TABLE I.
Summary of Data.

Diet	No. of animals	Avg initial wt, g	Initial avg total cholesterol mg/100 ml	Final avg total cholesterol mg/100 ml*	Avg N.P.N. mg/100 ml*	No. animals with aortic atheroma
Normal	10	2543	55 (29-80) §	—	20	0
Normal + uranium acetate 10 mg/kg†	10	2760	56 (35-84)	—	48	2 +1, +4
Cholesterol 3 wks	10	2968	59 (26-100)	1321 (647-1995)	—	3 all +1
Cholesterol 2 wks then uranium acetate 10 mg/kg‡	14	2647	44 (19-67)	499 (197-884)	118	12 Avg +2.6

* Determined just prior to sacrifice.
† From 3 to 14 days elapsed between uranium dose to sacrifice, avg 6.1 days.
‡ From 2 to 14 days elapsed between uranium dose to sacrifice, avg 5.9 days.
§ Numbers within parentheses indicate range.

age by this agent, further substantiates Holman's observation that "Arterial disease may be a matter of days not decades."(1)

Summary. Rabbits with dietary hypercholesterolemia of short duration develop aortic atherosclerosis abruptly after the production of renal damage by the administration of uranium acetate.

Received April 3, 1950. P.S.E.B.M., 1950, v74.

Joint Action of Penicillin with Chloramphenicol on an Experimental Streptococcal Infection of Mice.* (17820)

E. JAWETZ AND R. S. SPECK.
From Division of Bacteriology, University of California School of Medicine, San Francisco.

In the course of studies on the effects of antibiotics on enterococci it was observed that chloramphenicol interfered with the early bactericidal action of penicillin(1). In the presence of both penicillin and chloramphenicol the rate of bactericidal action was considerably slower than with penicillin alone. It was of interest to determine whether this apparent antagonism between two antibiotic agents is limited to an *in vitro* phenomenon or whether it could be demonstrated *in vivo*. Since enterococci are essentially nonpathogenic for small laboratory animals, a highly fatal infection with beta hemolytic streptococci was used in the present experiments.

Materials and methods. White Swiss mice weighing about 20 g, from a heterozygous stock, were used in all experiments. The infections were produced with a beta hemolytic, group A, type 3, streptococcus, strain C 203, kindly supplied by Dr. H. Eagle. The virulence of this organism was kept at high level by passage through mice once or twice weekly. Cultures were grown in 5% sheep blood broth at 37°C for 18 hours. The LD_{50} for intraperitoneal injection of mice consisted of 60-100 organisms contained in 0.2 ml of a 10^{-6} dilution of an 18 hour cul-

* Supported by a grant from the Research Committee of the University of California, School of Medicine.

1. Jawetz, E., Gunnison, J. B., and Coleman, V. R., *Science*, 1950, v111, 254.

ture. In all experiments 0.2 ml of a 10^{-4} dilution of culture, about 100 LD_{50}, was injected intraperitoneally as the infecting dose. Commercial crystalline sodium penicillin G was freshly dissolved in saline prior to each experiment and suitably diluted. Chloramphenicol (Chloromycetin lot No. 129416†) was dissolved in saline in a concentration of 4 mg/ml and sterilized by Seitz filtration. Such stock solutions were kept at 4°C for 2-3 weeks without apparent deterioration. Final dilutions were prepared in saline just prior to each experiment. Mice were treated one hour after infection. Each drug was injected separately in a volume of 0.2 ml into the peritoneal cavity. Mice receiving no drug were injected with 0.2 ml saline. The 2 intraperitoneal injections took place within 1 minute. No further treatment was administered. The animals were observed for 10 days and a few of those dying in each group were autopsied to ascertain the cause of death. Heart blood cultured on blood agar plates in all instances yielded beta hemolytic streptococci in pure culture.

Results. Under the conditions of these experiments the estimated 50% curative dose of penicillin alone was 2-5 μg (0.1-0.25 mg/kg), and of Chloromycetin alone 400-600 μg (20-60 mg/kg). All untreated animals died within less than 3 days after infection. A basic observation on the joint effect of penicillin and chloramphenicol is shown in Fig. 1. Groups of 20 mice were infected in the standard manner with streptococcus C 203, and were treated with either penicillin 30 μg alone, or chloramphenicol 800 μg alone, or both drugs. Fig. 1 illustrates how either drug alone protected all but 20% of the animals, whereas the administration of both drugs resulted in a mortality of 60%. Thus apparently the two drugs interfered with each other's efficacy as therapeutic agents. This type of experiment was repeated many times, always with essentially the same results.

