mg A.C.T.H. for mice, 60 to 80 days of age and 10 mg of A.C.T.H. for 4-month-old rabbits, it was considered that weight for weight 3½ to 4 mg of A.C.T.H. would be a suitable dose for the guinea pigs used. The animals were carefully observed for the appearance of anaphylactic shock which was graded as follows: mild shock, severe shock, and death.

Results. The results of this experiment are summarized in Table I. The animals in both groups showed a similar response to the shocking dose of the antigen. Group I, the control group, all showed varying degrees of shock, which was fatal in 12 of 17 animals, with 3 showing severe shock and 2 showing symptoms of mild shock. Group II, the experimental group, also showed 100% reactivity, there being 12 instances of fatal shock, 3 cases of severe shock, and mild shock in 3 animals. The time of death occurred at approximately the same time interval following the shocking dose in both groups of animals. Therefore, one may conclude that A.C.T.H. in the dose administered, and under these experimental conditions, does not seem to have any effect on anaphylactic shock as elicited in guinea pigs by the procedure outlined above.

Summary. The administration of adrenocorticotrophic extract to sensitized guinea pigs prior to injection of a shock dose of antigen failed to influence the course of anaphylactic shock in a group of 18 animals.

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In vitro Studies of Aureomycin, a New Antibiotic Agent.*

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Samples of a new antibiotic, designated as A377 and later named aureomycin, were received from the Lederle Laboratories Division of the American Cyanamid Company, in November 1947. Since preliminary trials showed that the product had considerable activity *in vitro* against both a gram negative bacillus and a gram positive coccus, it was decided to test it further.

Description. According to information received from the Lederle Laboratories, aureomycin is derived from a strain of *Streptomyces aureofaciens.* The partially purified hydrochloride which was sent us is a bright yellow, fluffy, crystalline material. A 10% solution can be prepared in distilled water but the solubility is lowered by the presence of sodium chloride. The pH of a 0.1% aqueous solution is approximately 4. Dilute broth solutions pass through Berkefeld N and Seitz filters with but little loss of activity.

Range of Action. The least amount of aureomycin which prevents visible growth for 24 hours was determined for a variety of gram positive and gram negative bacteria. Polymyxin[1] and penicillin were used as standards of comparison in parallel tests. Serial 2-fold dilutions of the drugs in Difco Heart Infusion Broth, with 0.075% dextrose added, were inoculated with equal volumes of 18 hour old cultures so diluted as to result in approximately 200,000 organisms per cc. The diluent for the gram negative bacilli was

* These investigations were supported by grants from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories Division of the American Cyanamid Company, Parke, Davis and Company, and The Upjohn Company.

[1] Stansly, P. G., Shepherd, R. G., and White, H. J., *Bull. Johns Hopkins Hosp.*, 1947, **81**, 43.

TABLE I.
Comparative Activity of Aureomycin (A377), Penicillin, and Polymyxin (B71) for Various Bacteria.

Bacterium	Group or type	Medium	Inoculum, cc	Minimal inhibitory conc., γ/cc (24 hr) A377	Peni.	B71	A/P ratio
Str. hemolyticus	A-C203	Blood HIB	.5 × 10-3	.3125	.006		50
" "	D-Zymog*	"	"	1.25	2.5	>(20)	.5
" "	F-For	"	"	.625	.05		12.5
" "	G-Dog	"	"	1.25	.0125		100
D. pneumoniae	I SV1	"	"	.3125	.025		12.5
" "	I Bailey	"	"	.3125	.0125		25
" "	II	"	× 10-4	.3125			
" "	III	"	"	.156			
Str. faecalis	D-Tarr*	"	× 10-3	1.25	>1		< 1
Staphylococcus	Barlow	"	"	.625	.025		25
(all are *aureus*)	Zeut	"	"	.62	.05		12.5
	Zorn	"	"	.62	.05		12.5
B. subtilis	Lederle	"	"	.08			
" "	FDA	"	"	2.5			
" "	"	Plain HIB	"	2.5			
E. coli	4 SI	"	× 10-4	5.0		.156	32
" "	9	"	"	5.0		.156	32
A. aerogenes	7	"	"	5.0		.3125	16
" "	8	"	"	5.0		.625	8
K. pneumoniae	Lederle	"	"	1.25		.156	8
" "	Cephus	"	"	5.0		.625	8
Ps. aeruginosa	16	"	"	>20		2.5	> 8
" "	Calloway	"	"	>20		2.5	> 8
Proteus	17	"	"	>20		>20	
"	18	"	"	>20		>20	
H. influenzae	b-Pittman	Fildes-HIB	—	2.0		.35	6

* Four additional strains of Group D streptococci (α and β) were tested. All were inhibited by 1.25 γ/cc A377 and were less sensitive to penicillin.

heart infusion broth, that for the cocci contained 4% of washed rabbit red cells. The tests were read after 18 hours incubation at 37°C.

