A COMPARISON OF EIGHT ANTIBIOTIC AGENTS, IN VIVO AND IN VITRO[1]

ELEANOR A. BLISS AND H. PATRICIA TODD

Department of Preventive Medicine, The Johns Hopkins University, School of Medicine, Baltimore 5, Maryland

Received for publication April 8, 1949

During the past year a number of new antibiotic agents have been studied by members of this department. Some of the observations, on separate agents, have already been published (Bliss *et al.*, 1948; Schoenbach *et al.*, 1948; Bryer *et al.*, 1948; Bliss and Chandler, 1948; Chandler and Bliss, 1948). In the present report, comparisons of the agents with respect to antibacterial activity, *in vitro* and in experimental infections in mice, are presented.

MATERIALS AND METHODS

Agents. Polymyxin D and aureomycin were received through the courtesy of The American Cyanamid Company and the Lederle Laboratories, Inc., during the fall of 1947. Polymyxin D, first described by Benedict and Langlykke (1947) and Stansly, Shepherd, and White (1947), is derived from filtrates of cultures of *Bacillus polymyxa.* The material used here is the hydrochloride, Lederle lot nos. 7-7795 and 7-8244.

Aureomycin is produced from *Streptomyces aureofaciens.* Its antibiotic properties were discovered by Dr. B. M. Duggar (1948) of the Lederle Laboratories and were first publicly described at a meeting in July, 1948. Lots 7-8020 A, 7-8071 A, 7-8254, and 7-8411 of the dried hydrochloride of this agent were used for the work that will be described. These lots were about 80 per cent pure aureomycin, according to a note from the manufacturers.

We are indebted to Burroughs Wellcome and Company for a supply of polymyxin B. The vials are labeled "Aerosporin-Brand." The history of the polymyxins is somewhat confusing. The one that was first described by Benedict and Langlykke and by Stansly, Shepherd, and White was derived, as mentioned above, from an organism identified as *B. polymyxa.* It is the one now known as polymyxin D. Almost simultaneously with its discovery, Ainsworth, Brown, and Brownlee (1947) announced that extracts of *Bacillus aerosporus* (Greer) had antibacterial activity. They named this product aerosporin but noted that *B. aerosporus* was called, by many investigators, *B. polymyxa.* Subsequently, Brownlee discovered that *B. aerosporus* (or *B. polymyxa*) produced at least two other agents besides the original polymyxin and aerosporin. This finding was reported at a conference of the Section on Biology of the New York Academy of Sciences held in May, 1948. Unfortunately the transactions of this meeting have not yet been published. After some discussion it was agreed that

[1] This study was supported by a grant from the Public Health Service, Division of Research Grants and Fellowships.

Brownlee's three aerosporins should be called polymyxin A, B, and C, whereas the original polymyxin should be given the suffix D. According to a personal communication from Dr. Gladys Hobby, pure polymyxin B has 10,000 units per milligram and the hydrochloride, lot no. 0-896, studied here, has a potency of 7,050 units per milligram.

Sodium penicillin O (allylmercaptomethyl penicillin), research no. 8809, produced by The Upjohn Company, was received from the Antibiotics Study Section of the Public Health Service and was included in the study for comparison with penicillin G. A number of different samples of sodium crystalline penicillin G were used—Winthrop Chemical Company, lot 2326C, potency: 1,500+ u per mg, and Lederle lots 3811-421A and 3811-10,031, potencies: 1,532 and 1,533 u per mg. respectively.

A 2-gram sample of chloromycetin was kindly furnished by Dr. E. A. Sharp of Parke, Davis and Company, in October, 1948. This is labeled lot X3176. Chloromycetin, isolated from cultures of *Streptomyces venezuelae* by Burkholder of Yale and Ehrlich and his associates of Parke, Davis and Company (Ehrlich *et al.*, 1947), was shown to have antibacterial properties by the same investigators, and to be effective in certain rickettsial and viral infections by Smadel and Jackson (1947).

Circulin (Q-19) was received from The Upjohn Company in November, 1948. It was first described by Murray and Tetrault (1948), who reported that lyophilized preparations from a sporeforming, aerobic soil bacillus inhibited the growth of a number of gram-negative and a few gram-positive bacteria. According to these authors, the organism belonged to the same genus as *B. polymyxa* but was not identical with that bacillus; its cultural reactions most closely resembled those of *Bacillus circulans*. Independently, McLeod (1948) discovered another antibiotic from a strain of *B. circulans* and called it, likewise, circulin. In a footnote to her paper she stated that her agent differs from the one described by Murray and Tetrault in that the latter is "water-soluble, relatively nontoxic and more active against gram-negative than gram-positive organisms." The material studied here is the Murray and Tetrault product, and, to avoid confusion, it will be designated as Q-19 in the present paper. According to the manufacturers' statement there are 2,500 units of active material per milligram of drug in the first lot received—research no. 8836. Later a new lot was received, having double the potency of the first. It is designated as Q-19-1 in the tables.

