THE ACTION OF BACITRACIN AND SUBTILIN ON TREPONEMA PALLIDUM IN VITRO AND IN VIVO[1]

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Received for publication December 2, 1947

Bacitracin is an antibacterial substance discovered by Johnson, Anker, and Meleney (1945) in culture filtrates of the so-called Tracy strain of *Bacillus subtilis*. The toxicity of the drug and its absorption and excretion in dogs have been described by Scudi and his coworkers (Scudi and Antopol, 1947; Scudi, Clift, and Krueger, 1947). The blood levels obtained after the intramuscular administration of bacitracin, its urinary excretion, and its renal clearance in rabbits and man have been reported in a previous communication from this laboratory (Eagle, Newman, Greif, Burkholder, and Goodman, 1947).

Subtilin is another agent produced by *B. subtilis*, described by Jansen and Hirschmann (1944). Its antibacterial activity *in vitro* and *in vivo* has been studied by Sallé and Jann (1945). It differs from bacitracin both in its antibacterial spectrum and in its chemical properties.

There have been no previous reports about the activity of these two antibiotics in the treatment of syphilis. As will be here reported, both were active *in vitro* against a cultured strain (Reiter) of *Treponema pallidum*, bacitracin being considerably more active than subtilin in this respect. *In vivo* also, bacitracin caused the disappearance of treponemata from testicular chancres in syphilitic rabbits and the prompt healing of the lesions. Subtilin, however, failed to effect permanent healing of the lesions in the largest doses used. The amount of bacitracin necessary to produce a permanent cure in rabbits is under present study, as is its activity in the treatment of the human disease.

METHODS AND MATERIALS

Bacitracin and subtilin. The courtesy of the Western Regional Research Laboratories at Albany, California, and of Drs. Eugene F. Jansen and Howard D. Lightbody in providing the subtilin used in these studies is gratefully acknowledged. Most of the bacitracin was prepared by the Ben Venue Laboratories at Bedford, Ohio.

The effective concentrations of bacitracin are expressed in the tables and figures in terms of the unit defined by Johnson, Anker, and Meleney, a unit of bacitracin being that "amount which when diluted 1:1024 in a series of two-fold dilutions in 2 cc. of beef infusion broth, completely inhibits the growth of a stock strain of Group A hemolytic streptococcus when the inoculum used to seed the tubes is 0.1 cc. of a 10^{-2} dilution of an overnight culture in blood broth." The

[1] This work was supported in part by a grant from the National Institute of Health, U. S. Public Health Service, Bethesda, Maryland.

lots of bacitracin used in these experiments varied in activity from 18 to 40 units per mg, and there is reason to believe that the activity of the pure material may be at least twice the latter figure.

The activity of subtilin is expressed in terms of milligrams, preliminary experiments in this laboratory with the Craig counter-current distribution apparatus (Craig, Hogeboom, Carpenter, and du Vigneaud, 1947) having indicated it to be a reasonably homogeneous material, at least as determined by its partition coefficient between water and butanol.

The crystalline penicillin G used in these studies for comparison with bacitracin and subtilin was Lot no. V31 supplied by the Squibb Institute for Medical Research of E. R. Squibb and Sons. Their co-operation is gratefully acknowledged.

Inhibition of growth of treponemata in vitro. The Reiter strain of so-called *T. pallidum*[2] used in the *in vitro* experiments was grown on Brewer's thioglycolate medium, enriched with 10 per cent rabbit or human serum. Sufficient additional agar was added (0.075 to 0.15 per cent) to thicken the medium and thus promote the formation of individual colonies in subculture.

For the determination of the inhibitory effect of the antibiotics on growth, decreasing amounts of their solutions (0.8, 0.6, 0.4, 0.3, 0.2, etc.) were added to 9 ml of the thioglycolate-serum medium, the tubes were then inoculated with just 1,000 organisms from a growing, actively motile 48-hour culture, and the total volume was adjusted to 10 ml. The number of colonies developing in each tube was counted after 7 to 14 days' incubation in an anaerobe jar at 37 C. In the absence of antibiotic, from 2 to 4 colonies were usually obtained for each 10 organisms inoculated into the medium. The control tube therefore contained on the order of 200 to 400 colonies, too many to count; and the experimental end point indicated in the tables was either that concentration which completely prevented growth, or that which permitted the development of only 20 colonies.