The results are shown in Table I. It is apparent that, in the test tube and with the inocula used here, aureomycin is less effective than polymyxin and penicillin against the gram negative bacilli and the gram positive cocci, respectively. The only exception noted was in the case of the Group D streptococci. Both α and β strains of these organisms were more susceptible to aureomycin than to penicillin. In the concentrations tested here, neither aureomycin nor polymyxin checked the growth of *Proteus*.

Comparison was also made with streptomycin in a few instances, with the results shown in Table II. Aureomycin was less effective than streptomycin in suppressing, for 18 hours, the growth of *E. coli* and *K. pneumoniae*. The strain of staphylococcus which was used in this test was more susceptible to the new agent than to streptomycin, while the two agents were equally effective against *H. influenzae b*.

Bacteriostatic vs. Bactericidal Activity. In the course of the above tests it was noted that on further incubation the end-points for aureomycin moved up day by day. Moreover, subcultures on blood agar plates made after the first day of incubation showed the presence of

TABLE II.
Comparative Activity of Aureomycin (A377) and Streptomycin for Various Bacteria, at the End of 24 and 48 Hours Incubation.

Organism	Drug	End-point—γ/cc Turbidity 24 hr	Turbidity 48 hr	Subculture 24 hr	A/S ratio 24 hr
E. coli	A377	10.0	20.0	>30.0	2.0
	Streptomycin	5.0	5.0	5.0	
Staph. aureus	A377	1.0	10.0	>20.0	0.1
	Streptomycin	10.0	10.0	10.0	
K. pneumoniae	A377	2.5	10.0	15.0	2.0
	Streptomycin	1.25	1.25	1.25	
H. influenzae	A377	2.0	2.0	2.0	0.67
	Streptomycin	3.0	3.0	3.0	

Note: Medium—Heart infusion broth, except in case of *H. influenzae* where Fildes broth was used.

TABLE III.
Growth Rate of *E. coli* and Beta Streptococcus in the Presence of Aureomycin (A377), Polymyxin (B71), and Penicillin.
(a) *E. coli*—Heart Infusion Broth.

Time of plating Hr after inoculation	Broth control	A377 20 γ/cc*	B71 0.2 γ/cc‡
	Organisms per cc		
	× 1000	× 1000	× 1
0	220	230	310,000
1	430	90	20
3	16,000	35	<10
6	300,000	12	< 2
24	5,000,000	13	< 2
72	—	++++†	—

(b) Beta Streptococcus C203—Heart Infusion Broth + Blood.

	Broth control		A377 1.25 γ/cc*		Penicillin 0.025 γ/cc*	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
	Organisms per cc × 1000					
0	140	275	40	305	120	290
1	200	545	90	95	40	30
3	4000	—	42	—	5	—
4	—	19,000	—	12	—	.3
5	115,000	—	23	—	1.8	—
24	550,000	—	6	—	.03	—
48	—	++++	—	++++	—	0
72	—	—	++++	—	0	—

* 4 times minimal inhibitory concentration for test organism.
† Maximum turbidity.
‡ This test was run on another occasion; 0.2 γ/cc polymyxin is only slightly more than the minimal inhibitory concentration for *E. coli*.

viable organisms in tubes containing 20 times the amount of antibiotic which had prevented visible growth.

These observations, illustrated in Table II, indicated that aureomycin is bacteriostatic rather than bactericidal in its action. This point has been confirmed by studies of the rate of growth of *E. coli* and the C203 strain of hemolytic streptococcus in the presence of 4 times the inhibitory concentration of the agent. In the case of C203 comparison was made with penicillin. Ten cc volumes of the

TABLE IV.
Deterioration of Aureomycin (A377) after Incubation Alone and in Presence of Test Organism.

