THE RATE OF BACTERICIDAL ACTION OF PENICILLIN IN VITRO AS A FUNCTION OF ITS CONCENTRATION, AND ITS PARADOXICALLY REDUCED ACTIVITY AT HIGH CONCENTRATIONS AGAINST CERTAIN ORGANISMS

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(Received for publication, April 2, 1948)

Penicillin has been shown to be directly and rapidly bactericidal (1–9), and not merely bacteriostatic. Whether that direct action is augmented *in vivo* by the natural defense mechanisms of the body has not yet been conclusively determined; but in any case, it is a reasonable surmise that the concentration of penicillin which is most rapidly effective against a given organism *in vitro* may also be the most effective *in vivo*. Further, if different organisms vary not only with respect to the effective concentrations of penicillin, but also in the maximal rate at which they can be killed by the drug, there may be a corresponding variation in the time for which treatment with penicillin must be continued in order to effect cure.

The present paper will describe experiments relating to the effect of the concentration of penicillin on the rate of its bactericidal action *in vitro* against a number of bacteria. As had previously been indicated by Hobby, Meyer, Dawson, and Chaffee (1, 2), Eagle and Musselman (6), and Demerec (7), the rate at which the bacteria were killed by penicillin *in vitro* was found to vary strikingly within relatively narrow zones of concentration. For every organism here studied, one could define (1) a concentration at which the net rate of multiplication was significantly reduced, (2) a somewhat higher concentration at which the organisms were killed faster than they multiplied, so that the net number of viable organisms slowly decreased, and (3) a maximally effective concentration, at which the organisms died at a maximum rate which was not further increased even by a 32,000-fold increase in penicillin concentration (*cf.* references 1, 2, 6, 7). However, the several bacterial species differed not only with respect to the magnitude of this maximally effective concentration of penicillin, but also with respect to the maximal rate at which they could be killed by the drug; and there was no necessary relationship between the two values. Finally, with many bacterial species there was a sharply defined concentration of penicillin at which it was optimally effective, and in excess of

which the rate at which the organisms were killed was paradoxically reduced rather than increased (*cf.* reference 9).

The therapeutic implications of these observations are discussed in the text.

I. METHODS AND MATERIALS

The organisms studied in the present paper included 14 strains of β-hemolytic streptococci, of Lancefield groups A, B, and C; 11 strains of α-hemolytic streptococci, including 7 strains of *Streptococcus fecalis*; 7 strains of *Staphylococcus aureus*, and 2 of *Staphylococcus albus*; *Diplococcus pneumoniae*, Types I, III, VIII, XI, XII, XIV, and XXIV; and the so called Reiter strain of cultured *T. pallidum*.[1]

Fifteen hour cultures grown at 30°C. were usually used as the starting material except in the case of the slow growing *T. pallidum*, for which 48 hour cultures at 37°C. on Brewer's thioglycollate medium were used. Penicillin G was added in varying concentrations to an appropriate dilution of the culture, and the mixture placed in a water bath at 37°C. Aliquot portions were taken out at intervals and (the spirochete cultures excepted) the number of surviving viable organisms was determined[2] by plating out serial 40-fold dilutions (0.3 cc. and 11.7 cc.) in an agar (or blood-agar) medium containing an excess of "clarase" (Takamine) in order to terminate the action of the penicillin. The number of such 40-fold dilutions was adjusted to the anticipated number of organisms. With the treponemal culture, the number of surviving and viable organisms was determined in fluid Brewer's thioglycollate medium enriched with 10 per cent rabbit serum, and containing enough agar (0.1 to 0.2 per cent) to cause the organisms to grow out in discrete colonies.

When the concentration of penicillin in the reacting mixture was in excess of 1 microgram per cc. (but less than 256), the clarase was added to the first tube of the serial subcultures to a final concentration of 0.083 per cent; all other tubes contained 0.017 per cent of clarase. When the penicillin concentration was 256 to 2048 micrograms per cc., the higher concentration of clarase was used in the first 2 tubes of the serial subcultures. Although Takamine clarase was used in most of the present experiments, a preparation of penicillinase obtained through the courtesy of the Schenley Laboratories has given equally satisfactory results

In the tables the number of surviving and viable organisms is expressed as the percentage of the original inoculum, determined prior to the addition of the penicillin. In the figures, the proportion of survivors has been expressed as a decimal fraction, referred to the original inoculum as 1.

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[1] Most of these strains were obtained through the courtesy of Dr. George F. Mirick, Dr. J. Howard Brown, and Dr. Martin Frobisher, Jr., of the Johns Hopkins Schools of Medicine and Public Health, and Dr. W. F. Verwey of the Sharp and Dohme Laboratories, Glenolden, Pennsylvania. It is a pleasure also to acknowledge the cooperation of the Squibb Institute for Medical Research, the Merck Company, Inc., and the Commercial Solvents Corporation in supplying the penicillin G used in these studies, of the Charles Pfizer Company in supplying penicillin K, and of the Lederle Laboratories in supplying the penicillin X (*cf.* page 128).

[2] These experiments determine the proportion of surviving organisms still viable after a given exposure to penicillin. Those bacteria still alive at the time of subculture, but which had been so damaged by penicillin that they failed to grow out in subculture, therefore appeared as dead organisms. This factor tended to make the apparent rate of bactericidal action somewhat greater than was actually the case.

A further complication was introduced by the fact that with those organisms which associated in clumps or chains, just one viable organism surviving out of a group of 10 for example, sufficed to form a colony on subculture, and the fact that the other 9 were dead or dying

II. THE RATES AT WHICH BACTERIA ARE KILLED BY PENICILLIN

A. β-Hemolytic Streptococci

1. Group A (Lancefield).—One of 5 similar experiments with the C-203 strain of *Streptococcus pyogenes* is summarized in detail in Table I and in Fig. 1. As is there shown, as little as 0.004 microgram per cc. of penicillin G had a

TABLE I

The Rate at Which Streptococcus pyogenes (C-203) Is Killed at Varying Concentrations of Penicillin G

(Inital number of organisms in reacting mixture = 750,000 per cc.)

Time at 37°C.	Concentration of penicillin G, *micrograms per cc.*											Concentration o penicillin (*micrograms per cc.*) which		
	0	0.004	0.006	0.008	0.012	0.016	0.024	0.032	0.048	0.064	512	reduced growth	effected net reduction in no. of viable organisms	killed organisms at maximal rate
	Percentage* of organisms still viable after indicated time period													
hrs.														
1½	700	—	—	—	10	0.88	0.28	0.12	0.087	0.063	0.04			
3	3,300	1,400	680	100	1	0.03	0.02	0.013	0.013	0.008	0.007			
6	15,000	1,700	21.6	4.7	0.02	0.006	0.0038	0.003	0.0026	0.002	0	0.004	0.006–0.008	0.048–0.064
12		—	—	0.024	0.0012	0.0003	0.00013	0.00013	0.00013	0	0			
24	4,500	15.3	0.5	0.002	0	0	0	0	0	0	0			
Time required to kill 99.9 per cent of organisms, *hrs.*		>24	>24	10.5	4.8	2.5	2.2	1.9	1.6	1.5	1.4			

* Referred to original inoculum as 100.

significant effect in diminishing the net rate of growth of the organisms as compared with that in a penicillin-free control. At concentrations of 0.006

was thereby masked. This factor would tend to make the rate of bactericidal action seem less than was actually the case.

These two important sources of error have of necessity been ignored in the following discussion. The number of colonies formed on subculture after a given exposure to penicillin has been taken as the number of organisms surviving at that moment, and has been used as a measure of the rate of bactericidal action. Within a single experiment, the comparative results with a series of penicillin concentrations nevertheless remain a valid measure of their *relative* activity.

and 0.008 microgram per cc., the number of viable organisms at first increased over a 3 hour period, or remained stationary (growth approximately equal to rate of death), and gradually fell off thereafter. A slight increase in concentration, to 0.012 microgram per cc., had a striking effect on the rate of death.

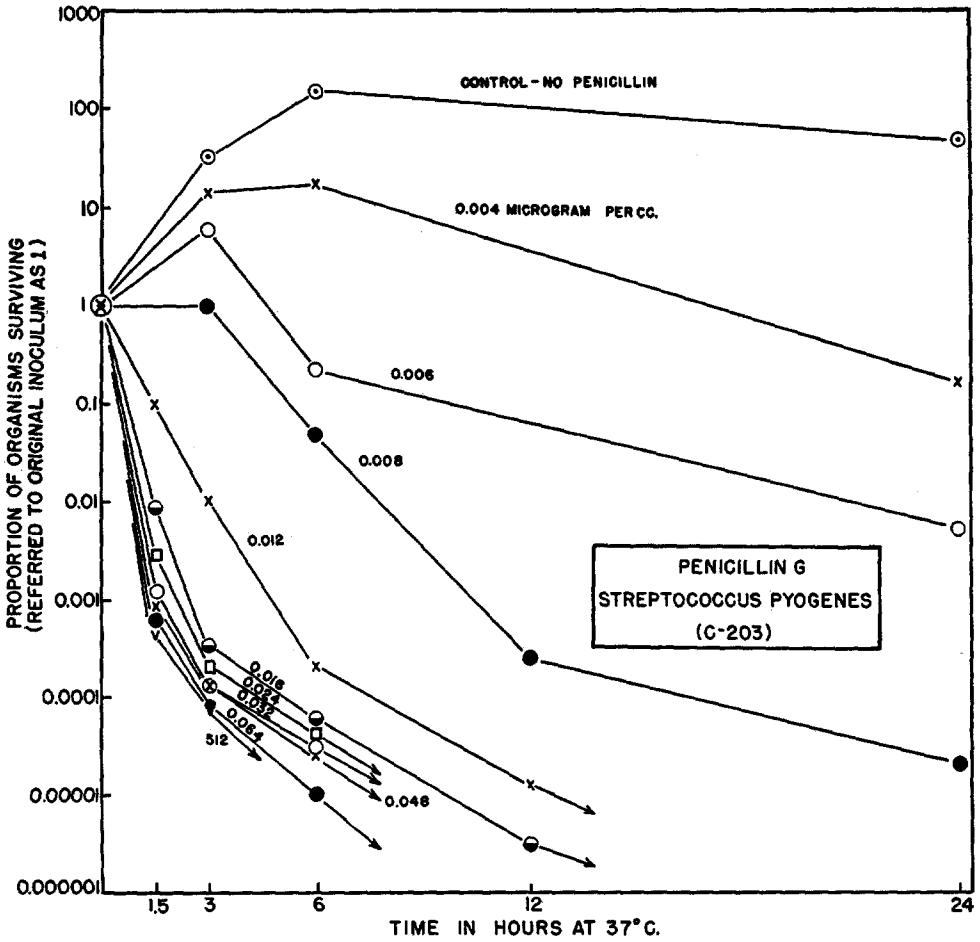

FIG. 1. The effect of the concentration of penicillin on the rate at which *Streptococcus pyogenes* (C-203) was rendered non-viable by penicillin G. (After data of Table I.)