Inhibition of growth of Streptococcus pyogenes (C-203). The concentrations of antibiotic necessary to prevent the growth of this organism were determined by a modified Rammelkamp-Kirby technique (Eagle, Newman, *et al.*, 1947), using inhibition of hemolysis as the end point.

Rate of treponemicidal action in vitro. Bacitracin or subtilin was added in varying concentration to tubes of thioglycolate-serum medium at 37 C. These were then inoculated with an actively growing 48-hour culture, to a final count of 10 million organisms per ml as determined by direct enumeration (Magnuson, Eagle, and Fleischman, 1948). At varying intervals thereafter (e.g., 3, 6, 12, 24, or 48 hours), aliquot portions were removed from the tubes, and the number of remaining organisms was determined by subculture in serial tenfold dilutions on thioglycolate medium. In the absence of a specific method for the inactivation of the bacitracin or subtilin, dependence had to be placed on the serial dilutions to dilute the material to the point that it would not affect the growth in subculture of the surviving organisms.

[2] The identification of these organisms as *T. pallidum* is debatable. They are probably saprophytic organisms that happened to be present in the syphilitic lesions when attempts were made at cultivation.

In some experiments, the effect of the antibiotics on the number of organisms visible by dark-field examination, and on the proportion of those which were motile, was also determined.

EXPERIMENTAL PROCEDURE AND RESULTS

Concentration of bacitracin and subtilin necessary to inhibit the growth of T. pallidum (Reiter) and of Streptococcus pyogenes (C-203) in vitro. Table 1 summarizes a number of experiments with four different lots of bacitracin and the cultured Reiter strain of so-called *T. pallidum*. With inocula of 100 organisms per ml, 0.004 Johnson-Meleney units of bacitracin per ml completely inhibited the growth of the organisms, and an average of 0.002 units per ml permitted the

TABLE 1

The inhibitory effects of bacitracin, subtilin, and penicillin G on the growth of T. pallidum (Reiter) in vitro

	INHIBITORY CONCENTRATIONS OF			
	Bacitracin*	Penicillin	Subtilin†	Penicillin
	units/ml	*μg/ml*	*μg/ml*	*μg/ml*
Complete inhibition‡				
Range	0.0024–0.0072	0.025–0.06	2; 3.5	0.02; 0.03
Mean	0.0041	0.047		
Partial inhibition‡				
Range	0.0015–0.0036	0.015–0.35	1.1; 1.95	0.015; 0.025
Mean	0.0023	0.027		
Conclusions as to relative activity	1 mg penicillin equivalent to an average of 90 units of bacitracin		1 mg penicillin equivalent to approximately 75–100 mg of subtilin	

* Eleven experiments with 4 lots of bacitracin, varying in activity from 18 to 36 units per mg, each tested in parallel with penicillin G.

† One experiment with each of 2 lots tested in parallel with penicillin G.

‡ Complete inhibition, no colonies in 10-ml tube inoculated with total of 1,000 organisms, and yielding 200 to 400 colonies in a control tube containing no antibiotic. Partial inhibition, 20 colonies in a 10-ml tube similarly inoculated.

development of 20 colonies in a 10-ml tube. In simultaneous experiments with penicillin G, which was the most active of the four natural penicillins against this particular strain of treponema (Eagle, 1946), an average of 0.047 micrograms per ml prevented growth, and 0.027 micrograms per ml permitted the development of 20 colonies. One milligram of crystalline penicillin G, therefore, had an average activity equivalent to that of 90 units of bacitracin, i.e., penicillin G was three times as active as a crude preparation of bacitracin containing 30 units per mg.

As shown in the same table, subtilin was far less effective than either bacitracin or penicillin G. The totally inhibitory concentration was on the order of 2 to 4 micrograms per ml, and 1 to 2 micrograms per ml permitted the formation of

20 colonies. Milligram for milligram, the two lots of subtilin tested were therefore approximately 1/70th to 1/100th as active as penicillin G against the Reiter strain, and 1/20th to 1/30th as active as a preparation of bacitracin assaying at 30 units per mg.

