TABLE III. Effects of Virus Multiplication on Relative Specific Activity of Phosphorus Fractions at 24 Hr Incubation.

Fraction	Relative specific activity*	
	Non-infected	Infected
Phospholipid	3.15	5.86
Desoxyribonucleic acid	.65	.67
Ribonucleic acid	3.60	4.20

* Rel. spec. activity—Specific activity (counts/minute/μg of P^{31}) of each fraction as % of specific activity of inorganic P fraction.

could ascertain the site of the reacting phospholipid in the cell. It has been shown by Friedkin(7) that mitochondria, which contain large amounts of phospholipids, take up P^{32} and incorporate it into their phospholipid fraction. In view of Friedkin's work, it is an interesting consideration that the observed changes in P^{32} uptake of infected tissue may occur in the mitochondria.

Since it is also known that considerable phospholipid is found in cell membranes, there may be a relation between phospholipid liberation and the penetration of the virus particle into the cell. The work of Howe(8) who found large amounts of the red cell receptor in a stroma complex rich in phospholipid, seems pertinent.

Because it has been shown(9) that large differences in the proportion between the hemagglutinating and infectious properties of influenza virus may occur in de-embryonated eggs, nothing can as yet be postulated concerning these findings and the infectious form of the virus.

Summary. It has been shown that infection of the chorioallantoic membrane by the PR-8 strain of influenza virus results in: 1. The decrease in the phospholipid phosphorus concentration of the membrane; 2. The presence of large amounts of lipid-bound phosphorus in the fluid medium; and 3. A more rapid uptake of P^{32} into the membrane.

The author wishes to express his sincere gratitude to Dr. Boris Magasanik for his assistance in the course of this work, and to Dr. A. K. Solomon for his help in the procurement and analysis of the radioactive materials.

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Lack of Interference of Chloramphenicol with Penicillin in a Hemolytic Streptococcal Infection in Mice. (19449)

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(Introduced by R. H. Williams.)
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Considerable interest has been shown in the recent demonstration by Jawetz and coworkers that aureomycin, terramycin, and chloramphenicol interfere with the early bactericidal action of penicillin(1-6). When any of the 3 antibiotics was combined with penicillin *in vitro,* susceptible organisms were destroyed more slowly than when exposed to penicillin alone. Interference also occurred in mice infected with group A streptococci; animals that received both chloramphenicol and penicillin had a higher mortality rate than those treated with penicillin alone. Effective blood levels were present for less than 3 hours, however, so that death or survival of the animals depended upon brief antibiotic

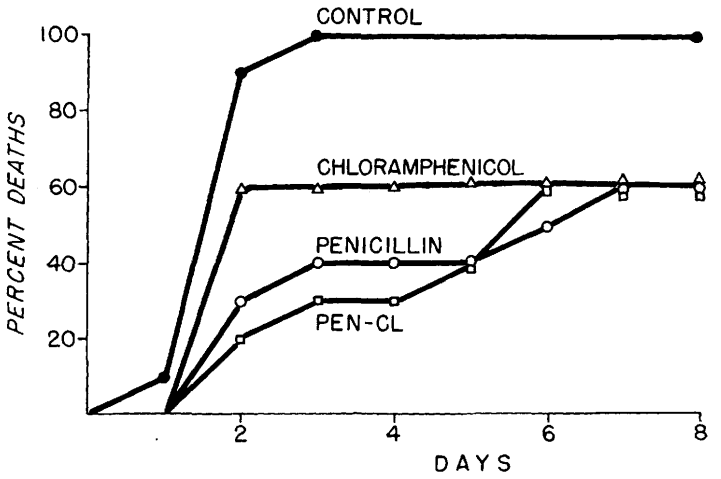

FIG. 1. Lack of interference of chloramphenicol with penicillin when treatment was continued for 3 days. Intramuscular injection of 25 units of procaine G penicillin twice daily, and 600 μg of chloramphenicol 3 times daily.

action. In other words, the animal experiments reflect the interference of chloramphenicol with the early action of penicillin.