Thus, the number of viable organisms surviving after 6 hours' exposure to 0.012 microgram per cc. was 1/5000th of the original inoculum, and was 1/240th of the number surviving in the tube containing just 33 per cent less penicillin (0.008 microgram per cc.). As the concentration of penicillin was further increased to 0.016, 0.024, and 0.032 microgram per cc., the rate of

bactericidal action also increased, but to a smaller degree with each succeeding increment in concentration. A maximum effect was obtained with a concentration of 0.064 microgram per cc., at which 99.998 per cent of the organisms were killed in the first 6 hours, and 99.94 per cent in the first 90 minutes, with no

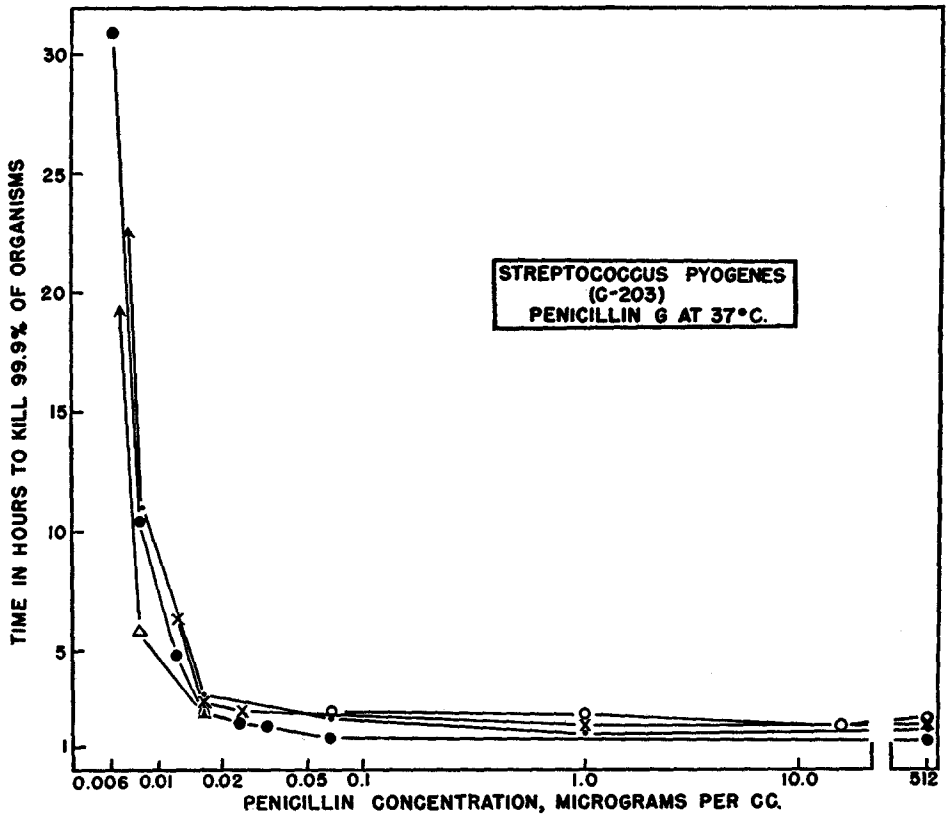

FIG. 2. The effect of the concentration of penicillin G on the time required to kill 99.9 per cent of the organisms in a suspension of *Streptococcus pyogenes* (C-203). (Summary of 5 experiments resembling that of Fig. 1 and Table I.)

indication of an initial "lag" period in the action of penicillin. Further increase in the concentration of penicillin, to as high as 512 or 2048 micrograms per cc. (32,000 times the maximally effective level of 0.064 microgram per cc.) did not further accelerate the rate at which the organisms were killed (*cf.* Hobby, Meyer, and Chaffee (1); Demerec (7)).

Five similar experiments with the same C-203 strain, all with qualitatively and quantitatively similar results, are graphically summarized in Figs. 2 and 3. In Fig. 2 the results are plotted to show the effect of the concentration on

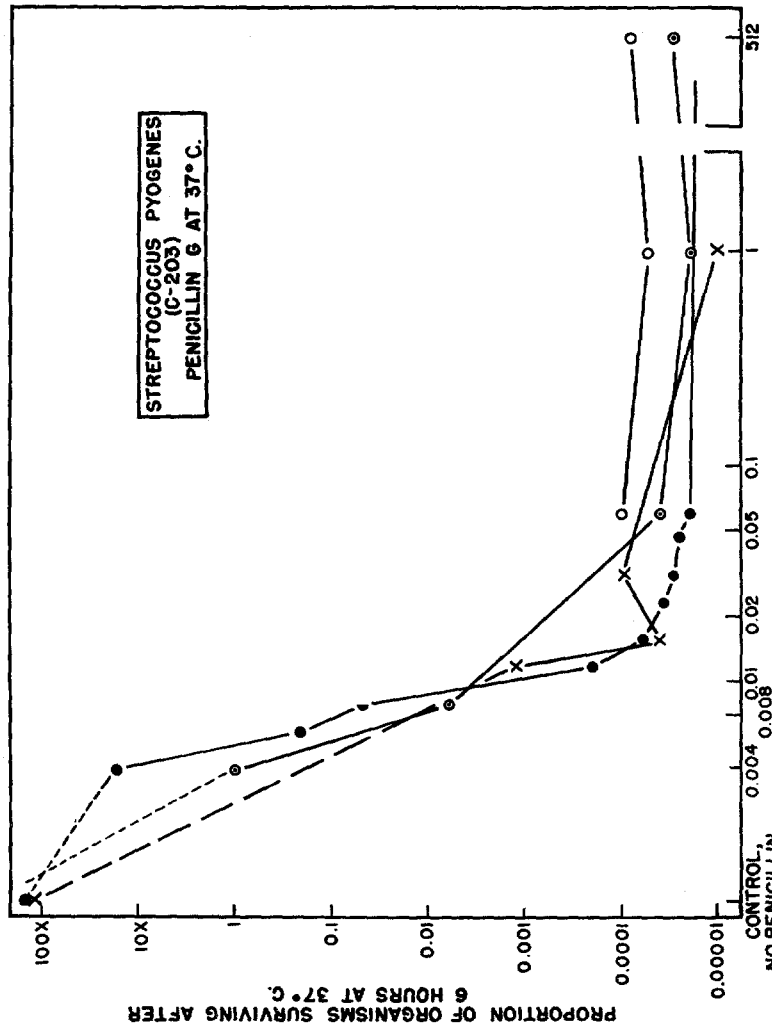

FIG. 3. The effect of the concentration of penicillin G on the proportion of organisms surviving after 6 hours at 37°C. (*Streptococcus pyogenes* (C-203)). (Summary of 4 experiments resembling that of Fig. 1 and Table I.)

the time required to kill 99.9 per cent of the organisms; and Fig. 3 shows the effect of penicillin concentration on the proportion of viable organisms after 6 hours' incubation at 37°C. In both of these figures, the graph with filled circles (•—•) refers to the experiment of Table I and Fig. 1.

With this strain of streptococcus, a 15-fold difference in penicillin concentration therefore comprised the total range of bactericidal activity, from concentrations which served only to reduce the net rate of multiplication (0.004 microgram per cc.), to concentrations at which the organisms were killed at a maximal rate (0.064 microgram per cc.).

TABLE II

The Susceptibility of 5 Strains of Group A β-Hemolytic Streptococci to Penicillin G

Strain No.	Concentration of penicillin G, *micrograms per cc.*							Concentrations of penicillin (*micrograms per cc.*) which			Concentration of penicillin G, *micrograms per cc.*					
	0	0.004	0.008	0.016	0.032	0.064	256	decreased net rate of multiplication	caused net decrease in no. of viable organisms	killed organisms at maximal rate	0.008	0.016	0.032	0.064	0.128	256
	Percentage of organisms surviving after 6 hrs. at 37°C.										Time required to kill 99.9 per cent of organisms, *hrs.*					
F24*	9,500	3,800‡	1,440‡	1.9	0.022		0.022	0.004	0.016	0.032	∞	4.4	2.6	3.7	4.1	2.8‡
M24	4,500	9,000 (?)	430	0.05	0.03	0.1	0.02	0.004–0.008	0.016	0.032	∞	5.4	4.7	6	4	
M25	17,200		425	0.018	0.015		0.004	0.004–0.008	0.016	0.032	∞	3.8	4			4
M27	57,100		1,100	0.87	0.89	0.31	0.016 (?)	0.004–0.008	0.016	0.032	∞	8.3	8.3	7.8		4.5 (9)‡
C-203§	15,000	1,700	4.7	0.006	0.003	0.0026	—	0.004	0.016	0.032–0.064	10.5	2.5	1.9	1.5	—	1.4

* This strain was killed more rapidly than the others, and the values given are the percentage of viable survivors after 3 hours, rather than 6.

‡ These fill-in values were obtained in a second experiment.

§ Experiment of Table I.

Four other group A strains gave results qualitatively similar to those obtained with the C-203 strain described above: concentrations of 0.008 microgram per cc. regularly reduced the net rate of multiplication; a maximal rate of bactericidal action was effected by concentrations of 0.016 to 0.032 microgram per cc.; and that rate was not affected by further increase in the concentration of penicillin up to as much as 256 micrograms per cc. (*cf.* Table II).

2. Group B β-Hemolytic Streptococci.—A single experiment with a group B β-hemolytic streptococcus is given in detail in Table III, and another experiment with the same strain is shown graphically in Fig. 4. Similar experiments

with 4 different strains are summarized in Table IV. As there shown, with all 4 strains, concentrations of 0.016 microgram per cc. sufficed to reduce the rate of multiplication, and concentrations of 0.032 to 0.064 microgram per cc. effected a net bactericidal action which was maximal at concentrations of 0.064 to 0.25 microgram per cc.