The relative activities of bacitracin, subtilin, and penicillin against the C-203 strain of *Streptococcus pyogenes* (C-203) are shown in table 2. Measured by the concentrations necessary to inhibit growth, bacitracin was somewhat less active against this organism than it was against the Reiter strain of *T. pallidum;* penicillin was several times more active; but subtilin was almost a hundred times more active. Subtilin was half as active as penicillin G against this strain of streptococcus *in vitro*, but only 1/70th to 1/100th as active as G against the Reiter strain of *T. pallidum*.

TABLE 2

The inhibitory effects of bacitracin, subtilin, and penicillin G on the growth of the C-203 strain of Streptococcus pyogenes in vitro

	GROWTH-INHIBITING CONCENTRATIONS AGAINST STREPTOCOCCUS PYOGENES OF			
	Bacitracin	Penicillin G	Subtilin	Penicillin G
	units/ml	*μg/ml*	*μg/ml*	*μg/ml*
Range.....	0.006–0.01*	0.0093–0.015	0.031, 0.037, 0.033†	0.015, 0.015, 0.014
Mean.....	0.008	0.013	0.034	0.015
Conclusions as to relative activity	1 mg penicillin equivalent to 600 units of bacitracin		1 mg penicillin equivalent to 2 mg of subtilin	

* Eleven experiments with 4 lots of bacitracin. In each experiment penicillin G was tested simultaneously.

† Three experiments with 2 different lots of subtilin, each tested simultaneously with penicillin G.

The rate of treponemicidal action of bacitracin and subtilin in vitro. A number of experiments designed to establish the rate at which the treponemata were killed by bacitracin are summarized in table 3. One of those experiments is graphically illustrated in figure 1. With the much larger inocula used in these experiments (10 million per ml instead of 100), and over the shorter time period (24 to 48 hours instead of 7 to 14 days), it required larger concentrations of bacitracin to render the organisms nonviable than was the case in the experiments summarized in the previous section. In experiment 5 of that table a minimum concentration of 0.025 units per ml was necessary to effect a significant treponemicidal action, and a somewhat higher concentration (>0.025 but <0.1 unit per ml) was necessary in experiment 6. The smallest effective concentration of penicillin G similarly tested was 0.032 micrograms per ml (Eagle and Musselman, 1944; Eagle, 1946). By this criterion 1 mg of G therefore had an activity equivalent to that of 800 units of bacitracin, and it was some 25 times more effective than were crude preparations of bacitracin assaying at 30 units per mg.

Unlike the case of penicillin (Eagle and Musselman, 1944) there was no indication of a maximally effective level of bacitracin. The rate at which it killed the organisms increased with its concentration up to the highest level tested (64 units per ml, equivalent to 2 milligrams per ml of the particular preparation used).

TABLE 3

The rate at which "T. pallidum" (Reiter) was rendered nonviable by bacitracin in vitro

EXP. NO.	UNITS/ML	TIME IN HOURS				TIME REQUIRED TO KILL 99.9 PER CENT OF ORGANISMS
		6	12	24	48	
		Proportion of organisms surviving (referred to viable organisms in original inoculum as 100)				
						hr
1	1.15	8.3		0.32	0.029	36
	0.144	18		2.2	0.18	53
	0.0022	200		440	2,500	∞
	0	—		680	2,000	—
2–4	0.144	15, 17, 17	1, 2.2, 2.4	0.17, 0.016, 0.018	—	55, 39, 40
	0			415, 255, 255	—	—
5	4	8.8		<0.005	<0.005	<24
	0.2	66		0.38	0.02	35
	0.1	55		5.5	0.11	48
	0.05	88		5.5	1.3	>50
	0.025	100		22	9.4	>50
	0	150		1,150	6,650	—
6	64	0.03	—	—	—	6
	4	7.5	0.3	0.01	—	14
	1	9	1.5	0.018	0.015	18.5
	0.1	48.4	12.1	3	—	53±
	0.025		240	150	600	∞
	0.0125		180	480	3,000	∞
	0.0062	210	400	1,120	4,240	∞
	0	—	570	1,600	8,200	∞

Conclusion: Although a bacitracin concentration of 0.0062 units per ml caused a decreased net rate of multiplication over the first 48 hours, it required 0.025 units per ml to effect a net reduction in the number of viable organisms. Since the minimal effective concentration of penicillin G is 0.032 micrograms per ml (Eagle, 1946), 1 mg of penicillin was equivalent to approximately 800 units of bacitracin by this method of assay.

