TABLE I. Titration of Plague Toxin in Mice Pretreated with Antibiotics.

Antibiotic* (2 mg/ml, i.p.)	Survivors/total mice, 48 hr after toxin inj. Toxin dilution (0.5 ml, i.v.) 1:40	1:80	1:160	1:320	1:640	1:1280
Aureomycin	0/10	1/10	8/10	9/10		
Terramycin	1/10	5/10	10/10	10/10	10/10	
Chloramphenicol		3/10	3/10	8/10	9/10	
Controls		0/10	1/10	1/10	6/10	10/10

* Given 4 hr before toxin. Controls were given physiologic sodium chloride sol.

TABLE II. Duration of Protection of Mice Against *P. pestis* Toxin.*

Antibiotics (2 mg/ml, i.p.)	Hr before toxin inj.	No. mice	Survival rate 8-12 hr	20-24 hr	48 hr
Aureomycin	24	20	10	10	10
	12	20	95	95	95
	1	20	90	75	75
	12, 6, 1	20	100	100	100
Terramycin	12	10	80	70	70
	1	10	80	80	80
Chloramphenicol	12, 6, 1	20	70	65	60
	1	20	60	35	35
Streptomycin	12, 6, 1	20	50	40	40
	1	20	50	35	25
Controls		20	35	35	25

* 2 LD_{50} of plague toxin given i.v.

venously. The results are presented in Table II. The protective action of aureomycin and terramycin lasted at least 12, but not 24 hours. Only when 6 mg of chloramphenicol was given did the survival rate reflect protection. Streptomycin, the most effective therapeutically, exerted no antitoxic effect, even when 6.0 mg was given.

Summary. Intraperitoneal administration of aureomycin, chloramphenicol and terramycin in 2.0 mg doses within 12 hours of intravenous injection of plague toxin conferred definite, but limited, antitoxic protection to mice. Streptomycin was without this protective action. Aureomycin injected 1 hour or 12 hours prior to toxin exerted the greatest protective effect. Chloramphenicol was not as effective as either aureomycin or terramycin against *Pasteurella pestis* toxin.

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Interference of Aureomycin and of Terramycin with Action of Penicillin *in Vitro.** (18262)

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Recent studies have established that one antibiotic may interfere with the action of another both *in vitro* and *in vivo*(1-6). It has been shown that chloramphenicol antagonizes penicillin in the test tube and in a streptococcal infection of mice(3,4,6). The interference *in vitro* of aureomycin with the bactericidal action of penicillin against enterococci has also been demonstrated(6).

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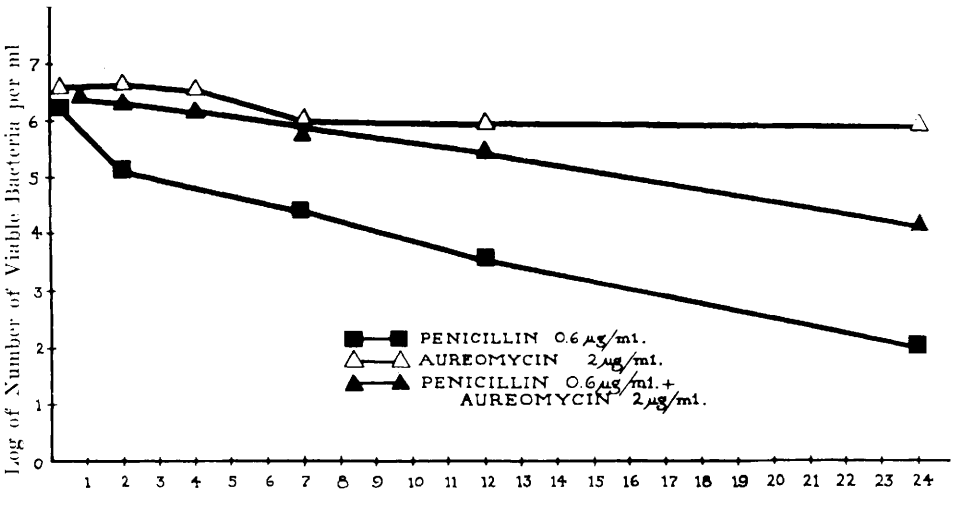

FIG. 1.
Interference of aureomycin with action of penicillin on *Streptococcus pyogenes*.