With further increase in the concentration of penicillin, however, the rate of death did not stabilize at that maximum as in the case of the group A organisms previously studied. Instead, the maximal effect was observed only within a narrow optimal range of penicillin concentration. With further increase, the organisms were killed more slowly, and progressively more slowly the higher the concentration of penicillin. Thus, with each of the 4 strains there were

TABLE III

A Paradoxical Zonal Effect in the Susceptibility of a Group B β-Hemolytic Streptococcus (Strain F20) to Penicillin G

Time	Concentration of penicillin, *micrograms per cc.*				
	0	0.032	0.064	0.128	256
	Percentage of organisms still viable (inoculum = 100)				
hrs.					
3	2,600	42	4	1.8	55
6	16,600	32	0.056	0.079	15
12	35,600	52	0.0048	0.016	4
24	47,700	87	0	0.0004	0.08
Time required to kill 99.9 per cent of organisms, *hrs*.....		>	5.6	5.8	23

from 10 to 200 times as many survivors after 6 hours' exposure to 256 micrograms of penicillin per cc. as there were after similar exposure to the optimal concentration of 0.064 to 0.25 microgram per cc. (*cf.* Table IV). This zonal effect, which is illustrated for the F20 strain in Fig. 4, was also apparent when the results were expressed in terms of the time required to sterilize the culture. At the optimal concentration of 0.1 microgram per cc., it required an average of 6, 7, 3.5, and 6 hours to kill 99.9 per cent of strains M22, F20, F21, and F22, respectively. The corresponding times required at concentrations of 1 microgram per cc. were 16, 21, 8, and 11 hours. This retarded activity of penicillin in high concentration was noted by Garrod (23), while studying the rate at which staphylococci were killed by the drug. However, he ascribed this phenomenon to the presence of impurities in the penicillin, an interpretation which is not consistent with the data here presented (*cf.* page 128).

3. Group C.—Of the 5 strains of group C streptococci listed in Table V, 2 (M20 and M26) were killed at a maximal rate by relatively low concentrations

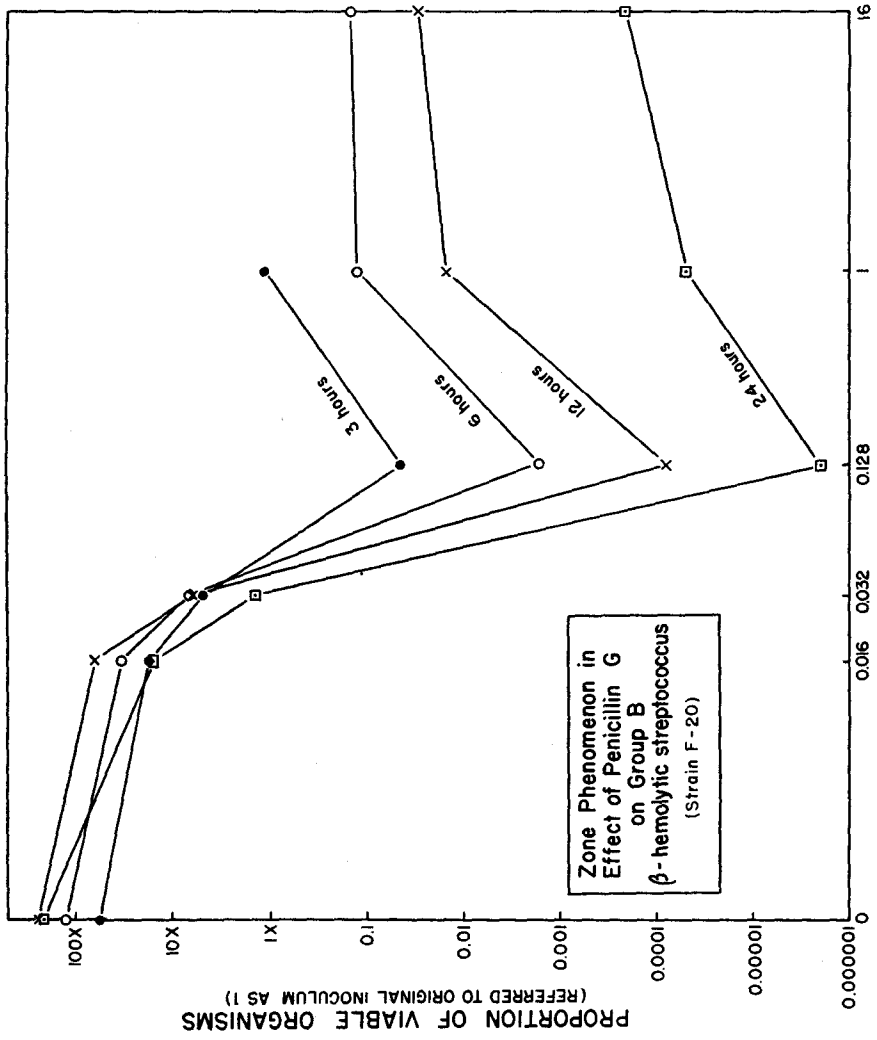

FIG. 4. The effect of the concentration of penicillin on the proportion of surviving viable organisms after 3, 6, 12, and 24 hours (experiment with a group B β-hemolytic streptococcus, illustrating the paradoxically decreased rate of death at higher concentrations of penicillin).

of penicillin (0.016 to 0.032 microgram per cc.), and that maximal rate was unaffected even by an 8,000-fold increase in the concentration of the drug. The other 3 strains, however, showed a pronounced zonal susceptibility to penicillin, similar to that described for the group B organisms in the preceding

TABLE IV

A Paradoxical Zonal Effect in the Susceptibility of Group B β-Hemolytic Streptococci to Penicillin (Illustrative Experiments with 4 Strains)

Strain No.	Concentration of penicillin (*micrograms per cc.*) which			Zone phenomenon at higher concentrations	Percentage of organisms surviving after 6 hrs. exposure to		Time required to kill 99.9 per cent of organisms at	
	reduces growth	effects net reduction in no. of viable organisms	kills organisms at maximal rate		optimal concentration of penicillin	256 micrograms per cc.	optimal concentration of penicillin	256 micrograms per cc.
							hrs.	*hrs.*
M22	0.016	0.064	0.128–0.25	+	0.04	6.6	6–7	16
F20	0.016	0.032–0.064	0.064	+	0.08	15	6–7	21–23
F21	<0.016	0.064	0.064–0.128	+	0.018	1.1	3.5	8.5
F22	0.016	0.064	0.064–0.128	+	0.11	1.26	6	12

TABLE V

The Susceptibility of 5 Strains of Group C β-Hemolytic Streptococci to Penicillin G

Strain No.	Concentration of penicillin (*micrograms per cc.*) which			Zone phenomenon at higher concentrations	Percentage of organisms surviving after 6 hrs. exposure to		Time required to kill 99.9 per cent of organisms at	
	reduces growth	effects net reduction in no. of viable organisms	kills organisms at maximal rate		optimal concentration of penicillin	256 micrograms per cc.	optimal concentration of penicillin	256 micrograms per cc.
							hrs.	*hrs.*
M20	0.004	0.016	0.016–0.032	±	0.03	0.39	3.9	>9
M26	0.004	0.016	0.032	0	0.022	0.02	3.8	4.7
F23	0.004	0.016	0.032	+	0.017	1.8	4	13.2
M23	0.004	0.016–0.032	0.032	+	0.71	15	9.1, 8	14, 21
M31*	1	4	4–8	+	1.7	74	17	80

* Identification as group C β-hemolytic streptococcus questionable.

section. These 3 strains were killed most rapidly within a narrow range of concentrations, which centered at 0.032 microgram per cc. for strains F23 and M23, and 4 to 8 micrograms per cc. for strain M31. (The identification of the latter discrepant strain as a group C organism is, however, questionable; *cf.* footnote to Table V.) A relatively slight increase beyond those optimally effective levels markedly retarded the rate of death. This is shown in Fig. 5, which relates to strain F23 of Table V. The zone phenomenon was further

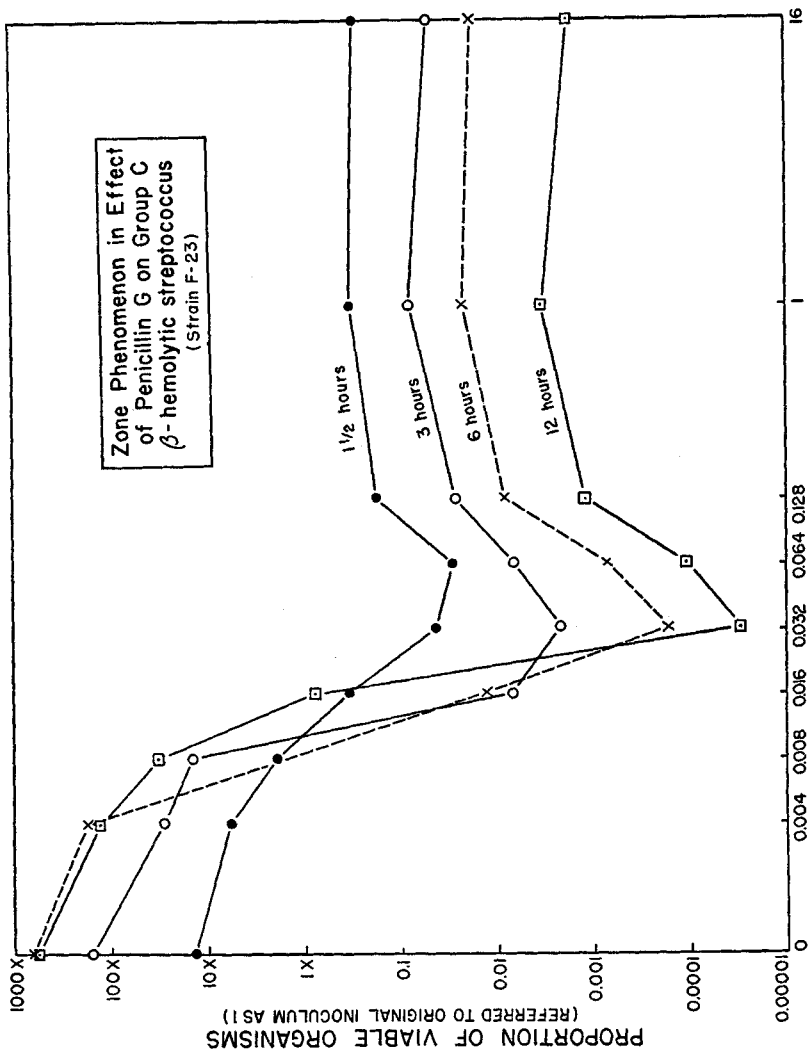

FIG. 5. The effect of the concentration of penicillin on the proportion of surviving viable organisms after 3, 6, 12, and 24 hours (experiment with a group C β-hemolytic streptococcus, illustrating the paradoxically decreased rate of death at higher concentrations of penicillin).

evidenced in the times required to kill 99.9 per cent of the organisms, as summarized in the last section of Table V.