Unlike penicillin, there was no maximally effective concentration of bacitracin. The rate at which the organisms were killed increased with the concentration of antibiotic up to the highest level tested (64 units per ml).

At that concentration, 99.9 per cent of the organisms were killed in less than 6 hours, as compared with an average of 26 hours required at maximally effective concentrations of penicillin (Eagle and Musselman, 1948).

The progressive death of the organisms was reflected, not only in the decreasing number able to grow out as colonies in subculture, but also in the decreasing

number visible by dark-field examination and in their progressive loss of motility. One of several such experiments is illustrated in figure 2. A certain number of the organisms apparently undergo lysis; others are immobilized; and

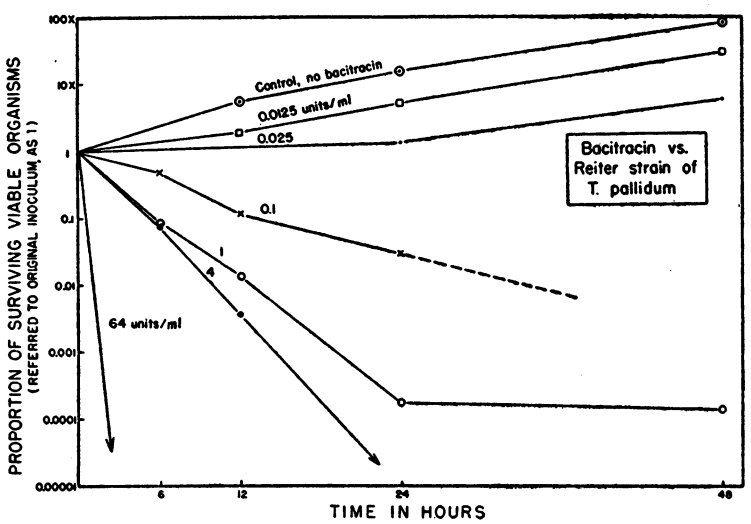

FIG. 1. THE EFFECT OF THE CONCENTRATION OF BACITRACIN ON THE RATE OF ITS TREPONEMICIDAL ACTION (CULTURED REITER STRAIN)
Experiment 6 of table 3

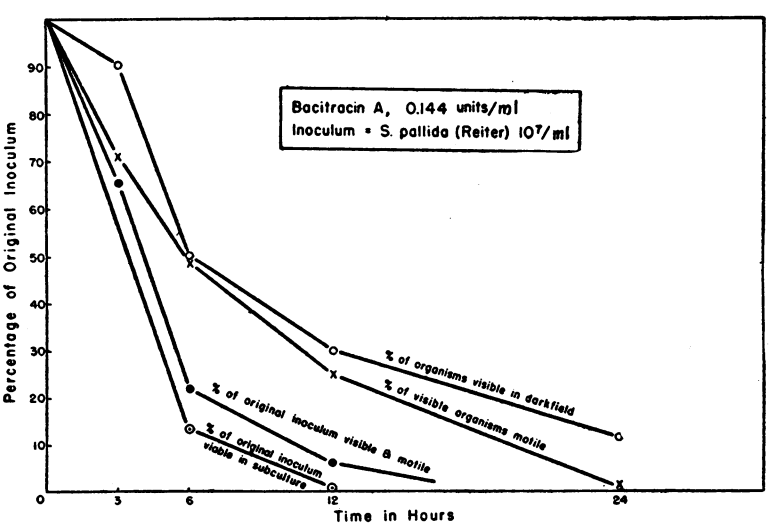

FIG. 2. THE TREPONEMICIDAL ACTION OF BACITRACIN IN VITRO (CULTURED REITER STRAIN)
Lysis, immobilization, and loss of viability in subculture

the number able to grow out in subculture corresponds essentially to the number of visible and motile organisms, corrected for the fact that in control tubes only 20 to 40 per cent of the motile organisms inoculated develop into colonies on subculture.