The streptomycin used was E. R. Squibb and Sons' hydrochloride, control 11392.

Preparation of solutions. Except in the case of chloromycetin, the solutions for injection were made up in sterile distilled water. With chloromycetin the limit of solubility in water is about 2 mg per ml. When higher concentrations are required another solvent must be used. Smith *et al.* (1948) used 20 to 50 per cent propylene glycol but, since Prigal and his associates (1947) observed antibacterial substances in the blood of animals treated with 20 per cent propylene glycol, it was thought best here to use alcohol as the solvent for chloromycetin. With 20 per cent ethyl alcohol a 1 per cent solution could be prepared,

but even then crystallization occurred on standing. For this reason solutions of chloromycetin, of over 0.1 per cent concentration, were left at room temperature and, if necessary, were warmed to 55 C before being used. This may be done with impunity because as reported by Bartz (1948) and confirmed by us, the agent is stable to heat. All of the other solutions were stored in a "deep freeze."

The agents were compared on a weight basis, penicillin, polymyxin B, Q-19, streptomycin, and chloromycetin invariably being weighed out. Solutions of polymyxin D and aureomycin were usually so prepared, but this was not necessary as the vials had been filled on a weight basis rather than on a unitage or equivalence basis.

In vitro tests. The basic medium used in the *in vitro* studies was Difco heart infusion broth to which 0.05 per cent of glucose was added. One-half-ml volumes of serial twofold dilutions of the agents were inoculated with 0.5 ml of a 1:10,000 dilution of the test organism. In the case of the cocci the final dilution was made in broth containing 4 per cent of fresh, or washed, rabbit's red cells. The tests were read after 18 to 20 hours' incubation at 37 C, and the presence or absence of visible turbidity or hemolysis was noted.

The organisms tested were stock laboratory strains and others recovered from patients during the year. The authors are indebted to Miss Minnie Schreiber and Miss Rosemary Stokes of the Biological Division, The Johns Hopkins Hospital, for isolating a number of these organisms, and to Dr. Horace W. Smith for identifying the strains of *Proteus*.

Two or more agents were tested simultaneously, and polymyxin D or penicillin G were usually included as standards of comparison. These agents, therefore, and aureomycin were the subjects of repeated trials. The end points for a given organism and drug were not always the same, but, in order to simplify the tables, the one noted most frequently is shown as the minimal inhibitory concentration.

The effect of certain of the agents on the rate of multiplication of *Escherichia coli* or the C203 strain of beta hemolytic streptococcus was studied. This was done by inoculating 10-ml volumes of drug-broth solutions with 0.1 ml of 1:100 dilutions of the cultures and plating out after 1, 3, 5, and 24 hours of incubation at 37 C.

In order to test the stability of the agents to heat and changes in the hydrogen ion concentration, a batch of heart infusion broth was divided into three lots, which were titrated to pH 5, 7, and 8 with hydrochloric acid or sodium hydroxide. To 25- to 30-ml aliquots at each pH sufficient antibiotic was added to make the desired concentration, namely, 200 μg per ml, for aureomycin, 100 μg per ml for streptomycin and chloromycetin, and 20 μg per ml for polymyxin D and Q-19. Each portion was subdivided in five 5-ml lots. One of these was placed in the "deep freeze" for 24 hours; one was held at room temperature and another at 37 C for the same period; one, after 22 hours in the refrigerator, was placed in a 56 C water bath for 2 hours; the last, refrigerated for 23 hours, was heated in a boiling water bath for 10 minutes. The solutions were then diluted out in broth, at pH 7.2, in twofold steps, using 0.5-ml volumes, and were inoculated with 0.5 ml of a 1:10,000 dilution of the test strain of *E. coli*.

Therapeutic tests. White Swiss mice weighing 18 to 22 grams were used. For

the first 3 months the mice came from a mixed stock; later, the purebred CF_1 mice were used. Since the results with the two kinds of mice were quite different, they will be discussed separately.