B. α-Hemolytic Streptococci

1. Streptococcus fecalis.—One of 5 experiments with a single zone-sensitive strain of *Streptococcus fecalis* is summarized in Table VI. Of 7 strains of *Streptococcus fecalis* similarly tested, 5 showed the same zonal phenomenon in the action of penicillin, and were remarkably uniform in their susceptibility to the drug. As is indicated in Fig. 6, the smallest concentration with a net bactericidal action was 3 micrograms per cc.; all 5 strains were most rapidly

TABLE VI

The Effect of the Concentration of Penicillin G on the Rate of Its Direct Bactericidal Action on Streptococcus fecalis

(Illustrating one of 5 similar experiments with the same strain: original inoculum = 667,000 per cc.)

Time	Concentration of penicillin G, (*micrograms per cc.*)										
	0	2	3	4	6	8	12	16	32	512	2,048
	Percentage of organisms still viable after indicated time period										
hrs.											
1½	1,950	67	4.7	3.5	2.2	3.24	7.2	10.4	20	60	65
3	7,900	49	0.36	0.18	0.28	0.44	1.32	2.0	5.6	48	60
6	29,400	26	0.046	0.037	0.031	0.049	0.16	0.14	1.1	13	29
12	47,400	700	0.026	0.007	0.0075	0.009	0.044	0.054	0.36	5.6	6.4
24	64,000	34,000	3,200	>100	0.005	0.0015	0.0075	0.0025	0.006	0.56	1.12
Time required to kill 99.9 per cent of organisms, *hrs.*		∞	5.4	4.1	4.4	5	8	8.1	16	33±	40

killed at concentrations of 4 to 6 micrograms per cc.; and the higher the concentrations in excess of that level to which the organisms were exposed, the more slowly they were killed. Thus, after 6 hours' exposure to concentrations of 64 micrograms per cc., there were, on the average, 20 times as many survivors as there were at concentrations of 4 to 6 micrograms per cc.; and at 512 micrograms per cc. the ratio was more than 400-fold. At 4, 16, 64, and 512 micrograms per cc., it required an average of 5.1, 13, 27, and 40 hours, respectively, to kill 99.9 per cent of the organisms, a progressive decrease in the rate of bactericidal action of penicillin as its concentration was increased.

The remaining 2 strains did not show the zone phenomenon under discussion. Like the other 5 strains (*cf.* top curves of Fig. 6), they were killed at a maximal rate at a concentration of 4 to 6 micrograms per cc.; but as with group A β-hemolytic streptococci, that maximal rate was not thereafter affected by a

128-fold increase in dosage. It is of interest that these 2 "non-zonal" strains were killed only slowly by penicillin even at optimal concentrations. More than 48 hours were required to kill 99.9 per cent of the organisms, as contrasted with an average of 5 hours for the "zone-sensitive" organisms.

2. Other Strains of α-Hemolytic Streptococci.—Five other strains of α-hemolytic organisms were similarly studied (Table VII). In all, growth was significantly inhibited at concentrations of 0.016 to 0.032 microgram per cc.; with

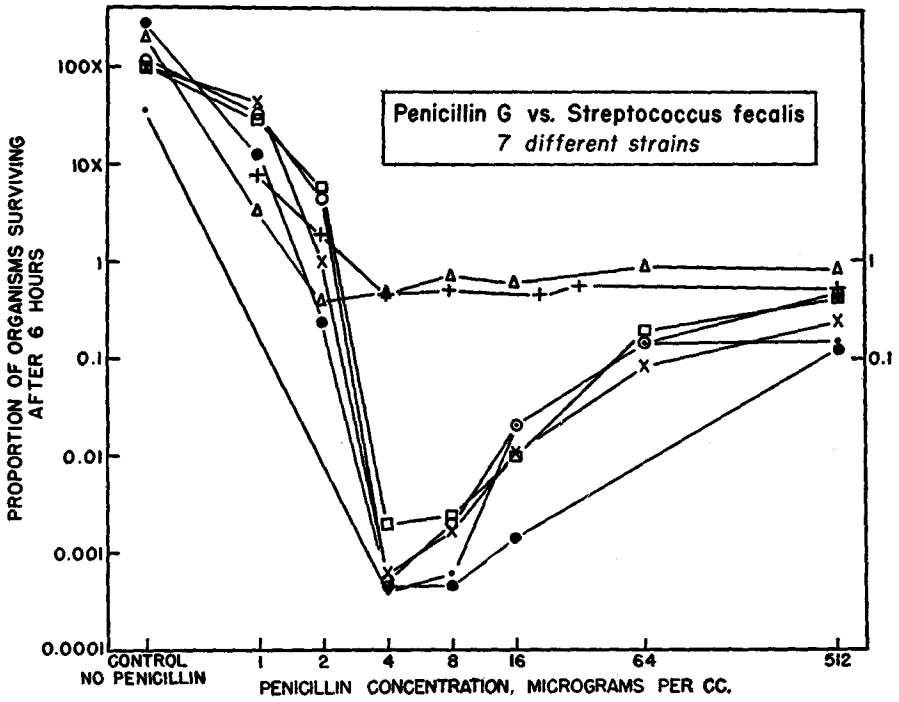

FIG. 6. The effect of the concentration of penicillin on the proportion of organisms surviving after 6 hours at 37°C. (7 strains of *Streptococcus fecalis*, of which 5 show the zone phenomenon).

all, twice that concentration was clearly bactericidal, and concentrations of 0.064 to 0.0128 microgram per cc. were maximally effective. With 3 of the 5 strains, further increase in penicillin concentration beyond the optimally effective level caused the typical retardation of penicillin action, graphically shown in Fig. 7.

As in the case of *Streptococcus fecalis* (see above) there was no necessary correlation between the concentration of penicillin necessary to kill a given strain, and the absolute rate of that bactericidal action. Thus, there was only a 2- to 4-fold difference in the optimum concentration of penicillin for strains F25 and M29 at the bottom of Table VII. At those optimal concentrations,

TABLE VII

The Susceptibility of 5 Strains of α-Hemolytic Streptococci to Penicillin G

Strain No.	Concentration of penicillin (*micrograms per cc.*)* which			Zone phenomenon at higher concentrations	Percentage of organisms surviving after 6 hrs.' exposure to		Time required to kill 99.9 per cent of organisms at	
	reduces growth	effects net reduction in no. of viable organisms	kills organisms at maximal rate		optimal concentration of penicillin	256 micrograms per cc.	optimal concentration of penicillin	256 micrograms per cc.
							hrs.	*hrs.*
F26	0.016–0.032	0.032–0.064	0.064	+	1.12	30.6	6.5	34
F27	0.016–0.032	0.032–0.064	0.064	+	0.12	10.5	7.0	26
M28	0.016–0.032	0.032–0.064	0.128	+	0.67	40	10	45
F25	0.016	0.032	0.064	0	0.029‡	0.05‡	2.8	2.4
M29	0.016–0.032	0.064–0.128	0.128	0	15.8	20.2	25	29

* Based on a number of experiments with each strain, each similar to that of Tables I and III.

‡ This strain was killed more rapidly than the others, and the values given are the percentage of viable survivors after 3 hours, rather than 6.

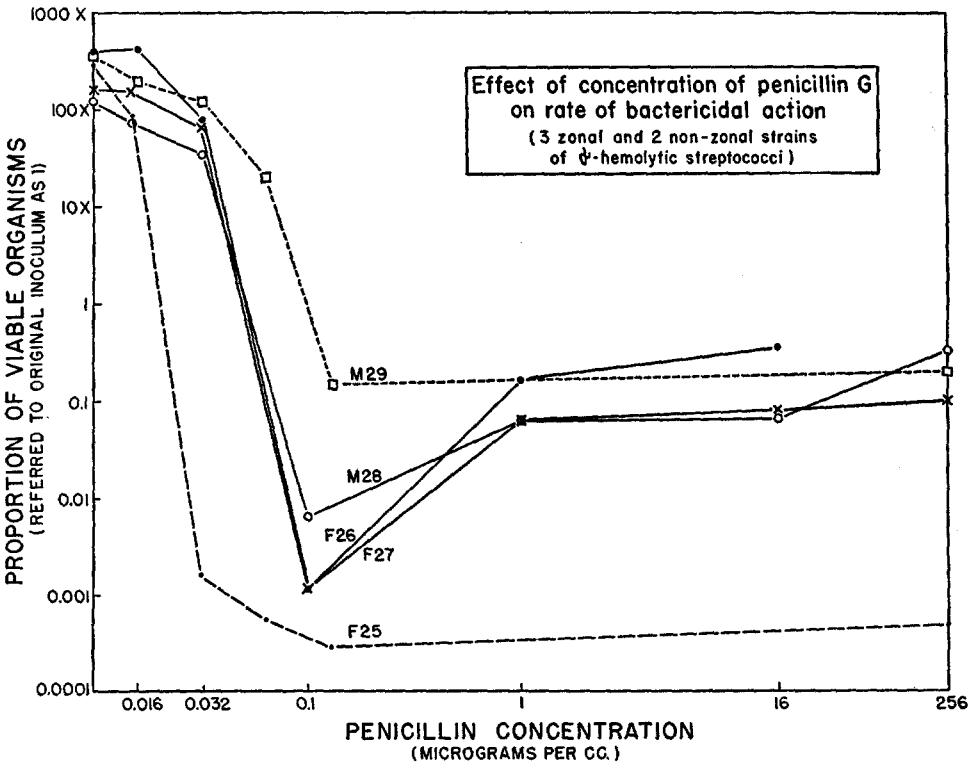

FIG. 7. The effect of the concentration of penicillin on the proportion of surviving viable organisms after 6 hours at 37°C. · (5 strains of α-hemolytic streptococci, 3 of which showed a zone phenomenon; *i.e.*, decreased rate of death at higher concentrations of penicillin).

however, the proportion of survivors after 6 hours differed more than 1500-fold (<0.01 and 16 per cent, respectively), and the times required to kill 99.9 per cent of the organisms differed 10-fold (2.4 and 25 hours, respectively).

C. Diplococcus pneumoniae

One of 5 similar experiments with a Type I strain of pneumococcus is summarized in Table VIII. A concentration of 0.016 microgram per cc. significantly reduced the rate of multiplication; the smallest concentration which

TABLE VIII

The Effect of the Concentration of Penicillin G on the Rate of Its Pneumococcidal Activity in Vitro

(Illustrating one of 5 experiments with a Type I strain of *Diplococcus pneumoniae:* original inoculum = 1.08×10^6 per cc.)