Although the combination killed organisms more slowly *in vitro,* Jawetz also noted that the bactericidal action continued progressively until, in many instances, the cultures were sterile(4). This progressive killing action of the combinations did not play a role in the mouse experiments because of the short duration of antibiotic blood levels. However, in patients treated with antibiotics, effective levels are present in body fluids for prolonged periods. It seemed desirable, therefore, to repeat Jawetz's *in vivo* experiments under conditions that would provide more sustained antibiotic levels.

Materials and methods. Preliminary studies. Three experiments were done under the exact conditions outlined by Jawetz. Mice were infected with group A streptococci, and shortly afterward treated with a single injection of penicillin, chloramphenicol, or both antibiotics. In every case the mortality rate of the animals that received both antibiotics was significantly higher than that of those getting only penicillin. The results were clear-cut, and it was not felt necessary to pursue these confirmatory studies further. *Inoculum.* A Group A streptococcus was passed through mice twice weekly to maintain virulence. An overnight culture was diluted to 10^{-4} in broth, and each animal was given 0.2 cc intraperitoneally. Plate counts revealed that this infecting dose contained approximately 5,000 organisms, and produced a consistently high mortality rate in control animals. *Antibiotics. Chloramphenicol* was dissolved in sterile 0.85% saline in concentrations of 1.0 to 6.0 mg/cc, dispensed in sterile tubes, and stored at —20°C. It was necessary to warm mixtures containing more than 2.5 mg/cc to obtain complete solution, and these solutions usually remained supersaturated when thawed. If not, they were warmed again. *Penicillin* was administered as procaine penicillin G in peanut oil gelled with 2% aluminum monostearate in concentrations ranging from 125 to 500 units/cc.*

Procedure. The antibiotics were given in a constant volume of 0.1 cc, and were administered intramuscularly in some experiments and subcutaneously in others. It was found that somewhat smaller amounts of antibiotics were needed if the subcutaneous route was used. The animals treated with both chloramphenicol and penicillin received the 2 injections within a few seconds of one another, but at different sites. Those receiving only one antibiotic were given simultaneous injec-

* We are indebted to the Bristol Laboratories for the penicillin preparations.

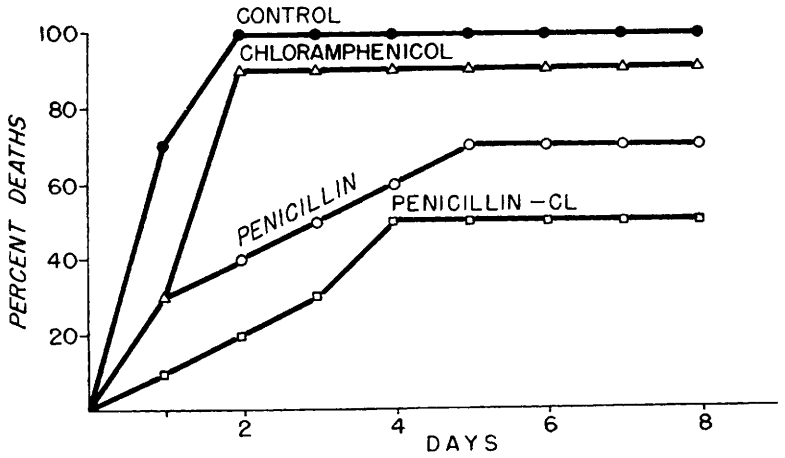

FIG. 2. No interference demonstrable with a relatively ineffective dosage of chloramphenicol (100 μg 3 times daily). Penicillin dosage as in Fig. 1. Therapy was administered intramuscularly for 3 days.

tions of sterile saline. The control animals were given no injections other than the original intraperitoneal inoculum. The chloramphenicol was injected 3 times each day at 9 a.m., 3 p.m., and 9 p.m. Penicillin was given simultaneously 3 times daily in 4 experiments, and in the other 8 penicillin was given only twice each day, at 9 a.m., and 9 p.m. Therapy was instituted one to 2½ hours after infection, and was continued for either 2 or 3 days. Deaths were recorded for 5 to 10 days following therapy. Autopsies were performed on some of the animals as they died, and pure cultures of beta hemolytic streptococci were invariably obtained from the heart's blood. Twelve experiments were performed in all.