TABLE 1

Effect of size of inoculum on titration end points

DRUG	E. COLI				HEMOLYTIC STREPTOCOCCUS, C203			
	Inoculum = 0.5 ml of dilution							
	1:5	1:500	1:50T	1:50M	1:5	1:500	1:50T	1:5M
	End points—μg/ml							
Penicillin G					0.1	0.012	0.012	0.006
Aureomycin					2.5	0.625	0.312	0.312
Chloromycetin	6.25	6.25	3.12	1.56	12.5	3.12	1.5	0.8
Streptomycin	100	6.25	3.12	1.56	100	50	12.5	6.25
Polymyxin D	10	0.625	0.15	0.04				
Polymyxin B	5	1.25	0.625	0.15				
Q-19	10	2.5	1.25	0.625				

TABLE 2

Minimal inhibitory concentrations of antibiotics for gram-negative bacilli

(20-hour readings)

ORGANISM	AUREO.	CHLORO.	STREPTO.	Q-19	POLY. B	POLY. D
	Minimal inhibitory concentration, μg/ml					
E. coli, 4	5	10	6.25	0.625	0.312	0.156
E. coli, 9	5	10	6.25	0.625	0.312	0.156
E. communior, 14	5	5	2.5	1.25	1.25	0.625
Citrobacter, 6	5	5	5	1.25	0.625	0.312
Aerobacter, 10	5	10	>100	1.25	2.5	0.625
Aerobacter, 12	2.5	5	2.5	1.25	2.5	0.625
Aerobacter, 8	5	—	10	0.625	2.5	0.625
Aerobacter, 15	—	—	—	—	0.625	0.312
K. pneumoniae A (Lederle)	1.25	1.25	0.62	0.625	0.625	0.156
K. pneumoniae B (Cephus)	5	5	5	1.25	0.625	0.625
Proteus vulgaris, 11	6.25	2.5	5	>100	>100	>100
Proteus mirabilis, 18	100	25	5	>100	>100	>100
Proteus mirabilis (Buck)	100	6.25	25	>100	>100	>100
Proteus mirabilis (Herr)	100	12.5	12.5	>100	>100	>100
Proteus unidentified, 17	50	12.5	5	>100	>100	>100
P. aeruginosa (Butts)	100	100	100	5	1.25	2.5
P. aeruginosa (Callo)	100	>100	>100	1.25	2.5	2.5
P. aeruginosa (Herr)	100	>100	25	2.5	1.25	1.25
P. aeruginosa, 16	100	50	50	5	1.25	1.25

Three organisms were used in the therapeutic comparisons: the C203 strain of hemolytic streptococcus, the SV1 strain of type I pneumococcus, received some years ago from Dr. Colin McLeod, and a strain of *Klebsiella pneumoniae* type A, received from the Lederle Laboratories and designated by them as KpnA-D.

Cultures were prepared by mouse passage the day before they were to be used. From the mouse they were transferred to blood broth and grown for 18 hours at 37 C.

The mice were infected by the intraperitoneal injection of 0.5 ml of a plain broth dilution of the culture. For C203 a 1:1,000 dilution was used, for SV1 the culture was diluted 1:5,000, and for *K. pneumoniae*, 1:500. These amounts resulted in inocula averaging 100,000 bacteria in the case of the first two organ-

TABLE 3

Minimal inhibitory concentrations of antibiotics for gram-positive cocci

(20-hour readings)