Time	Concentration of penicillin G, *micrograms per cc.*										
	0	0.012	0.016	0.024	0.032	0.048	0.064	1	16	512	2,048
	Percentage* viable after indicated time period										
hrs.											
1½	220	93	120	97	61	9.5	4	5.5	13		24
3	3,300	400	200	1.2	0.25	0.16	0.1	0.09	0.8		3
6	6,900	1,600	320	1.1	0.042	0.036	0.054	0.04	0.05		0.075
12	12,000	620	—	—	—	0.0022	0.005	0.008	0.005		—
24	—	—	840	0.72	0.125	0	0.0006	—	—		
Time required to kill 99.9 per cent of organisms, *hrs.*	∞	∞	∞	>24	4.5	3.7	3	3	5.3		5.8

* Referred to original inoculum as 100.

effected a net decrease in the number of viable organisms was 0.024 microgram per cc.; and essentially the maximal rate of killing was observed at 0.064 microgram per cc. At the maximally effective concentration it required from 2.5 to 5.1 hours to kill 99.9 per cent of the organisms, averaging 3.7 in the 5 experiments; and 0.004 to 0.072 per cent were still viable after 6 hours.

Of particular interest was the indication in some, but not all, of the experiments with this strain of pneumococcus of a slight zonal effect similar to that previously described for some strains of streptococci. This is evident in Table VIII.

Seven strains of pneumococci, of as many different types, were tested by the

same technic. As shown in Fig. 8, 6 of the 7 strains did not differ significantly with respect to either the effective concentrations of penicillin or the optimal rate of killing. One strain, a Type XI organism, was much less sensitive to penicillin, and was killed more slowly, than the other 6. The maximally effective concentration was 1 to 16 micrograms instead of 0.064; and the maxi-

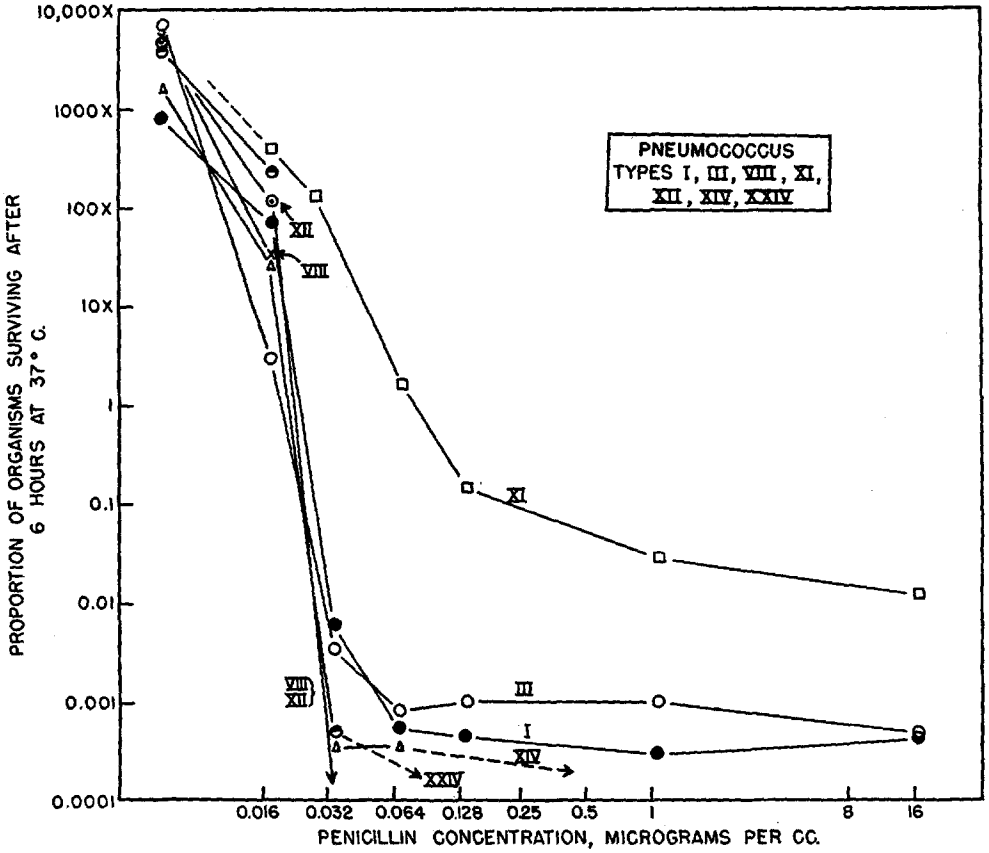

FIG. 8. The effect of the concentration of penicillin G on the proportion of organisms surviving after 6 hours at 37°C. (*Diplococcus pneumoniae*, types I, III, VIII, XI, XII, XIV, and XXIV).

mal rate at which the organisms could be killed by penicillin was also low, 1.3 per cent of the organisms surviving after 6 hours' exposure to 16 micrograms per cc.

D. Staphylococcus aureus and Staphylococcus albus

An experiment with a single strain of *Staphylococcus aureus* is summarized in the 2 sections of Fig. 9. As shown in Fig. 9 *a*, as the concentrations of

penicillin were increased up to the optimal concentration of 0.096 microgram per cc., there was a progressively more rapid bactericidal action. However, when the concentration of penicillin was increased beyond that optimal level, this trend was reversed, and the rate at which the organisms were killed fell off progressively (*cf.* Fig. 9 *b*). At concentrations of 1 microgram per cc., there were 110 times as many survivors after 6 hours as there were at 0.1 microgram, and it required 19 hours instead of 5 to kill 99.9 per cent of the organisms. Qualitatively similar results were obtained in every one of 5 experiments with this particular strain.

Of a total of 9 strains of staphylococci tested, 7 of *Staphylococcus aureus* and 2 of *Staphylococcus albus*, in 2 there was a well defined concentration of penicillin which was maximally effective, with diminished activity at concentrations in excess of that optimum; and in 2 others the zone phenomenon was less pronounced but none the less definite and reproducible (Table IX). With the other 5 strains the rate of bactericidal action increased with the concentration of penicillin to a maximum which was thereafter unaffected even by a 1000-fold increase.

It is to be noted in Fig. 9 *a* that at borderline concentrations of penicillin (0.024 and 0.032 microgram per cc. in that experiment) an initial decrease in the number of viable organisms was followed by an increase, the number of organisms ultimately reaching the same level attained in the control tube. This phenomenon was relatively uncommon with the group A streptococci, pneumococci, and treponemata, and then only within a narrow threshold range of penicillin concentrations; it was observed not infrequently with *Streptococcus fecalis;* but was a regular occurrence in experiments with staphylococci. In some of the experiments, as in that of Fig. 9 *a*, this remultiplication of organisms became manifest within a few hours, and at a time when only a relatively small proportion had been killed; in other experiments the remultiplication began only after 24 or 48 hours, sometimes after more than 99.99 per cent of the organisms had been killed. In the case of staphylococci, and unlike any of the other organisms here studied, the daughter cells of the last few resistant survivors were found to have a significantly increased resistance to the action of penicillin. It is therefore probable that remultiplication began when the average multiplication rate of the resistant survivors (which presumably developed as a mutation in the course of the experiment (7, 12)) exceeded the rate at which those surviving organisms could be killed by penicillin.

E. Treponema pallidum (Reiter)

One of 5 similar experiments with the cultured Reiter strain of so called *T. pallidum* is summarized in Table X, and a second in Fig. 10. In those experiments, a concentration of 0.016 microgram per cc. significantly decreased

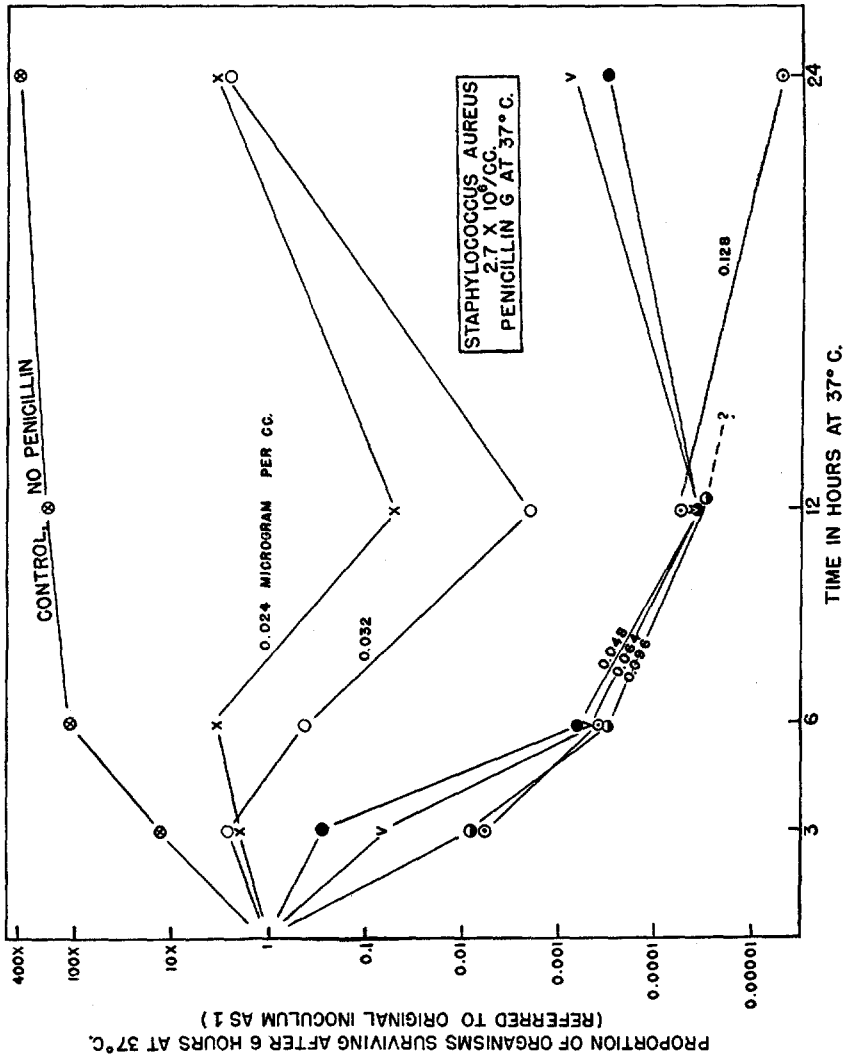

FIG. 9*a*

FIG. 9 *a* and *b*. The effect of the concentration of penicillin on the rate of its bactericidal action against the Smith strain of *S. aureus*. (*a*) The progressive *increase* in the rate of bactericidal action as the concentration of penicillin was increased from 0.024 to 0.096 microgram per cc. (*b*) The progressive *decrease* in the rate of bactericidal action as the concentration was increased from 0.096 to 2048 micrograms per cc.