Results. The control animals were usually dead within 72 hours. Surprisingly, an occasional animal remained entirely well. The results of a typical experiment are presented in Fig. 1. In this instance penicillin alone, and chloramphenicol alone, each protected 40% of the animals. When both antibiotics were given, the survival rate was also 40%, *i.e.*, there was neither synergism nor antagonism.

When chloramphenicol was less effective than penicillin, the mortality with the combination was approximately the same as with penicillin alone. This was observed when the amounts of antibiotics employed protected relatively few animals (Fig. 2), and also when highly effective amounts were used. In the other 10 experiments the results were essentially the same as those described. At no time was antagonism demonstrated.

Comment. Jawetz's observation of the interference of chloramphenicol with penicillin *in vivo* has been confirmed in the present studies. There was a higher mortality rate among mice treated with a single aqueous injection of both chloramphenicol and penicillin than when penicillin was administered alone. However, when significant blood levels of chloramphenicol and penicillin were maintained for 2 or 3 days, interference was not demonstrable.

The explanation for these divergent results undoubtedly lies in the *in vitro* observation that although the early killing action of penicillin is antagonized by chloramphenicol, there is a progressive bactericidal action with the combination which often results in eventual sterilization of the cultures(4). Thus, when mice were exposed to both antibiotics for only 2 or 3 hours, fewer organisms were destroyed than with penicillin alone, and the mortality rate was higher. On the other hand, when both antibiotics were present in tissues for 2 or 3 days, there was a progressive bactericidal action and, in addition, time was allowed for effective phagocytosis to occur.

Interference of any of the newer antibiotics

with penicillin appears to be of importance only when recovery is dependent upon the early, rapid killing action of penicillin. A recent report of apparent interference of aureomycin with penicillin in pneumococcal meningitis is therefore of great interest(7). This is a condition which, untreated, is associated with a high mortality rate, and in which phagocytosis is relatively ineffective(8).

The importance of the interference of the newer antibiotics with the early killing action of penicillin can be determined only by extensive clinical studies. However, on the basis of the present experiments and the evidence cited above, it is our impression that there are probably few clinical conditions in which the phenomenon is of practical importance.

Summary. 1. Mice were infected with group A streptococci and treated with chloramphenicol and penicillin, separately and in combination. Jawetz's observation that chloramphenicol interfered with penicillin when animals were given a single aqueous injection of both antibiotics was confirmed. 2. The interference was not demonstrable, however, when antibiotic blood levels were maintained for 2 or 3 days. Thus, despite early interference, the combination caused a progressive bactericidal action which, in conjunction with phagocytosis, protected the mice as well as penicillin alone. 3. These experiments imply that the phenomenon of antibiotic interference is seldom of clinical significance.

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Inhibition of Mouse Encephalomyelitis Virus, *in vitro*, by Certain Nucleoprotein Derivatives.* (19450)

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Previous work showed that various substances including heavy metals, enzyme inhibitors such as dinitrophenol, cyanide, azide and iodoacetate as well as certain amino acids and analogues inhibit the propagation of Theiler's GDVII strain of murine encephalomyelitis virus in tissue cultures of minced one-day-old mouse brain(1-3). The present report gives the results of tests with certain purines, pyrimidines and substituted pyrimidine nucleosides(4-6). Preliminary data have been presented in part elsewhere(7).

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Materials and methods. Cultures were prepared in 50 ml Erlenmeyer flasks closed with rubber stoppers and containing 3 ml of Simms' X7 solution and 50-100 mg of minced mouse brain from one-day-old mice. The initial reaction of the medium was adjusted to pH 8-9. Cultures were incubated for 1-2 days at 35°-36°C. The supernatant fluid obtained after centrifugation of the pooled material from 3 flasks was tested for virus content by hemagglutination of human red blood cells with serial twofold dilutions(3).

Results. Several of the compounds tested