ORGANISM	AUREO.	CHLORO.	STREPTO.	PENI. O	PENI. G	Q-19
	Minimal inhibitory concentration, μg/ml					
Streptococcus group A, C203	0.31	5	12.5	0.008	0.008	100
Streptococcus group A, NY5	0.16	2.5	25	0.006	0.006	
Streptococcus group A, Cotton	0.16	2.5	12.5	0.006	0.006	
Streptococcus group B, 090	1.25	5	50	0.10	0.006	
Streptococcus group B, 19	0.62	2.5	100	0.10	0.031	
Streptococcus group C, K61	0.62	2.5	6.2	0.025	0.016	
Streptococcus group D, Zymog	1.25	10	50	5.0	2.5	>100
Streptococcus group D, H69	0.62	5	50	—	2.5	
Streptococcus group D, 22A	0.62	10	50	5.0	2.5	
Streptococcus group F, For	0.62	2.5	6.2	0.10	0.05	
Streptococcus group F, H59	1.25	2.5	12.5	0.10	0.016	
Streptococcus group G, Dog	1.25	2.5	12.5	0.10	0.012	
Streptococcus faecalis, Weston	1.25	10	50	5.0	2.5	
Streptococcus faecalis, Black	0.62	10	50	5.0	2.5	>100
Streptococcus faecalis, Tarr	1.25	10	50	5.0	2.5	>100
Streptococcus faecalis, Twyman	0.62	5	10	5.0	2.5	
Streptococcus viridans, Dopkin	1.25	2.5	3.1	0.625	0.625	
Streptococcus viridans, Keel	0.62	10	12.5	5.0	2.5	
D. pneumoniae type I, SV1	0.31	2.5	12.5	0.016	0.016	>100
D. pneumoniae type I, Bailey	0.12	1.2	2	0.025	0.012	>100
D. pneumoniae type III, Bayer	<0.15	2.5	12.5	0.025	0.025	
Staphylococcus albus, Heatly	0.62	5.0	2	0.062	0.016	>100
Staphylococcus aureus, Zeut	0.62	5	12.5	0.125	0.062	>100
Staphylococcus aureus, Zorn	0.62	5	2	0.125	0.062	>100
Staphylococcus aureus, Gelb	0.62	5	—	0.062	0.062	
Staphylococcus aureus, Gibb	0.62	10	—	0.062	0.031	

isms, and 600,000 in that of *K. pneumoniae*. The virulence of the specific culture was determined each time by injecting two mice each with three higher dilutions of the culture, from 1:1 million to 1:1 billion. These tests showed a variation in virulence that appeared to be unrelated to difference in the bacterial count. However, in most of the experiments with C203 and *K. pneumoniae* the infecting dose was approximately 10,000 MLD. The inoculum of 10^{-4} ml of pneumococcus culture also represented 10,000 MLD for the mixed breed of mice, but 100,000 for the pure CF_1 strain.

Immediately after each mouse in the treatment series was infected it was

given a subcutaneous injection of 0.2 ml of one of the drug dilutions. In most of the experiments two additional treatments were given, at 5½ and 23 hours after the infecting dose. Polymyxin B and D were compared at first on the basis of a single immediate treatment. The mice were observed for 7 days after the last treatment.

RESULTS

Stability of agents. Chloromycetin was completely stable to heat at pH 5, 7, and 8. Even subjection to a temperature of 100 C for 2 hours did not raise the titration end point above that shown by solutions stored in the "deep freeze."

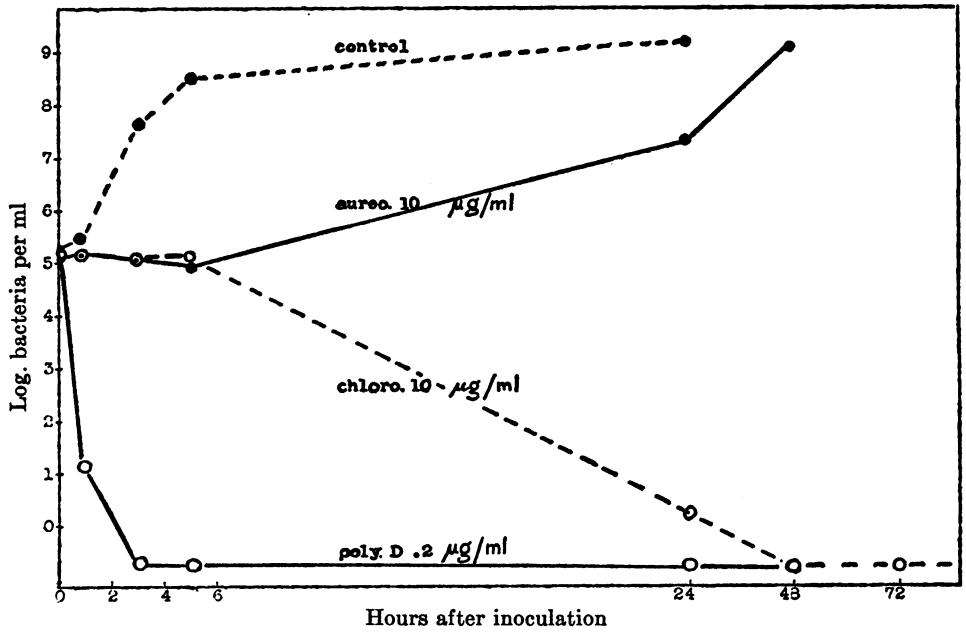

Figure 1. The effect of aureomycin, chloromycetin, and polymyxin D on the rate of multiplication of *E. coli*.