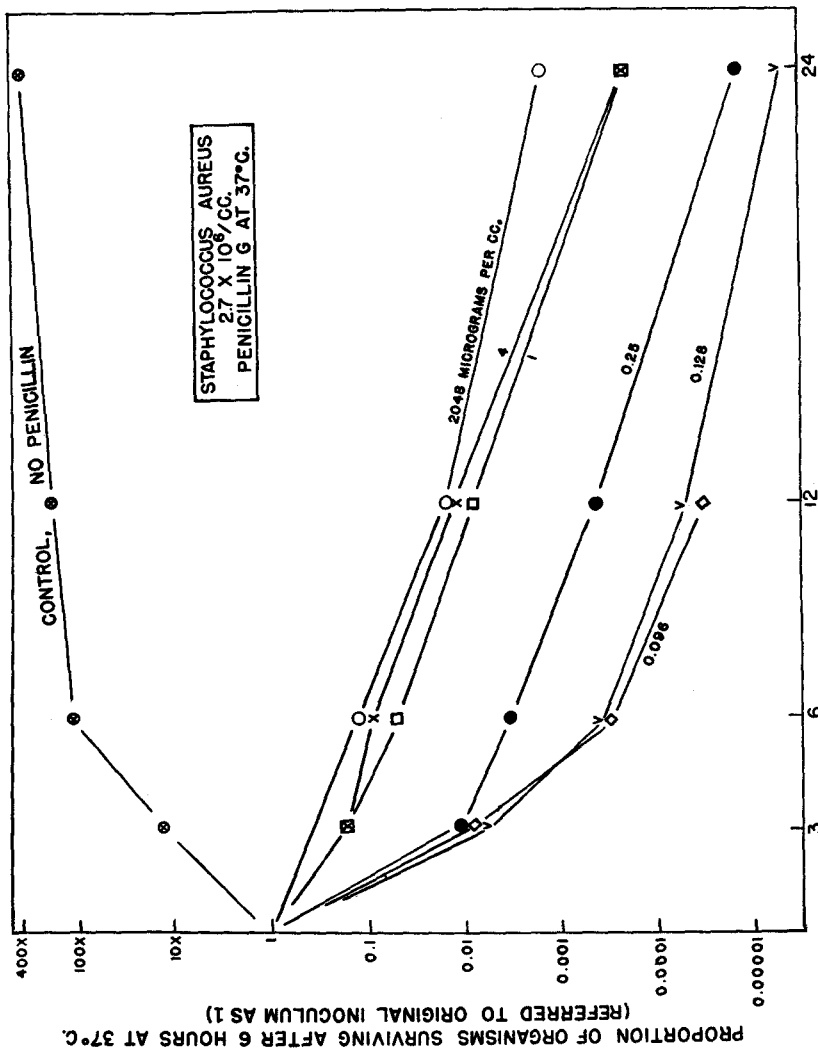
STAPHYLOCOCCUS AUREUS
2.7 X 10⁸/CC.
PENICILLIN G AT 37°C.
CONTROL, NO PENICILLIN
2048 MICROGRAMS PER CC.
1
0.25
0.125
0.062
400X
100X
10X
1
0.1
0.01
0.001
0.0001
0.00001
3
6
12
24
TIME IN HOURS AT 37° C.
PROPORTION OF ORGANISMS SURVIVING AFTER 6 HOURS AT 37°C.
(REFERRED TO ORIGINAL INOCULUM AS 1)

FIG. 9*b*

TABLE IX

The Susceptibility to Penicillin G of 9 Strains of Styphylococcus (Strains M14 and M15 Were Staphylococcus albus; the Other 7 Were Staphylococcus aureus, 3 of Which Showed a Paradoxical Zonal Susceptibility (Slower Rate of Killing at Higher Concentrations of Penicillin))

Strain No.	Concentration of penicillin (*micrograms per cc.*) which			Zone phenomenon at higher concentrations	Percentage* of organisms surviving after 6 hrs.' exposure to		Time required to kill 99.9 per cent of organisms* at	
	reduces growth	effects net reduction in no. of viable organisms	kills organisms at maximal rate		optimal concentration of penicillin	256 micrograms per cc.	optimal concentration of penicillin	256 micrograms per cc.
							hrs.	*hrs.*
Smith	0.016–0.032	0.032	0.064–0.128	+	0.036	13	5	27
M10	0.016	0.032	0.064–0.14	+	0.08	3	5.8	13‡
M12	0.016	0.064	0.128	+	0.07	3.2	5.8	9.7
M15	0.016	0.032	0.128	+	0.23	8.0	9	23
F10	0.016	0.064	0.25	0	7	18	16	21
M11	0.25	1	1–16§	0	6	6‡	12	11
B	0.016	0.032–0.064	0.064	0	0.19	0.69	8	10
M13	0.032	0.128	0.25–1	0	1.1	0.54	11	11
M14	0.016	0.032	0.064–0.128	0	0.9	3.9	23	19

* Results in single illustrative experiment.

‡ 2,048 micrograms per cc.

§ An initial decrease was followed by late regrowth at concentrations of 1, 4, and 16 micrograms (*cf.* text) but not at higher concentrations.

TABLE X

The Rate at Which T. pallidum (Reiter) Is Killed at Varying Concentrations of Penicillin G

(Initial number of organisms in reacting mixture = 750,000 per cc.)

Time at 37°C.	Concentration of penicillin G, *micrograms per cc.*						Concentration of penicillin* (*micrograms per cc.*) which		
	0	0.016	0.064	0.25	1	256	reduced growth	effected net reduction in No. of viable organisms	killed organisms at maximal rate
	Percentage‡ of organisms still viable after indicated time period								
hrs.									
6	—	127	73	27	7	11			
24	370	54	1.5	0.54	0.26	0.13	0.016	0.032–0.064	1–4
48	1,450	—	0.28	0.038	0.017	0.015			
Time required to kill 99.9 per cent of organisms, *hrs.*.....	∞	?	65±	39	32	27			

* Based on this and 4 similar experiments.

‡ Referred to original inoculum as 100.

the net rate of multiplication; the minimal concentration with a net bactericidal action was 0.032 microgram per cc.; and almost the maximum effect was produced by a concentration of 0.25 to 1 microgram per cc. Thereafter, a 2000-

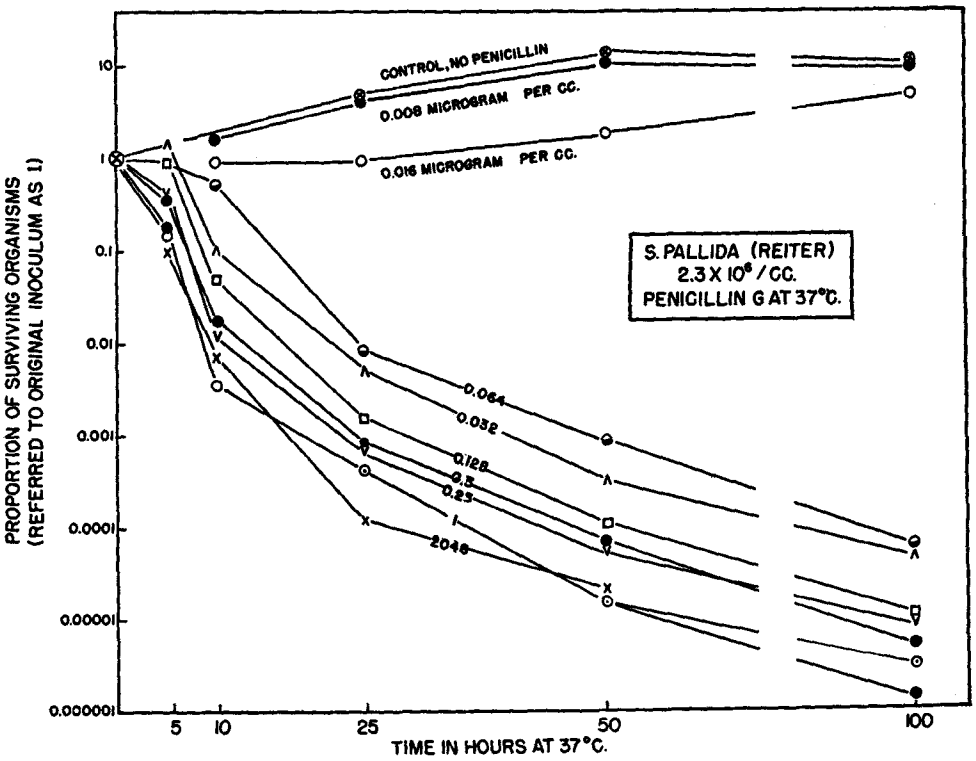

FIG. 10. Effect of the concentration of penicillin G on the rate at which treponemata (Reiter strain) are rendered non-viable *in vitro*.

fold increase in penicillin concentration, up to 2048 micrograms per cc. only slightly accelerated the rate at which the organisms were killed. These results with crystalline penicillin G agree qualitatively and quantitatively with those obtained in previously reported studies (2) with a sample of commercial penicillin known to contain significant amounts of the other penicillin species.[3]

[3] With amorphous penicillin, longer periods of time were necessary to effect 99.9 per cent reduction than with crystalline G; and the maximally effective concentration was significantly higher. Further, with commercial penicillin there was a greater tendency for the organism to grow out after an initial decrease. These differences may reflect the greater stability of crystalline penicillin in comparison with that of the amorphous commercial product, under the conditions of the experiment.

The slow rate at which these treponemal cultures were killed, as compared with for example *Streptococcus pyogenes,* is particularly to be noted (compare Fig. 10 with Fig. 1, or Table X with Table I). To kill 99.9 per cent of the treponemata at the maximally effective level of 1 microgram per cc. required from 23 to 33 hours, against 1.4 to 2.2 hours for the C-203 strain of *Streptococcus pyogenes;* and the proportion of organisms surviving after 6 hours was 1 to 10 per cent, as compared to 0.002 to 0.01 per cent for the streptococcus.

III. THE DIFFERENCES IN RESISTANCE TO PENICILLIN AMONG BACTERIAL POPULATIONS

The susceptibility of bacteria to penicillin may be defined in terms of two variables: the concentrations of the drug which are necessary to kill the bacteria, and the rate at which the organisms are killed at these concentrations. Although the former factor has been stressed in most previous studies, the maximal rate at which a given bacterial strain can be killed by penicillin may also be of major therapeutic significance. Both factors varied widely among the strains here studied, and there was no necessary correlation between the two.

