated by Thiol broth after 1 h, and less than 10% of the activity remained after 12 h. Exposure of penicillin G to tryptic soy broth did not affect the antimicrobial activity.

The effect of aeration on Thiol broth was determined by (i) chronically venting a Thiol bottle for 24 h, (ii) vigorously mixing small samples of broth (5 to 10 ml) on a mechanical Vortex mixer, and (iii) exposing 100 ml of broth in a 500-ml Erlenmeyer flask to room air for 1 h with gentle mixing every 5 to 10 min. After aeration, penicillin G (final concentration, 100 μg/ml) and then the *S. aureus* indicator organism (final concentration, 10^3 colony-forming units per ml) were added to the broths. The broths were incubated at 35°C for 5 days and examined daily for macroscopic growth. The ability of Thiol broth to neutralize penicillin G was abolished by each method of aeration. Penicillin G remained active and inhibited the growth of the indicator organism. However, if the Thiol broth was chronically vented after the penicillin G and indicator organism were added (a situation analogous to what would happen with a clinical specimen), then the penicillin was inactivated by the Thiol broth.

The precise mechanism by which Thiol broth inactivates penicillins is not known. In preliminary experiments we have been able to duplicate the Thiol reaction by supplementing tryptic soy broth with cysteine hydrochloride (final concentration, 100 μg/ml). Penicillin G is completely inactivated within 24 h by this concentration of cysteine. However, if the cysteine-tryptic soy broth is autoclaved, the effect of cysteine on penicillin G activity is abolished. This is consistent with the observation that commercially prepared Columbia broth does not inactivate penicillins, even though it is supplemented with cysteine. The Thiol reaction is not specifically due to cysteine because autoclaved Thiol broth and filter-sterilized Thiol broth have similar activities. The activity of Thiol broth is most likely due to the reduction of penicillin by a component(s) in the thiol complex of the broth. However, the composition of this ingredient is the proprietary information of the manufacturer.

These experiments establish that Thiol broth inactivates penicillins (e.g., penicillin G, carbenicillin, oxacillin, and nafcillin) but not cephalosporins or the commonly used non-β-lactam antibiotics. This reaction occurs in the presence or absence of blood and in vented and nonvented bottles. The results of testing different commercially available blood culture broths indicate that only Thiol broth is capable of inactivating penicillin. Although Thiol broth with sodium polyanetholesulfonate inactivated penicillins and aminoglycosides, we were unable to corroborate the manufacturer's claim that cephalosporins were neutralized.

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