Streptomycin was less active when kept in an acid environment than when it was kept at pH 7, but polymyxin D and Q-19 were most stable at pH 5. All three of these agents were slightly affected by temperatures over 37 C. Aureomycin proved highly susceptible to heat, especially in the presence of alkali.

Effect of size of inoculum. Previous experience (Bliss *et al.*, 1948) showed that end points with polymyxin D depended upon how many bacteria were inoculated. The other agents were tested in like manner, with the results shown in table 1. There it is seen that the behavior of all of the agents is affected by the size of the inoculum. Chloromycetin appears to be least subject to this factor, whereas polymyxin D is the most sensitive to it. For this reason, a standard inoculum of 0.5 ml. of a 1:10,000 dilution of the culture was used in all of the titrations, except those involving the strains of *Proteus*. These organisms had been found

so resistant to polymyxin D, in the earlier studies, that their cultures were diluted 1:100,000.

Minimal inhibitory concentrations. In tables 2 and 3 are shown the lowest concentrations of the agents which inhibited the growth of the various strains for

TABLE 4

Therapy of K. pneumoniae infection in mice, with polymyxin B and D, Q-19, streptomycin, aureomycin, and chloromycetin

(Strain KPnAD—type A)

B½: SINGLE AT 0 HR			THREE, AT 0, 5½, AND 23 HOURS AFTER INFECTION										
	Mixed-strain mice					CF_1 mice						
Dose of drug per B½	Poly. D	Poly. B	Poly. D	Aureo.	Chloro.	Poly. D	Poly. B	Q-19	Q-19-1	Strepto.	Aureo.	Chloro.
	Per cent survival (10–20 mice per dose)											
mg/kg												
80				80	90					100	90	85
70											90	100
50										100	70	65
35											70	80
25				10	10	100	100	100	90	100	20	0
10										100		
8				0	0	100		100	100	90		
4			80			90	100	100	100	60		
2.4						85	100	95	100	60		
1.6										40		
1.2			90			70						
1.0	70	85	30									
0.8						90	100	70	90	30		
0.6							60					
0.4	20	20	25			27.5	65	38	90			
0.3			0				20	0	80			
0.2	0	5	0			0	0	0	10			
0.1						0	0	0	0			
Median protective dose—mg/kg/treatment												
PD_{50}	0.8?	0.7?	1.0	58?	52?	0.7	0.4	0.5	0.3	2.3	38	42
95% confidence limits						0.5–0.95	0.33–0.53	0.35–0.81	0.18–0.51	1.6–3.2	30–46.8	37.6–47.0

20 hours. In general, the most effective agent against the gram-negative bacilli, *in vitro*, was polymyxin D. The least active was chloromycetin. This last, however, was more effective than either polymyxin D or aureomycin against the five strains of *Proteus*. These strains, all extremely resistant to the polymyxins and Q-19, varied in sensitiveness to aureomycin and were all moderately re-

sponsive to chloromycetin and streptomycin. Strains of *Pseudomonas* required high concentrations of aureomycin, chloromycetin, and streptomycin for inhibition.

Against the gram-positive cocci the two penicillins were generally most active; aureomycin occupied a middle position, and chloromycetin and streptomycin were the least effective of the agents in this class. Q-19 and polymyxin D (not shown in table 3) did not prevent the growth of any of the nine strains of cocci that were tested at concentrations under 100 μg per ml. It is to be noted that

TABLE 5

Therapy of hemolytic streptococcal infection in mice with penicillin G and O, aureomycin, chloromycetin, and streptomycin

(R$\frac{1}{2}$: 3, at 0, 5$\frac{1}{2}$, and 23 hours after infection. Strain: C203, group A)

DOSE OF DRUG PER R$\frac{1}{2}$	MIXED-STRAIN MICE				CF1 MICE				
	Peni. G	Peni. O	Aureo.	Chloro.	Peni. G	Peni. O	Aureo.	Chloro.	Strepto.
	Per cent survival (10–20 mice per dose)								
mg/kg									
100									70
80								0	43
50				33				10	30
25									10
10				0			85		
5				0			60		
3							30		
2			30				20		
1	100	100	0						
0.5	100	90	0		70	90			
0.4					73	60			
0.2	60	40			55	10			
0.16					0	10			
0.1	0								
0.06					10	0			
Median protective dose—mg/kg/treatment									
PD_{50}	0.18?	0.26?			0.22	0.33	4.3	>100	80
95% confidence limits					0.15–0.31	0.28–0.51	3.2–5.8		57–111.7

aureomycin inhibited the strains of *Streptococcus faecalis* and other enterococci at slightly lower concentrations than did either penicillin O or G.