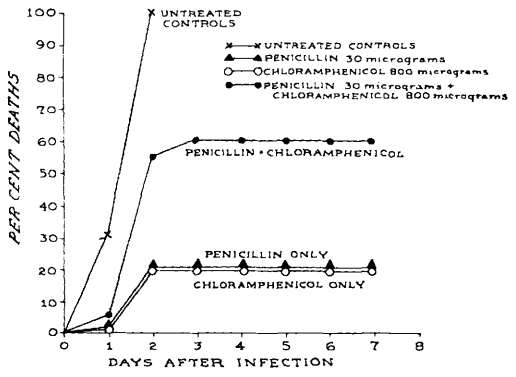

FIG. 1.
Joint action of penicillin (30 μg) with chloramphenicol (800 μg) on a streptococcal infection of mice.

† Kindly supplied in generous amounts by Dr. G. Rieveschl, Parke, Davis and Co., Detroit, Mich.

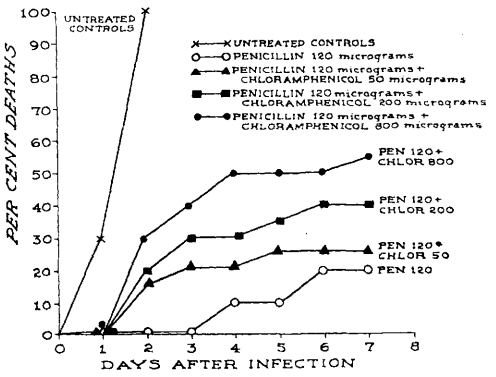

FIG. 2.
Joint action of a constant amount of penicillin (120 μg) with increasing amounts of chloramphenicol (50-800 μg) on a streptococcal infection of mice.

The quantitative relationships of this joint action of drugs was investigated by keeping the level of one agent constant and varying the other. The effect of increasing doses of chloramphenicol with a constant dose of penicillin is shown in Fig. 2. The more chloramphenicol is added to 120 μg of penicillin, the higher the death rate. With a smaller amount of penicillin, however, (*e.g.* 30 μg) this proportional increase was not observed. While 30 μg of penicillin alone protected all but 20% of the mice, the addition of 50, 200, or 800 μg of chloramphenicol resulted in 65, 60, and 60% deaths respectively.

When the dose of chloramphenicol was kept constant and the dose of penicillin varied, a different relationship was observed (Table I). Any combination of penicillin

TABLE I.

Joint Action of Penicillin with Chloramphenicol on a Streptococcal Infection of Mice.

20 g mice were given 100 LD_{50} of a culture of C 203 intraperitoneally. One hour later the drugs, or saline, were administered intraperitoneally, each in a volume of 0.2 ml, about 1 minute apart.

Streptococcus LD_{50}	Chloramphenicol, μg	Penicillin G, μg	Deaths/total in group	% deaths
100	800	0	9/26	34
100	0	6	3/16	19
100	0	120	2/26	8
100	800	6	11/16	68
100	800	30	12/20	60
100	800	120	18/36	50
100	50	6	11/16	68
100	50	30	13/20	65
100	50	120	13/35	34
0	800	120	0/16	0
100	0	0	26/26	100
10	0	0	12/12	100

with chloramphenicol resulted in a much larger number of deaths than the same dose of either drug alone, particularly penicillin alone. However, with any dose of chloramphenicol, within the experimental range, the more penicillin was administered, the lower was the rate of death.

Discussion. Chloramphenicol and penicillin, administered to uninfected mice in the doses used in these experiments, either alone, or together, never gave any evidence of toxicity. Consequently the higher death rate encountered with the joint use of both drugs could not be attributed to toxic depression of the host's defense mechanism, nor to direct toxic action of the drug. The evidence presented permits only the interpretation that in some manner penicillin and chloramphenicol interfere with each other's chemotherapeutic effect, in the experimental infection studied here.

This drug interference, or antagonism, might take place in one of 3 ways. (a) The two drugs could interact with one another in some physical or chemical process, so that the resulting complex was of lesser chemotherapeutic efficacy. (b) The 2 drugs might compete with one another for "receptors" on the susceptible bacterial cells. It is conceivable that through mass action the less effective drug might thus interfere with the more potent one. (c) Possibly one drug might so change developmental characteristics or properties of the infecting microorganisms that their susceptibility to other agents is greatly diminished. At present no definite choice can be made among these possible mechanisms. However, experiments are being designed to clarify this issue. The data at present available are compatible with the hypothesis that in some way chloramphenicol quantitatively interferes with penicillin effect.