Two experiments with subtilin are illustrated in table 4. The lowest concentration that decreased the net rate of multiplication was approximately 1 microgram, and the lowest concentration that had a demonstrable net bactericidal effect was 2 to 4 micrograms per ml. The latter concentrations were 64 to 128 times the similarly effective concentrations of penicillin G. As with bacitracin, and unlike penicillin, the treponemicidal activity of subtilin increased with its concentration up to the highest level studied (64 micrograms per ml). At that concentration, 99.9 per cent of the organisms were killed in 5½ hours, or 5 times faster than the maximal rate at which these organisms can be killed by penicillin G *in vitro*.

TABLE 4

The rate at which "T. pallidum" (Reiter) was rendered nonviable by subtilin in vitro

EXP. NO.	SUBTILIN	TIME IN HOURS AT 37 C				TIME REQUIRED TO KILL 99.9 PER CENT OF ORGANISMS
		6	12	24	48	
		Proportion of organisms surviving (referred to viable organisms in original inoculum as 100)				
	micrograms/ml					*hr*
1	64	0.055		<0.005	<0.005	5
	8	5.5		0.016	<0.0005	10
	4	11		1.3	0.055	43
	2	44		9.4	6.6	>50
	1	120		—	260	—
	0	150		1,150	6,650	—
2	16	0.36	0			6
	8	7.5	0.015	0.006	0.0006	10
	4	27.2	—	0.63	—	33
	2	150	—	150	—	—
	1	330	570	1,230	3,030	—
	0.05		540	1,570	7,500	
			570	1,600	8,200	

Conclusion: The lowest concentration of subtilin that effected a net decrease in the number of viable organisms within 48 hours was 2 to 4 micrograms per ml. Since penicillin G was similarly effective at a concentration of 0.032 micrograms per ml, it was 64 to 128 times more active per mg than the particular preparations of subtilin used in these experiments.

The treponemicidal action of bacitracin and subtilin in vivo. When bacitracin at dosages greater than 36 units per kg was injected intramuscularly into syphilitic rabbits with testicular chancres, motile treponemata usually disappeared from the lesions within 24 hours, and they were usually dark-field negative within 48 to 72 hours. As is shown in table 5, however, after single doses of as much as 2,560 units per kg, organisms would often reappear in the testis or epididymis days, weeks, or even months after treatment. In rabbits treated once daily for 4 days, similar relapses were noted in animals treated at doses up to 640 units per kg, but none in the seven animals treated at larger doses.

When penicillin was injected once daily for 4 days, treponemata reappeared

in the testes of two rabbits treated at 4,000 units per kg (2.4 mg per kg of penicillin G), but not in two treated at 8,000 units per kg. Gravimetrically, a crude preparation of bacitracin assaying at 30 units per mg was therefore on the order of one-tenth as active as penicillin in effecting the permanent healing of testicular chancres in rabbits.

The largest doses of subtilin so far used (10 mg per kg every 4 hours, repeated 4 times daily, and continued for 4 days) did not cause the permanent disappearance of organisms from the testicular chancres in any of the four rabbits tested.

TABLE 5

The effect of treatment with bacitracin on the presence of T. pallidum in testicular chancres of rabbits

NO. OF INJECTIONS	DOSAGE, UNITS/KG PER INJECTION	CHANCRE DID NOT BECOME DARK-FIELD-NEGATIVE AS RESULT OF TREATMENT	CHANCRE BECAME TEMPORARILY DARK-FIELD-NEGATIVE BUT ORGANISMS LATER REAPPEARED	CHANCRE BECAME AND REMAINED DARK-FIELD-NEGATIVE THROUGHOUT PERIOD OF OBSERVATION (2 TO 4 MONTHS)	DOSAGE OF BACITRACIN AT WHICH CHANCRE BECAME AND REMAINED DARK-FIELD-NEGATIVE
One	36	1	0	0	Single injection of 5,120 units/kg
	72	0	2	0	
	144	0	2	1	
	288	0	1	1	
	576	0	0	4	
	640	2	0	0	
	1,154	0	1	3	
	1,280	0	2	1	
	2,560	0	3	5	
	5,120	0	0	4	
One injection daily for 4 days	36	3	0	0	1,150 units/kg repeated once daily for 4 days
	72	0	3	0	
	144	0	0	4	
	160	0	1	0	
	288	0	1	1	
	320	0	1	2	
	576	0	0	3	
	640	0	2	1	
	1,154	0	0	2	
	1,280	0	0	3	
	2,560	0	0	2	