Effect on the multiplication of bacteria. The effects of aureomycin, chloromycetin, and polymyxin D on the rate of multiplication of *E. coli* are illustrated in figure 1. Polymyxin D appears to be immediately effective in reducing the bacterial population and, in concentrations close to the minimal inhibitory concentration, in sterilizing the culture. Polymyxin B and Q-19 were also rapidly lethal in some of the tests, but their action was irregular and in general was not as striking as that of polymyxin D. Streptomycin, as well as chloromycetin,

had a slow but steady bactericidal effect. Aureomycin, at twice its minimal inhibitory concentration for *E. coli*, was only temporarily bacteriostatic.

With a strain of streptococcus as the test organism, penicillin G at 0.025 μg per ml and chloromycetin at 5 μg per ml were slowly bactericidal, whereas aureomycin at 1.25 μg per ml delayed growth only for 24 hours. Even at 5 μg per ml, a concentration equal to 10 times that required to suppress visible growth of the streptococcus for 24 hours, aureomycin failed to sterilize the cul-

TABLE 6

Therapy of pneumococcal infection in mice, with penicillin G and O, aureomycin, chloromycetin, and streptomycin

(R̄: 3 subcutaneous injections at 0, 5½, and 23 hours after infection. Strain SV1, type I)

DOSE OF DRUG PER R̄	MIXED-STRAIN MICE		CF1 MICE				
	Peni. G	Aureo.	Peni. G	Peni. O	Aureo.	Chloro.	Strepto.
	Per cent survival (10–40 mice per dose)						
mg/kg							
80						0	100
70							40
60			70	80			40
50						0	23.3
40			60	60			33.3
35							0
25			60	35	100	0	0
20		90					
10		40	30	3.3	90		
8		30			20		
6					20		
5	80		20	7.5			
4			10	10	0		
2.5			10	10			
2	60	10	10	5			
0.8	20		0	0			
Median protective dose—mg/kg/treatment							
PD_{50}	1.8	11.8	22.5	30.5	8.0	>80	56.2
95% confidence limits	1.19–2.72	9.64–13.9	14.0–36.2	16.8–55.2	7.2–8.9		53.8–58.7

ture; and, although there were fewer than 10 organisms per ml at 24 hours, at 48 hours the count was 30 per ml, and by 96 hours full growth had occurred.

Therapeutic activity. The results of treating infections induced in mice with *K. pneumoniae* A, a type I pneumococcus, and the C203 strain of hemolytic streptococcus are shown in tables 4, 5, and 6. The percentage survival at the different doses is indicated, and also the median protective dose for each drug, as determined by the method of Litchfield and Fertig (1941). Where possible the 95 per cent confidence limits, for the PD_{50}, have also been given. Chloromycetin and aureomycin showed the same order of activity against the *K. pneumoniae* infection, whereas streptomycin, the two Q-19's, and the two polymyxins were

effective at much lower doses. Although the differences between the last four agents were slight, they were statistically significant (with P less than 4 per cent) in the case of polymyxin D and polymyxin B and of polymyxin D and Q-19-1.

Chloromycetin, used in concentrations up to the limit of its solubility in 20 per cent alcohol, had no effect upon the pneumococcal and but little upon the hemolytic streptococcal infection. Aureomycin was effective against both infections when three treatments with 10 to 20 mg per kg were used. The two penicillins protected against the streptococcal infection at the lowest dosage levels of any of the five agents tested, and penicillin G was superior to aureomycin in the pneumococcal infection in the mixed breed of mice. In the purebred mice, however, much larger amounts of penicillin than of aureomycin were required to protect 50 per cent of the mice against the pneumococci. This difference between the two kinds of mice, in amenability to therapy, was not noted in the other infections, nor with the other drugs. Moreover, the dosage-survival curves were much steeper in the case of the CF_1 mice infected with pneumococci and treated with either of the penicillins than they were in any other combination of circumstances. (An impression of the steepness of the dosage-survival curves may be had from a study of the spread of the data in the "per cent survival" columns in the tables.)