Materials and methods. In the present study, *Streptococcus pyogenes* (C203)‡ and *Klebsiella pneumoniae* (A-D)§ were used as test organisms. Crystalline potassium penicillin G, aureomycin hydrochloride (No. 7-6116)‖, and terramycin hydrochloride¶ were dissolved in 0.85% sodium chloride solution to make a concentration of 2.5 mg/ml. The solutions of aureomycin and of terramycin were sterilized by Seitz filtration. The aureomycin solution was quickly frozen and stored at −20°C. Final dilutions of the antibiotics freshly prepared in broth in a volume of 19 ml were inoculated with 1 ml of an 18-hour broth culture containing 10^7-10^8 bacteria per ml. Samples of 0.5 ml were removed at intervals during incubation at 37°C and the number of viable bacteria was estimated by plate counts. The culture media used were Proteose Peptone No. 3 agar (Difco), to which 2% blood and 4% penicillinase solution (Bacto-Penase) were added for tests with *S. pyogenes*, and broth with the same base.

Results. Both aureomycin and terramycin interfered with the bactericidal action of penicillin against *S. pyogenes* and *K. pneumoniae*. The bactericidal rate of penicillin alone was reduced by the addition of certain concentrations of either aureomycin or terramycin. Representative experimental results are shown in Fig. 1 and 2.

Against *S. pyogenes*, penicillin was highly active, 0.006 μg/ml was rapidly bactericidal; aureomycin and terramycin were less active, 1-10 μg/ml were mainly bacteriostatic. When either of the less active drugs was added to penicillin the rate of death of the streptococci approximated that obtained with the less effective antibiotic alone (Fig. 1). Increase in the concentration of aureomycin or terramycin up to 10-50 μg/ml resulted in a slight increase in their antagonism toward penicillin. When the concentration of aureomycin was further increased to the point where it was moderately bactericidal by itself interference with penicillin action could no longer be demonstrated.

Against *K. pneumoniae*, each of the 3 antibiotics was moderately active in about equal

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§ Originally obtained from Dr. E. R. Jackson, American Cyanamid Co., Stamford, Conn.

‖ Kindly supplied by Dr. S. H. Hardy, Lederle Laboratories, Pearl River, N. Y.

¶ Kindly supplied by Dr. H. A. Anderson, University of California School of Medicine (Lot WBW 507019).

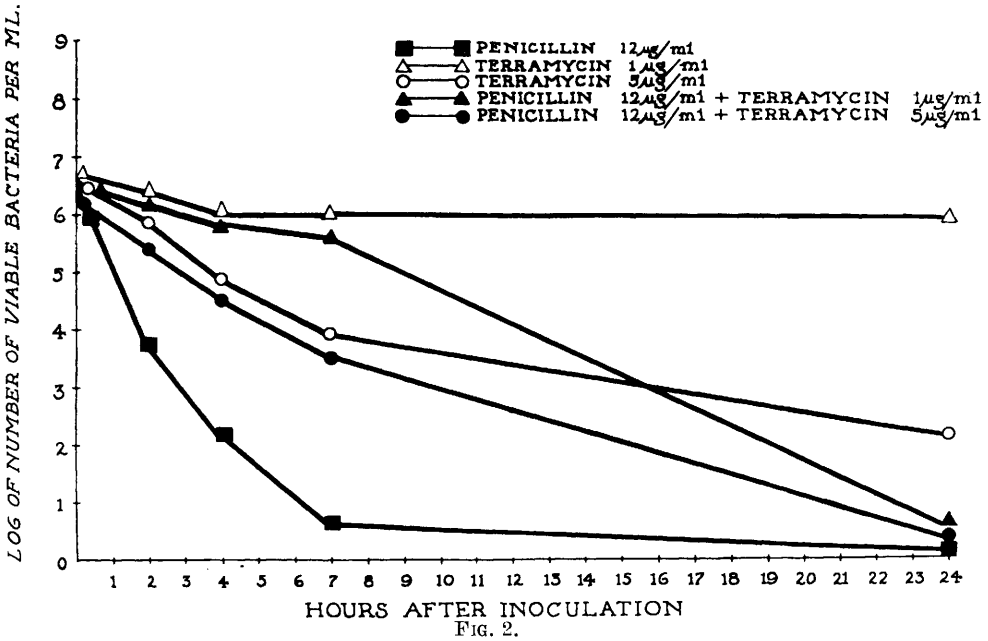

FIG. 2.