This antibiotic was therefore even less effective against pathogenic *T. pallidum in vivo* than was indicated by its direct bactericidal activity against the nonpathogenic, cultured strain *in vitro*.

Abortion of syphilitic infection If penicillin is administered a few hours to a few days after the inoculation of rabbits with *T. pallidum*, a minute fraction of the dosage necessary to cure the established disease then suffices to kill the small inoculum and to abort the syphilitic infection (Magnuson and Eagle, 1945; Eagle, Magnuson, and Fleischman, 1947). Rake, Dunham, and Donovick (1947)

have developed a rapid method, based on this fact, for the assay of antisyphilitic agents. Since the criterion of failure is the development of a chancre at the site of inoculation, the assay can be completed within 2 to 3 months, as compared with the 12 months necessary for the ordinary therapeutic assay in established syphilitic infection, in which the criterion of cure is the noninfectiousness of a lymph node on transfer to normal rabbits 6 months after the completion of treatment.

The relative activity of bacitracin and penicillin G in preventing the development of syphilitic infection is shown in table 6 (Eagle and Fleischman, 1948). As there indicated, when animals were inoculated with 2,000 organisms and

TABLE 6

The relative activity of bacitracin and penicillin G in the abortion of syphilitic infection in rabbits (after Eagle and Fleischman, 1948)

PENICILLIN G				BACITRACIN			
Mg/kg per injection	Nonsyphilitic	Syphilitic	PD_{50}, mg/kg per injection*	Units/kg per injection	Nonsyphilitic	Syphilitic	PD_{50}, mg/kg per injection*
0.125	0	4		18	0	4	
0.25	3 (2)	3		36–39	1 (1)	7	
0.5	5 (5)	1		72–78	5 (2)	4	
1	6 (2)	0	0.3	144–156	7 (5)	3	90
2	4	0		313	4 (4)	1	
4	2	0		625	5	0	

Rabbits were inoculated intradermally with 2,000 organisms. Treatment with intramuscular injections of penicillin G or bacitracin was begun 4 days later, and continued once daily for 4 days. The failure of the animals to develop a syphilitic lesion at the site of inoculation was taken to indicate that the infection had been sucessfully aborted. The numbers in parentheses represent the number of animals in which this was confirmed by lymph node transfer into a normal rabbit 4 to 6 months after treatment. The others were not tested.

Conclusion: One mg of penicillin was as effective as were 300 units of bacitracin in the abortion of syphilitic infection in rabbits. By this method of assay, penicillin was therefore ten times as effective as a crude preparation of bacitracin assaying at 30 units per mg.

* Dosage that protected 50 per cent of animals (calculated after Reed and Muench, 1938).

treated 4 days later by intramuscular injections repeated once daily for 4 days, a daily dose of 0.3 mg per kg of penicillin G sufficed to abort the infection in half the animals. The similarly effective dose of bacitracin was 90 units per kg. By this method of assay, 1 mg of penicillin G was therefore equivalent to 300 units of bacitracin, and penicillin was ten times as effective as a crude preparation of bacitracin assaying at 30 units per mg. The similarly abortive dose of subtilin was not determined.

Experiments are now in progress to determine the curative dose of bacitracin in established syphilitic infection of rabbits, using lymph node transfer 4 to 6 months after treatment as the criterion of cure, and to determine also whether it

exercises a synergistic action with penicillin. Preliminary trials of bacitracin in the treatment of human patients are also in progress.