DISCUSSION

Attention was called in previous reports (loc. cit.) to the rapidly lethal effect of polymyxin D upon cultures of *E. coli* and to the transiency of aureomycin's action. The latter appeared to be the result of deterioration of the drug *in vitro*. When fresh aureomycin was added every 24 hours (Chandler and Bliss, 1948), growth could be prevented indefinitely, but, if not replenished, even amounts of aureomycin greatly in excess of the minimal inhibitory concentration failed to prevent eventual outgrowth. Chloromycetin behaves like aureomycin in the early stages of a growth study, but the decline in bacterial population is maintained and by 24 or 48 hours no viable organisms are found. In contrast to aureomycin, chloromycetin is stable to heat even in an alkaline environment.

One of the interesting aspects of the *in vitro* comparisons was the grouping of the compounds in relation to their effects on organisms belonging to the *Proteus* and *Pseudomonas* genera. The agents derived from *Streptomyces* were almost without effect on the *Psuedomonas* strains, but showed moderate activity for *Proteus*. With the bacterial derivatives the reverse was true. This pattern was adhered to by 10 additional strains of each organism with only two exceptions. Two strains of *Pseudomonas* were inhibited by less than 6 μg per ml of streptomycin. It is believed that the divergent responses of the two kinds of bacteria to the two kinds of agents must reflect differences in the metabolic processes of *Proteus* and *Pseudomonas* and in the modes of action of the agents.

Of the agents tested, streptomycin was the most unpredictable in effect on the gram-negative bacilli. All the way from 0.6 to 100 μg per ml of this agent was required for the inhibition of members of the coli-aerogenes group. Aureomycin, as noted also by Paine, Collins, and Finland (1948), varied in its effect on strains of *Proteus*. These authors found the minimal inhibitory concentration of

aureomycin for 13 strains of *Proteus vulgaris* to be over 100 μg per ml. The single strain of *P. vulgaris* shown in table 3 of the present report was considerably more susceptible than the three strains of *Proteus mirabilis*, but among the additional strains studied jointly with Dr. Horace W. Smith there appeared to be no association between species and sensitivity to aureomycin.

Correlation between the *in vivo* and *in vitro* activity of the agents was good, but not perfect. In general, the order of effectiveness shown in the test tube was repeated in the animal experiments, but polymyxin D was effective *in vitro* at a lower concentration than any of the other agents, yet more of it was required for the successful therapy of *K. pneumoniae* infection in mice than of polymyxin B or the Q-19's. Moreover, there was a much greater difference between the therapeutic doses of aureomycin and streptomycin and of streptomycin and the polymyxin Q-19 group than was expected from the similarity of their minimal inhibitory concentrations for *K. pneumoniae in vitro*.

Chloromycetin, which *in vitro* seemed more active against the cocci than streptomycin, failed almost completely to protect mice infected with streptococci or pneumococci. The possibility that the doses of chloromycetin that were employed were approaching the toxic level was considered but was discarded in view of the relatively low toxicity of the agent for mice (Smith *et al.*, 1948). There is still the possibility that acute alcoholism may have contributed to the poor results with chloromycetin. Mice given 0.5-ml doses of 20 per cent alcohol by subcutaneous injection all survived but were quite ataxic for an hour. However, equally poor therapeutic results were obtained with a propylene glycol solution, administered by gavage in 50 mg per kg doses, to mice infected with streptococci. One can only conclude that chloromycetin is not effective in streptococcal and pneumococcal infections in mice at the doses to which we were limited by the low solubility of the agent.

Perfect agreement between the *in vivo* and *in vitro* orders of activity of a series of drugs is, of course, not expected because therapeutic properties are dependent upon the pharmacological behavior as well as upon the antibacterial activity of the agents. It would not have been surprising, therefore, had aureomycin, which is relatively slowly excreted (Dowling *et al.*, 1948), appeared more effective in the therapeutic trials than the rapidly excreted penicillins—and this was observed in the pneumococcal infection in the CF_1 mice. What is surpirising is the difference between the results with penicillin in the two kinds of infection. The explanation presumably lies in the greater virulence shown by the pneumococcus for the CF_1 mouse than for the mixed-breed line. It seems possible that two treatments were enough for the mixed-breed mice, and that the third treatment, essential for the CF_1 mice, came too late to be of any avail in the case of penicillin. Whatever the cause of the difference, it stresses the danger incurred in drawing conclusions from experiments run in just one kind of animal.

SUMMARY

Most gram-negative bacilli were considerably more sensitive, *in vitro*, to polymyxin D and B and circulin (Q-19) than to aureomycin, chloromycetin, or streptomycin. The striking exception to this was the behavior of strains of *Proteus*

which, as a group, proved extremely resistant to the first three agents. The polymyxins and Q-19 were more effective than the other agents in the control of an experimental *Klebsiella pneumoniae* infection in mice.