Duration of preliminary incubation, hr	Visible end-point γ/cc		
	24 hr reading	48 hr reading	72 hr reading
*			
0	1.25	5.0	10
24	10	20	20
48	20	>20	>20
†			
0	1.25	2.5	10
24	2.5	10	20
48	5	20	>20

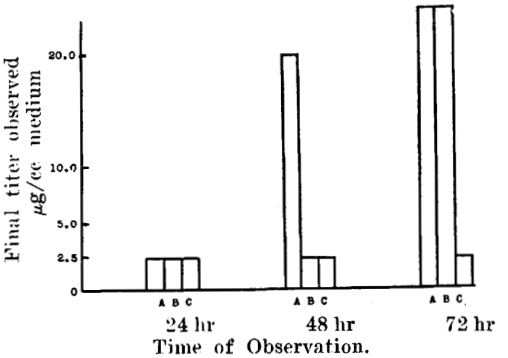
* 40 γ/cc A377 in heart infusion broth after 0 hr, 24 hr, 48 hr incubation—titrated against test organism (*Staph. aureus*).

† Supernatant of 40 γ/cc A377 after incubation with live *Staph. aureus* at 0 hr, 24 hr, 48 hr—titrated against test organism (*Staph. aureus*).

solutions were inoculated with 0.1 cc of 1:100 dilutions of the cultures. Plates were poured at intervals. The results, shown in Table III a and b, indicate that although aureomycin brings about a progressive decline in the bacterial population for the first 24 hours of exposure, the organisms eventually recover and reach maximum growth. In contrast, penicillin caused an immediate decline in the bacterial count which terminated in the sterilization of the cultures. Polymyxin, in a concentration only slightly above the minimal inhibitory concentration, has been observed, in other studies,[2] to exert an extremely rapid bactericidal effect upon *E. coli*. Aureomycin is clearly less decisive in its action than are penicillin and polymyxin.

It is not known at present whether the eventual outgrowth, in the case of aureomycin, is the result of the survival of resistant organisms or is due to the deterioration of the drug. As shown in Table IV, when aureomycin was titrated in the usual manner after incubation at 37°C in broth alone or in the presence of the test organism for 24 and 48 hours, there was progressive and rapid inactivation.

Confirmatory evidence of this phenomenon was obtained in another set of experiments in which *K. pneumoniae* was the test organism. Three sets of tubes, each containing 2-fold dilutions of aureomycin from 0.625 γ/cc through 20 γ/cc were inoculated in the usual manner. After 24 hours incubation, growth was found to have been inhibited by 2.5 γ/cc per tube of drug in all 3 sets of tubes. The first set (a) was allowed to incubate without further addition of drug through 72 hours. Fresh drug, to give an additional 2.5 γ/cc per tube, was added to the second set (b) at 24 hours, and to the third set (c) at 24 and 48 hours. As shown in Fig. 1, the inhibitory concentration in (a) was 20 γ/cc at 48 hours and more than 20 γ/cc at 72 hours. Growth in (b) was inhibited by 2.5 γ/cc for 48 hours and then titrated to more than 20 γ/cc at 72 hours. In (c), however, with drug replenished at 24 and 48 hours, the original inhibitory titre of 2.5 γ/cc was maintained throughout the 72 hour observation period. With no further addition of drug to set (c) at 72 hours, growth occurred through 20 γ/cc at 96 hours.



A: No addition of aureomycin.
B: Addition of 2.5 μg/cc at 24 hr.
C: Addition of 2.5 μg/cc at 24 and 48 hr.

FIG. 1.

Effect of adding increments of fresh aureomycin.

2 To be published.

TABLE V.
Effect of Normal Human Serum on the End-points of Titrations of Aureomycin.

Bacterium	Medium	Serum concentration, % 50	25	12½	6¼	3⅛	1½	0
		Minimal inhibitory conc. of A377, γ/cc						
E. coli	S1G1	25	12.5	6.25	6.25	5	1.56	1.25
	HIB	80	15	10				4
Strept. C203	HIB + Blood	6.25	1.25	0.625	0.312	0.312	0.125	0.125

TABLE VI.
Instability of Aureomycin to Heat. Minimal Inhibitory Concentrations for *E. coli* after Various Exposures.

Duration of exposure	Temperature, centigrade 10	24	37	56
	Minimal inhibitory conc., γ/cc			
0 min.			6.25	6.25
25 "		6.25		
40 "				6.25
1 hr				12.5
2½ "		6.25		
4 "				25.0
5 "		6.25		
18 "	6.25		20	
24 "	6.25	12.5		

Effect of Environmental Factors on Titration. Medium. In contrast to that of the sulfonamides, the activity of aureomycin for *E. coli* is but little better in the synthetic medium, S1G1,[3] than it is in heart infusion broth. The addition of serum, however, to either medium has a marked effect upon the titration end point. As shown in Table V, the end points vary directly with the concentration of serum. In this respect aureomycin resembles penicillin. The effect, however, is very much greater on the new antibiotic, 50% serum causing a 20- to 50-fold raising of the end-point of aureomycin and only a 4-fold increase in the case of penicillin.