Interference of terramycin with action of penicillin on *Klebsiella pneumoniae*. Effect of varying concentrations of terramycin.

degree; 5-10 μg/ml usually killed 99.9% of the inoculated bacteria within 24 hours. Mixtures of 10 μg/ml of aureomycin or terramycin with 0.6-6 μg/ml of penicillin showed no antagonism. The addition of smaller amounts of aureomycin or terramycin (0.1-5 μg/ml), which were mainly bacteriostatic, to a rapidly bactericidal concentration of penicillin (12 μg/ml) resulted in a sharp reduction in the bacterial death rate below that shown with penicillin alone (Fig. 2). This interference increased greatly with a decrease in the amount of aureomycin or terramycin; *e.g.*, 0.1 μg/ml of aureomycin showed more interference than did 1.0 μg/ml. Still smaller amounts of aureomycin (0.01 μg/ml) below its bacteriostatic level no longer interfered with the action of penicillin.

During the first 7 to 12 hours of incubation, mixtures of interfering concentrations of aureomycin or terramycin with penicillin killed *K. pneumoniae* at rates approximating those obtained with the interfering drug alone (Fig. 2). After 24 hours of incubation, however, the organisms exposed to single antibiotics were likely to multiply whereas those exposed to the mixtures were not viable. This ability of combinations of antibiotics to destroy all exposed bacteria ultimately *in vitro* even though the initial death rate is slower than with penicillin alone has also been noted with enterococci(3,6).

There is no evidence that penicillin interferes in any way with the bactericidal action of effective concentrations of either aureomycin or terramycin; *e.g.*, the rate of death of *K. pneumoniae* in the presence of 10 μg/ml of aureomycin was the same whether or not a bacteriostatic concentration of penicillin was added.

Discussion. Apparently aureomycin and terramycin interfere with the action of penicillin only in those concentrations which are at least bacteriostatic and yet not markedly bactericidal by themselves; *i.e.*, inactive concentrations and highly active concentrations do not antagonize the effect of penicillin. With *S. pyogenes* as test organism, 10 μg/ml of aureomycin interfered with 0.6 μg/ml of penicillin; in the case of *K. pneumoniae*, these same concentrations showed no interference. It seems unlikely, therefore, that the antagonism is due to a chemical inactivation of one drug by the other without reference to the sensitivity of the bacteria concerned.

Preliminary experiments indicate that the

interference of aureomycin with penicillin occurs not only *in vitro* but also in mice inoculated with either *S. pyogenes* or *K. pneumoniae*(7). Mice treated with both penicillin and aureomycin showed a higher death rate than did those treated with penicillin alone.

It is of interest that not only chloramphenicol but all 3 of the "newer" antibiotics that are widely used clinically, interfere with the bactericidal action of penicillin. While these agents have certain features in common there is no evidence that they resemble one another chemically. To date no evidence has appeared suggesting that the interference of these drugs with penicillin action is due to a direct physical or chemical interaction. Other possible mechanisms for this "antibiotic antagonism" have been previously suggested(4,6). It might be that the 2 interfering drugs compete with one another for "receptors" on the bacterial cell. No evidence has been produced directly favoring this possibility. One drug might alter the developmental characteristics of the microorganism in such a way as to diminish their susceptibility to other antimicrobial agents. All instances of antibiotic antagonism demonstrated thus far in this laboratory have dealt with an interference of bacteriostatic drugs with the bactericidal action of penicillin. Most of the data are compatible with the hypothesis that the presence of a bacteriostatic concentration of chloramphenicol, aureomycin, or terramycin may interfere with the multiplication of bacteria. Since penicillin principally affects dividing microorganisms such an interference with multiplication might result in interference with penicillin action. Future experiments will have to explore this hypothesis.

While striking manifestations of antibiotic antagonism have been produced experimentally in mice it cannot be inferred that the phenomenon may be of importance in the antibiotic therapy of human patients. It should be noted, however, that the drug levels showing antibiotic antagonism *in vitro* and in experimental animals fall in the range commonly obtained in human body fluids and tissues with ordinary therapeutic doses.

Summary. Aureomycin and terramycin interfered *in vitro* with the bactericidal action of penicillin against *S. pyogenes* and *K. pneumoniae*. The early bactericidal rate was slower with mixtures of aureomycin or terramycin with penicillin than with penicillin by itself. Ultimately, however, the mixtures were more likely to destroy all exposed bacteria than were the single antibiotics. Interference was most marked when bacteriostatic concentrations of aureomycin or terramycin were mixed with actively bactericidal concentrations of penicillin.

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A Serologic Study of Chorionic Gonadotrophin. (18263)

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The antigenicity of chorionic gonadotrophin* has been suggested by Leathem(1), Leathem and Rakoff(2) and others. Chase(3) has demonstrated that the serum of rabbits immunized with gonadotrophic hormones reacted with these hormones in a variety of immunological test procedures. CG in urine

* Hereafter designated as CG.

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