SUMMARY AND DISCUSSION

Bacitracin

Bacitracin has here been shown to have a definite treponemicidal action, both against the cultivated Reiter strain *in vitro* and pathogenic *Treponema pallidum in vivo* (cf. table 7).

TABLE 7

The relative activity of penicillin, subtilin, and bacitracin against Streptococcus pyogenes and treponemata: summary of all experiments

TEST ORGANISM	CRITERION OF ANTIBIOTIC ACTIVITY	ACTIVITY OF ANTIBIOTIC PER MG RELATIVE TO THAT OF PENICILLIN G	
		Bacitracin (impure prepn. assaying at 30 units/mg)	Subtilin
		%	%
Streptococcus pyogenes, C-203	Inhibition of hemolysis *in vitro* (table 2)	5	50
Reiter strain *in vitro*	Inhibition of growth in 7 to 10 days (table 1)	33	1
	Direct bactericidal action in 24 to 48 hours (tables 3 and 4)	35	1.5
Treponemata			
Pathogenic *T. pallidum in vivo* (rabbits)	Permanent disappearance of organisms from primary lesion (table 5)	10	<1
	Abortion of syphilitic infection (table 6)	10	?
	Cure of experimental infection	?	1

Conclusion: A crude preparation of bacitracin assaying at 30 units per mg was 5 per cent as active per mg as penicillin G against *Streptococcus pyogenes*, 35 per cent as active as G against the cultured Reiter treponema *in vitro*, and 10 per cent as active against the pathogenic *T. pallidum*.

Subtilin was 50 per cent as active per mg as penicillin G against the streptococcus, but only <1 to 1.5 per cent against the treponemata, either *in vitro* or *in vivo*.

Cultured Reiter strain in vitro. The drug caused the lysis and immobilization of the cultured organisms and a progressive loss of viability as judged by subculture. Approximately 0.004 units per ml completely inhibited growth, and 0.025 units per ml had a definite treponemicidal effect within 24 to 48 hours.

Unlike the case of penicillin, there was no indication of a maximally effective concentration of bacitracin; instead, the rate of its treponemicidal action increased progressively up to the largest concentrations feasible to use experimentally in the absence of a simple method of inactivation. Thus, at concentrations

of 0.1, 1, 4, and 64 units per ml, it required 53, 19, 14, and 6 hours to kill 99.9 per cent of the organisms.

Pathogenic T. pallidum in vivo. In syphilitic rabbits, small doses of bacitracin (36 units per kg) caused the rapid disappearance of the organisms from the primary lesion. The similarly effective doses of penicillin G are on the order of 0.11 mg per kg (Turner, Cumberland, and Li, 1947). Much larger doses were, however, necessary in order to effect the permanent healing of the chancre. The effective doses of bacitracin in this respect were 5,000 units per kg at one injection, or 1,150 units per kg repeated once daily for 4 days. The latter dosage (40 mg per kg of a crude preparation assaying at 30 units per mg) was approximately ten times the similarly effective dose of penicillin G.

In rabbits inoculated with 2,000 organisms 4 days previous to treatment with bacitracin, syphilitic infection could be aborted in half the animals by the administration of 90 units per kg of bacitracin, given once daily for 4 days. This dosage (3 mg per kg of a crude preparation assaying at 30 units per mg) was 10 times the similarly effective dose of penicillin G.

Streptococcus pyogenes. Against the C-203 strain of β-hemolytic streptococcus *in vitro*, penicillin G was twenty times as active as a 30-unit-per-mg preparation of bacitracin.

Subtilin

Subtilin was more active than bacitracin against the C-203 strain of *Streptococcus pyogenes*, approaching the activity of penicillin in this respect. Against treponemata, however, whether the cultivated Reiter strain *in vitro* or the pathogenic organisms *in vivo*, it was far less active than either penicillin or bacitracin. The effective concentrations *in vitro* of 2 to 4 micrograms per ml were 75 to 100 times those of penicillin G; and the largest doses so far used, approximately 80 times the curative dose of penicillin similarly injected, failed to cause even the permanent disappearance of treponemata from a primary lesion in rabbits.