Temperature. Aureomycin showed progressive loss of activity when held, in broth solutions; at higher than refrigerator temperatures. As shown in Table VI, at room temperature there was no change for 5 hours, but by 24 hours half the activity had been lost. Storage at 37°C for 18 hours before use reduced activity by 60%. At 56°C the rate of deterioration increased; 50% of the activity was lost in 1 hour, 75% in 4 hours.

Relation of Size of Inoculum to Minimal Inhibitory Concentration. Seven sets of serial 2-fold dilutions of aureomycin, containing 0 to 50 γ/cc, were set up in Wassermann tubes. Each set was inoculated with one of a series of 10-fold dilutions, in blood broth, of an 18 hour culture of C203. The tests were incubated at 37°C for 18 hours. It was found that the end-point depends, to a certain extent, upon the number of organisms inoculated. Inocula of 10^{-5} to 10^{-7} cc of culture were inhibited by 0.156 γ/cc aureomycin, while 0.312 γ/cc was required to delay the development of 10^{-2} to 10^{-4} cc. The end-point obtained with 1/10 cc of culture could not be determined because there was sufficient streptolysin present in the inoculum to hemolyze the red blood cells.

Summary. A new antibiotic, aureomycin, is effective *in vitro* against both gram positive and gram negative bacteria. Although its activity is of a lower order than that of penicillin for gram positive cocci or polymyxin for gram negative bacilli, it is effective against both classes of bacteria in concentrations close

[3] Bliss, E. A., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 14.

to those required of streptomycin. Inhibition at such concentrations, however, is fleeting in the case of aureomycin, much larger amounts being required for permanent suppression of growth. The new agent is bacteriostatic rather than bactericidal in its effect. Its antibacterial action is greatly diminished in the presence of serum, *in vitro*, and deterioration at room temperature is marked. Differences in the size of the inoculum have a moderate effect upon the minimal inhibitory concentration.

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Further Studies in Experimental Allergic Encephalitis in the Guinea Pig.

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It has been indicated[1,2] that an encephalitic process, presumably allergic in nature, results in guinea pigs inoculated with rabbit-brain tissue and "adjuvants". A brief report is herein given of additional experiments with brain-tissue inocula derived from various animal species, as well as on the effect of progressive dilutions of brain-tissue inocula, attempts at purifying the antigen, effect of intracerebral re-injection of the antigen, and on the development of antibrain antibodies in animals inoculated either subcutaneously alone, or subcutaneously and then followed by an intracerebral inoculation of the brain suspension.

1. *Inoculation of brain tissue from various animal species.* The technic followed was the same as reported previously[2] except that brain material from 5 different species was used for the subcutaneous inoculation. Paralysis of inoculated guinea pigs was observed usually 21 or more days after the injection. Death followed from 1 to 15 days after onset of paralysis and occasionally occurred in animals which had shown no neurological manifestations. The results obtained (Table I) do not lend themselves to a satisfactory comparison because of the low and unequal number of animals used in each group. In general, the percentage of animals which succumbed was proportionately similar in each group regardless of the source of the brain tissue used in the inoculum.

Lesions in the central nervous system (CNS), of the type already described,[2] were observed in all animals. In general no conspicuous differences were noted in the lesions which could be ascribed to the use of heterologous antigens, but in some guinea pigs more extensive lesions were obtained when guinea pig brain suspensions were used as inoculum.

2. *Effect of progressive dilutions of brain tissue inoculum.* Phenolized rabies vaccines prepared from calf and rabbit brain tissue, respectively, were used as source material for the inoculum. Starting with the 1:20 dilution of brain emulsion, serial twofold dilutions were made in saline, incorporated into adjuvants and injected into guinea pigs. For the sake of brevity, the results of the experiment (Table II) were grouped irrespective of the brain tissue used as inoculum. For comparison are included data summarizing the results of various experiments in which guinea pigs were inoculated with a suspension of normal rabbit brain tissue in 1:10 dilution. Up to and including the 1:80 dilution, there appeared to be no material difference in the

[1] Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

[2] Jervis, G. A., and Koprowski, H., *J. Neuropath. and Exp. Neurol.*, 1948, **7**, 309.