A. Differences in the "Effective" Concentrations of Penicillin for Different Bacterial Species and Strains.—Three "effective" concentrations of penicillin have been here defined for a number of bacterial species and strains (*cf.* section *a* of Table XI). (*a*) The first and lowest is that concentration of drug which suffices only to reduce the rate at which the net number of viable organisms increases. (This concentration may be of therapeutic significance if it should be proven that it renders bacteria *in vivo* more vulnerable to the body's natural humoral or cellular defense mechanisms, even though it does permit multiplication at a diminished rate *in vitro.*) (*b*) At a somewhat higher concentration, penicillin causes a slow progressive reduction in the number of viable organisms. This approximately equals the concentration which prevents visible growth *in vitro,* and thus approximates the "sensitivity" of a given strain to penicillin as ordinarily determined (1–4, 8, 13, 14). It is not, however, the concentration at which the organisms are killed at the maximal rate *in vitro.* (*c*) The latter maximally effective concentration was usually 2 to 20 times the minimal concentration which was effectively bactericidal, and probably represents the concentration which should be maintained at the focus of infection in order to kill the largest number of organisms in the shortest possible time. Even a 32,000-fold increase beyond this maximally effective concentration did not further increase the rate of bactericidal action of penicillin. Unless high concentrations of penicillin can be shown to have an effect *in vivo* over and above their direct bactericidal action, then in the case of most of these organisms a penicillin level of for example 0.1 to 1 microgram per cc. maintained at the focus of infection would be expected to be just as effective therapeutically as concentrations of 10, 100, or 1000 micrograms per cc. Extremely large doses of penicillin

TABLE XI

The Susceptibility of a Number of Organisms to Penicillin
(a) The Effective Concentration of the Drug
(b) The Presence of a Zone Phenomenon (Paradoxically Retarded Bactericidal Action) at High Concentrations of Penicillin
(c) The Time Required to Kill 99.9 Per Cent of the Organisms at Optimal and Excessive Concentrations
(d) The Proportion of Organisms Surviving after Exposure for 6 Hours to Optimal and Excessive Concentrations of Penicillin

Species		No. of strains studied	(a) Concentrations of penicillin G (*micrograms per cc.*) which			(b) No. strains showing zone phenomenon (slower bactericidal action at higher concentrations of penicillin)		(c) Time required to kill 99.9 per cent of organisms at		(d) Percentage of organisms surviving after 6 hrs.' exposure to	
			decreased net rate of multiplication	slowly killed organisms	killed organisms at maximal rate	Yes	No	optimum concentrations of penicillin)	256 micrograms per cc. and higher	optimal concentration of penicillin	256 micrograms per cc. and higher
								hrs.	*hrs.*		
β-hemolytic streptococci	Lancefield Group A	5	0.004–0.008	0.016	0.032–0.064	0	5	2–8	2–5	0.002–0.6	0.004–0.25
	Group B	4	0.016	0.032–0.064	0.064–0.128	4	0	4–10	15–23	0.08–0.56	1.1–44
	Group C	4	0.004	0.016	0.032	3		4–9	13, >9, 21, 14	0.011–0.27	0.19–15
							1	4	5	0.02–0.08	0.02–0.05
	M31*	1	1	4	4–8	1		11–17	54	0.17–0.6	19–80
α-hemolytic streptococci	*Fecalis*	7	1	3–4	4–6	5		4.6–5.2	30–40	0.029–0.062	23–44‡
							2	48	48	13, 48	?, 50
	Others	5	0.016–0.032	0.032–0.064	0.128	3		6.5–11	26–45	0.12–0.67	11–44
							2	2.4, 25	2.4, 29	0.03, 16	0.05, 20
Staphylococci	*S. aureus* and *S. albus*	9	(8) 0.016–0.032	0.032–0.064	0.064–0.128	4		5–9	13–27	0.07–0.9	3–22
			(1) 0.25	1	16		5	8–17	10–21	1.1–8.5	0.54–18
Pneumococci	Types 1, 3, 8, 12, 14, 24	6	0.006–0.016	0.032	0.032–0.064	1?	5	2.5–4.4§	4.6–6§	0.004–0.058	0.01–0.075
T. pallidum (nonpathogenic Reiter strain)		1	0.016	0.032–0.064	1–4	0	1	23–42	17–27	6.4–17	11–15

* Although this strain reacted with a group C typing serum, it was a lanceolate organism which grew in pairs or occasional short chains, liquefied gelatin, and fermented lactose, salicine, mannite, trehalose, and sorbitol. It probably falls in the *Streptococcus zymogenes* group.

‡ 512 micrograms per cc. instead of 256.

§ Values for Type I strain only.

would be of therapeutic advantage only to the degree that they provided this maximally effective concentration for longer periods of time, and not by virtue of the higher absolute levels attained.

The three effective levels of penicillin for the various bacterial species and strains here studied are summarized in section *a* of Table XI. In Figs. 11 and 12, the lowest point in each curve indicates the maximally effective concentration of penicillin for a single strain of each species, *i.e.* the concentration at

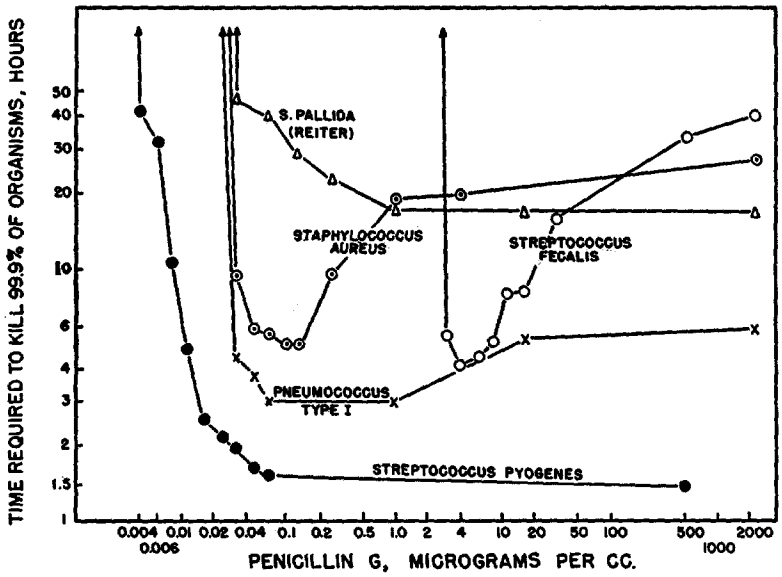

FIG. 11. Effect of the concentration of penicillin on the time required to kill 99 per cent of the organisms. Illustrative single experiment with single strain each of *T. pallidum* (Reiter), *Staphylococcus aureus*, *Streptococcus fecalis*, *Diplococcus pneumoniae* Type I, and *Streptococcus pyogenes*.

which for example 99.9 per cent of the organisms were killed in the shortest time (Fig. 11), or at which there was the smallest proportion of viable survivors after 6 hours for example (Fig. 12).

The large differences in the maximally effective concentrations for different species of bacteria are well known. Within a single species, different strains usually had approximately the same sensitivity. There were, however, occasional striking disparities (*cf.* the relatively resistant staphylococcal strain M11 in Table IX, and the Type XI pneumococcus in Fig. 8; the identification of the discrepant M31 strain in Table V as a group C streptococcus was questionable).

The Varying Resistance to Penicillin of Individual Organisms in the Same Bacterial Culture: Within the same bacterial culture, individual organisms

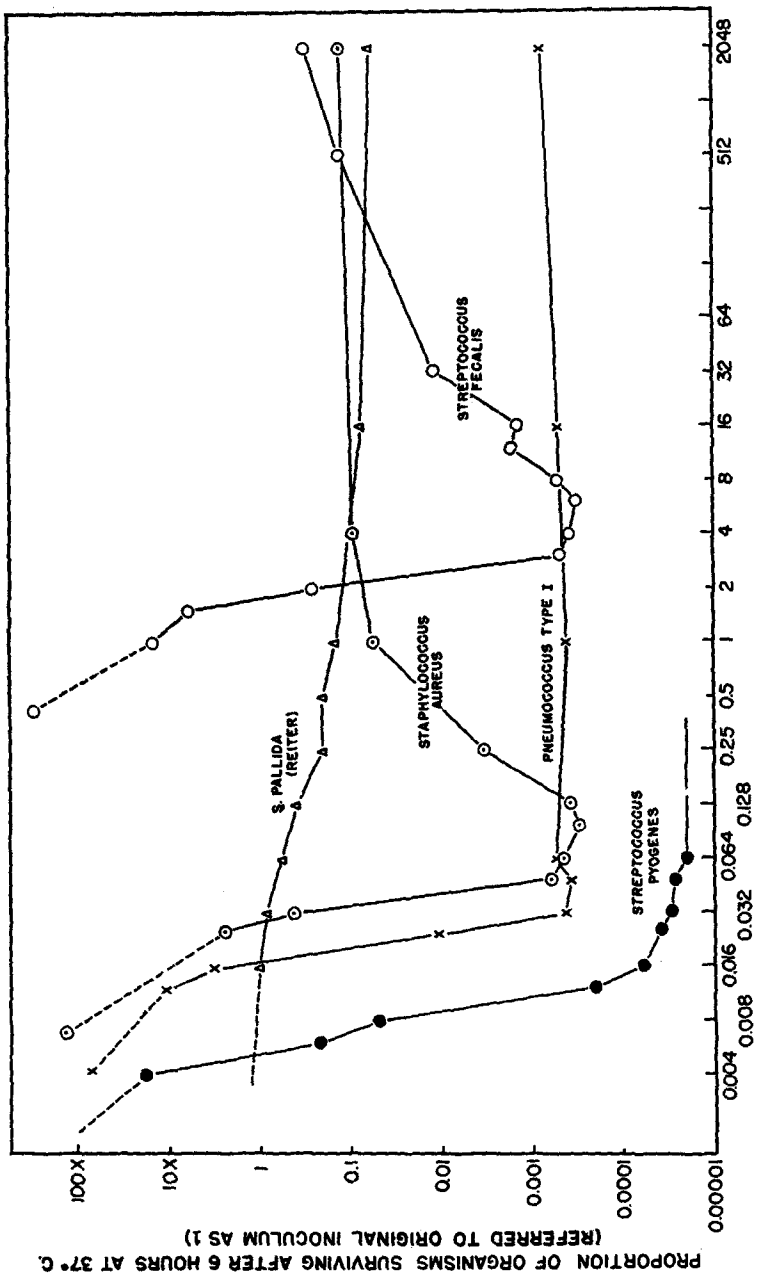

FIG. 12. Effect of the concentration of penicillin on the proportion of organisms surviving after 6 hours at 37°C. Illustrative single experiment with single strain each of *T. pallidum* (Reiter), *Staphylococcus aureus*, *Streptococcus fecalis*, *Diplococcus pneumoniae* Type I, and *Streptococcus pyogenes*.

also varied in their susceptibility to penicillin. At threshold concentrations of the drug only a fraction of the organisms might be killed; and an initial decrease in the number of viable organisms which reflected the death of the more susceptible, was often followed by the rapid multiplication of the relatively more resistant organisms.[4] All the organisms would, however, be killed by higher concentrations of penicillin. A 4-fold difference in concentration constituted the usual range of variation between the sensitivity threshold of the most susceptible and the most resistant organisms in a given suspension. The factor underlying this difference remains to be determined (*cf.* Foster (10); Foster and Wilker (11)).