Penicillin G and O inhibited the growth of most gram-positive cocci at lower concentrations than aureomycin, chloromycetin, or streptomycin, and were the most active agents in the treatment of an experimental hemolytic streptococcal infection. Although penicillin G protected one breed of mouse against a type I pneumococcal infection at low doses, in another breed both it and penicillin O were much less effective, ranking well after aureomycin.

Chloromycetin, which inhibited the growth of cocci *in vitro* at lower concentrations than streptomycin, was valueless as a therapeutic agent in the pneumococcal and streptococcal infections in mice.

REFERENCES

AINSWORTH, G. C., BROWN, A. M., AND BROWNLEE, G. 1947 'Aerosporin,' an antibiotic produced by *Bacillus aerosporus* Greer. Nature, **160**, 263–264.

BARTZ, Q. R. 1948 Isolation and characterization of chloromycetin. J. Biol. Chem., **173**, 445–450.

BENEDICT, R. G., AND LANGLYKKE, A. F. 1947 Antibiotic activity of *Bacillus polymyxa*. J. Bact., **54**, 24–25.

BLISS, E. A., AND CHANDLER, C. A. 1948 *In vitro* studies of aureomycin, a new antibiotic agent. Proc. Soc. Exptl. Biol. Med., **69**, 467–472.

BLISS, E. A., CHANDLER, C. A., AND SCHOENBACH, E. B. 1948 *In vitro* studies of polymyxin. Bull. Johns Hopkins Hosp., **82**, 635.

BRYER, M. S., SCHOENBACH, E. B., CHANDLER, C. A., BLISS, E. A., AND LONG, P. H. 1948 Aureomycin. J. Am. Med. Assoc., **138**, 117–119.

CHANDLER, C. A., AND BLISS, E. A. 1948 *In vitro* studies with aureomycin. Ann. N. Y. Acad. Sci., **51**, 221–227.

DOWLING, H. F., LEPPER, M. H., SWEET, L. K., AND BRICKHOUSE, R. L. 1948 Studies on serum concentrations in humans and preliminary observations on the treatment of human infections with aureomycin. Ann. N. Y. Acad. Sci., **51**, 241–245.

DUGGAR, B. M. 1948 Aureomycin: a product of the continuing search for new antibiotics. Ann. N. Y. Acad. Sci., **51**, 177–181.

EHRLICH, J., BARTZ, Q. R., SMITH, R. M., AND BURKHOLDER, P. R. 1947 Chloromycetin: a new antibiotic from a soil actinomycete. Science, **106**, 417.

LITCHFIELD, J. T., JR., AND FERTIG, J. W. 1941 On a graphic solution of the dosage-effect curve. Bull. Johns Hopkins Hosp., **69**, 276–286.

MCLEOD, C. 1948 Circulin, an antibiotic from a member of the *Bacillus circulans* group. I. Bacteriological studies. J. Bact., **56**, 749–754.

MURRAY, P. J., AND TETRAULT, P. S. 1948 A new antibiotic effective against gram-negative organisms. Soc. Am. Bact., Proc. Meetings, **1**, 20.

PAINE, T. F., COLLINS, H. S., AND FINLAND, M. 1948 Bacteriologic studies on aureomycin. J. Bact., **56**, 489–497.

PRIGAL, S. J., MCGAVACK, T. H., SPEER, F. D., AND HARRIS, R. 1947 Aerosol penicillin. J. Am. Med. Assoc., **134**, 932–938.

SCHOENBACH, E. B., BRYER, M. S., BLISS, E. A., AND LONG, P. H. 1948 Polymyxin. J. Am. Med. Assoc., **136**, 1096–1098.

SMADEL, J. E., AND JACKSON, E. B. 1947 An antibiotic with chemotherapeutic activity in experimental rickettsial and viral infections. Science, **106**, 418–419.

SMITH, R. M., JOSLYN, D. A., GRUHZIT, O. M., MCLEAN, I. W., JR., PENNER, M. A., AND EHRLICH, J. 1948 Chloromycetin: biological studies. J. Bact., **55**, 425–448.

STANSLY, P. G., SHEPHERD, R. D., AND WHITE, H. J. 1947 Polymyxin: a new chemotherapeutic agent. Bull. Johns Hopkins Hosp., **81**, 43–54.