REFERENCES

CRAIG, L. C., HOGEBOOM, G. H., CARPENTER, F. H., AND DU VIGNEAUD, V. 1947 Separation and characterization of some penicillins by the method of counter-current distribution. J. Biol. Chem., **168**, 665–686.

EAGLE, H. 1946 Relative activity of penicillins F, G, K, and X against spirochetes and streptococci *in vitro*. J. Bact., **52**, 81–88.

EAGLE, H., AND FLEISCHMAN, R. 1948 The relative antisyphilitic activity of penicillins F, G, K, and X and of bacitracin, based on the amounts required to abort early syphilitic infection in rabbits. J. Bact., **55**, 341–346.

EAGLE, H., MAGNUSON, H. J., AND FLEISCHMAN, R. 1947 Relation of the size of the inoculum and the age of the infection to the curative dose of penicillin in experimental syphilis, with particular reference to the feasibility of its prophylactic use. J. Exptl. Med., **85**, 423–440.

EAGLE, H., AND MUSSELMAN, A. D. 1944 The spirocheticidal action of penicillin *in vitro* and its temperature coefficient. J. Exptl. Med., **80**, 493–505.

EAGLE, H., AND MUSSELMAN, A. D. 1948 The kinetics of the bactericidal action of penicillin against streptococci, staphylococci, pneumococci and treponemata. I. The effect of the concentration of penicillin, and its paradoxically reduced activity at high concentrations. *In preparation.*

EAGLE, H., NEWMAN, E. V., GREIF, R., BURKHOLDER, L. M., AND GOODMAN, S. C. 1947 The blood levels and renal clearance in rabbits and man of an antibiotic derived from *B. subtilis* (bacitracin). J. Clin. Investigation, **26**, 919–928.

JANSEN, E. F., AND HIRSCHMANN, D. J. 1944 Subtilin. An antibacterial product of *Bacillus subtilis*. Culturing conditions and properties. Arch. Biochem., **4**, 297–309.

JOHNSON, B., ANKER, H., AND MELENEY, F. 1945 Bacitracin: a new antibiotic produced by a member of the *B. subtilis* group. Science, **102**, 376–377.

MAGNUSON, H. J., AND EAGLE, H. 1945 The retardation and suppression of experimental early syphilis by small doses of penicillin comparable to those used in the treatment of gonorrhea. Am. J. Syphilis, Gonorrhea and Venereal Diseases, **29**, 587–596.

MAGNUSON, H. J., EAGLE, H., AND FLEISCHMAN, R. 1948 The minimal infectious inoculum of *S. pallida* (Nichols strain), and a consideration of its rate of multiplication *in vivo*. Am. J. Syphilis, Gonorrhea and Venereal Diseases, **32**, 1–18.

RAKE, G. W., DUNHAM, W. B., AND DONOVICK, R. 1947 Evaluation of antibiotics by the prevention of experimental syphilis. J. Infectious Diseases. *In press*.

REED, L. J., AND MUENCH, H. 1938 Simple method of estimating 50 per cent endpoints. Am. J. Hyg., **27**, 493–497.

SALLÉ, A. J., AND JANN, G. J. 1945 Subtilin. An antibiotic produced by *Bacillus subtilis*. I. Action on various organisms. Proc. Soc. Exptl. Biol. Med., **60**, 60–64.

SALLÉ, A. J., AND JANN, G. J. 1946 Subtilin. III. Effect on type III pneumococcus in mice. Proc. Soc. Exptl. Biol. Med., **62**, 40–42.

SCUDI, J. V., AND ANTOPOL, W. 1947. Some pharmacological characteristics of bacitracin. Proc. Soc. Exptl. Biol. Med., **64**, 503–506.

SCUDI, J. V., CLIFT, M. E., AND KRUEGER, R. A. 1947 Some pharmacological characteristics of bacitracin. II. The absorption and excretion of bacitracin in the dog. Proc. Soc. Exptl. Biol. Med., **65**, 9–13.

TURNER, T. B., CUMBERLAND, M. C., AND LI, H.-Y. 1947 Comparative effectiveness of penicillins G, F, K, and X in experimental syphilis as determined by a short *in vivo* method. Am. J. Syphilis, Gonorrhea and Venereal Diseases, **31**, 476–484.