Antagonistic influences of one antibiotic agent upon another have been observed by others(2) using a different laboratory model. In the experiments of Price *et al.*(2) the differences between single and multiple drug action appeared relatively small, and a shift from apparent synergism to antagonism was observed with 4- to 10-fold changes in drug concentrations. No immediate comparison of their data with ours is therefore possible. The clear-cut antagonistic action between chloramphenicol and penicillin demonstrated here is of interest in several regards. It draws attention to the possibility of interfering with, rather than potentiating, the action of one chemotherapeutic agent by administering it together with others. It has been increasingly popular medical practice to administer several chemotherapeutic agents simultaneously to patients, in the expecta-

2. Price, C. W., *et al. Am. J. Publ. Health,* 1949, v39, 340.

tion that such therapy would more rapidly and effectively terminate an infectious process. Such multiple chemotherapy has often been employed in the absence of accurate bacteriological diagnosis. In some instances it has been conclusively shown that several antimicrobial drugs administered together are more effective than one alone. The material presented here strongly suggests that the opposite may sometimes be the case. If antagonistic effects between antibiotic agents play any role in clinical practice it may become necessary for physicians to use such drugs only for specific indications, and abandon "shotgun therapy".

The observed antagonism between antibiotic agents may also provide a useful laboratory tool for the investigation of antibiotic action, provided it can be shown that it is not due to a simple chemical inactivation of one drug by another, but rather to an interference between the drugs at their site of action.

Addendum. Since this paper was submitted for publication additional experiments have been carried out with a separation of the two antibiotic agents in space and in time. Similar antagonistic results were obtained when the two drugs were injected subcutaneously at widely separated sites, or when they were given in different sites one hour apart.

Received April 3, 1950. P.S.E.B.M., 1950, v74.

Resistance to Epinephrine Stress in the Dog.* (17821)

JULES H. LAST, PAUL H. JORDAN,† ISADORE PITESKY, AND BERNARD M. SIEGEL.
From the Departments of Pharmacology and Surgery, University of Illinois College of Medicine, Chicago, Ill.

Current concepts of the physiologic activation of the pituitary-adrenal mechanism suggest that epinephrine is the humoral agent responsible for the release of ACTH from the anterior pituitary(1,2). It is not known whether epinephrine acts directly on the anterior pituitary or by stimulating the hypothalamus(3,4). The hypothalamus in turn may secrete an adrenergic substance(5) into the hypothalamo-hypophysial portal system and thereby stimulate the anterior pituitary to release ACTH. Other evidence suggests that epinephrine released as a result of sympathico-adrenal discharge may act peripherally to increase the tissue utilization of 11-oxysteroids, leading to lowered plasma levels and activation of the anterior pituitary to release ACTH(6). The site of action of epinephrine is further disputed by conflicting reports in the hypophysectomized rat wherein epinephrine effects a lymphopenia in one laboratory(7) whereas another group reports that epinephrine effects no increase in adrenal cortical size or lipid content in the absence of the pituitary(8). It has been suggested that in man, large doses of epinephrine may act directly on the adrenals(2). Epinephrine is a key hormone in present thinking on pituitary-adrenal relationships. Pharmacologically speaking, it is believed that no true tolerance to epinephrine exists. This concept must be qualified. Continuous or repetitive administration of epinephrine fails to produce continuous hyperglycemia or gly-

* Aided by grants from the Office of the Surgeon General, Department of the Army, and The Chicago Heart Assn.

† Public Health Service Research Fellow of the National Heart Institute.

1. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.

2. Thorn, G. W., and Forsham, P. H., *Recent Progress in Hormone Research*, 1949, v4, 229.

3. Harris, G. W., *J. Endocrinol.* (London), 1949, v6, xvii.

4. Hume, D. M., *J. Clin. Invest.*, 1949, v28, 790.

5. Harris, G. W., personal communication.

6. Sayers, G., and Sayers, M. A., *Ann. N. Y. Acad. Sci.*, 1949, v50, 522.

7. Hungerford, G. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 356.

8. Vogt, M., *J. Physiol.*, 1945, v104, 60.