B. The Widely Varying Maximal Rates at Which Different Strains of Bacteria or Different Organisms within the Same Strain Can Be Killed by Penicillin.— The concentration of penicillin which suffices to kill a given bacterial strain *in vitro* affects what may be called the *intensity* of treatment; *i.e.*, the dosage of penicillin to be administered, and the frequency with which that dose should be repeated. The pronounced differences between bacterial species in this respect have been stressed by numerous workers. It is, however, apparent from Table XI and Figs. 11 and 12 that there are also pronounced differences in the maximal rate at which the various bacterial species can be killed by penicillin. This is indicated by the varying vertical position of the minima on the curves of Figs. 11 and 12. Thus, after 6 hours' exposure to the most effective concentration of penicillin, 1 to 10 per cent survived in the case of the Reiter treponeme (4 experiments), but <0.0004 per cent in the case of the F24 strain of group A β-hemolytic streptococci, a difference in this respect of more than 2500-fold. Similarly, the times required to kill 99.9 per cent of the organisms in suspensions of these 2 strains were respectively 17 to 37 and 2 to 3 hours. Even within the same species, different strains varied markedly. With the M4 strain of *Streptococcus fecalis*, only 0.029 per cent survived a 6 hour exposure to the optimally effective concentration of penicillin, but 48 per cent of strain F1 survived under similar conditions; and the times required to kill 99.9 per cent were 4.6 and >48 hours, respectively. These differences in the susceptibility of the bacterial strains to penicillin in terms of the rate at which they can be killed may well affect the length of time for which treatment must be continued to effect cure. An organism of which 99.9 per cent can be killed in for example 3 hours will probably be more susceptible to treatment than one which requires 48 hours' exposure even at maximally effective concentrations of the drug.

There was no *necessary* correlation between the concentration of penicillin necessary to kill a given organism, and the maximum rate at which that or-

[4] This may be observed in the absence of demonstrable mutation or adaptation. The daughter cells of these relatively resistant survivors may have the same distribution of resistance as the original culture.

ganism could be killed by the drug. This is indicated by the wide scatter of the points in Fig. 13. Organisms exquisitely sensitive to penicillin in terms of the low concentrations which sufficed to kill the organisms at a maximal rate might be killed only slowly at that optimum concentration (*cf.* △ and ▲ in lower left of Fig. 13); while others killed only by high concentrations might be killed quite rapidly (*cf.* × in upper right portion of figure). Nevertheless, Fig.

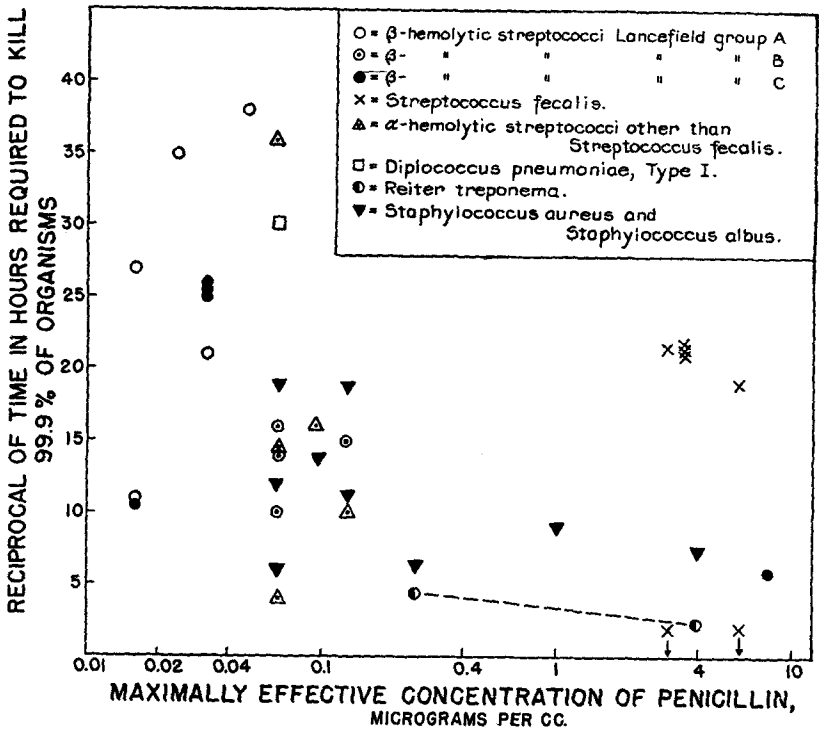

FIG. 13. The correlation between the maximally effective concentrations of penicillin for various bacterial species and the rate at which the organisms are killed at those maximally effective levels.

13 does suggest a rough correlation between the concentrations of penicillin maximally effective against the various strains, and the rates at which the organisms could be killed by those maximally effective concentrations. By and large, the more resistant a strain in terms of the amounts of penicillin required, the more resistant it was also in terms of the rate at which the organisms were killed, and in the times required to sterilize a given suspension.

C.—Yet a third factor which affects the "curability" of an infection with penicillin, over and above the effective concentrations of the drug, and the rate at which the particular strain of organism can be killed, is the initial number

of bacteria in the infected host. There are several reports which describe the bactericidal action of penicillin as a function of the number of organisms (15–17). In our own experiments, however, the initial number per cubic centimeter usually had no material effect on the rate of bactericidal action of penicillin. Although there were occasional exceptions (*cf.* staphylococcus experiment in Fig. 14) essentially the same proportion was usually killed within a given time period whether the initial number was; *e.g.*, 1000 or 1,000,000 per cc. (*cf.* Fig. 14).

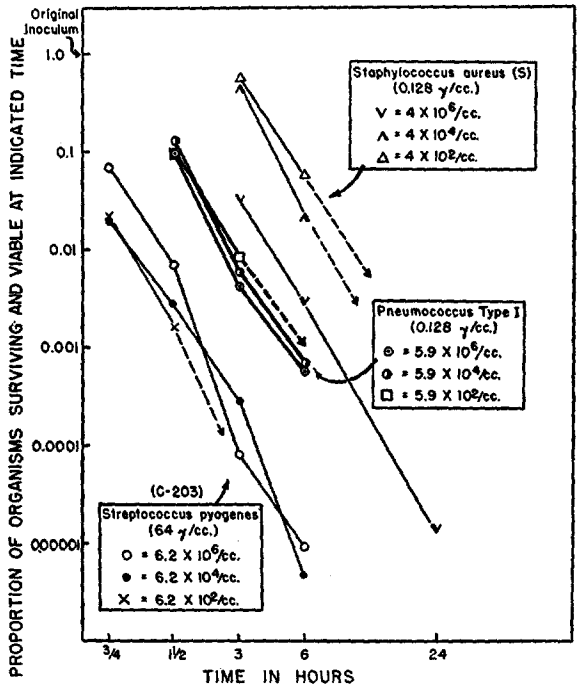

FIG. 14. The effect of the initial number of organisms on the rate at which bacteria are killed by maximally effective concentrations of penicillin.

It is nevertheless clear that the more organisms which are initially present in a culture or in an infected host, the longer will be the times required to effect sterilization or cure. Of an initial 1000 organisms, only > 99.9 per cent need be killed;[5] but with 1,000,000 organisms that proportion becomes > 99.9999 per cent. A longer time is then required, not only because a larger proportion must be disposed of, but also because the last few highly resistant organisms require a disproportionately long exposure to the drug (*cf.* Figs. 1, 9, 10, and 14). It usually took as long or longer to kill the last 0.01 per cent of the organisms as

[5] Assuming that the persistence of 1 organism implies the persistence of infection: the argument is not affected whatever one sets as the arbitrary threshold of cure.

it did to kill the entire first 99.99 per cent; and there was usually an 8-fold difference in the times required to sterilize suspensions of 1000 and 1,000,000 organisms.

It is perhaps unnecessary to point out that the foregoing considerations are based on *in vitro* experiments, and that the participation of the host may alter these relationships not only quantitatively, but perhaps also qualitatively. However, such data as are available indicate that these inferences may be valid also *in vivo*. In syphilitic rabbits inoculated with 20, 2000, and 200,000 organisms, and treated 4 days later with a single injection of penicillin suspended in oil and beeswax, the doses necessary to abort infection in half the animals have been shown to be approximately 200, 500, and 3500 units per kilo, respectively (18). The larger the inoculum, the longer was the time period for which the effective concentrations of penicillin had to be maintained in order to kill the very last organism and thus abort the infection; and the correspondingly larger was the dose of penicillin which had to be administered. Even more striking correlations between the number of organisms in the infected animal, and the curative dose of penicillin (*i.e.*, the period of time for which effectively bactericidal levels must be maintained) have more recently been observed in experimental pneumococcal infection in mice treated with penicillin in aqueous solutions (19, 20).

The foregoing discussion involves the several factors of effective penicillin *concentration*, the varying *rate* at which different bacterial strains can be killed even at maximally effective levels of the drug, the *number* of organisms to be killed, and the total *time* for which the bacteria are exposed to effective concentrations. A corollary to these factors is the therapeutic problem as to whether the exposure to penicillin should be continuous, and the penicillin concentrations *in vivo* therefore sustained at effectively bactericidal levels, or whether the penicillin levels may be intermittently allowed to fall below those effective levels without prejudicing the outcome of the treatment. Experiments on this point, with particular reference to the time required for various bacteria to recover from the toxic effects of penicillin *in vitro* and *in vivo*, will be described in a following paper ((19); *cf.* also (21)).

IV. ZONE PHENOMENON: THE PARADOXICALLY REDUCED ACTIVITY OF HIGH CONCENTRATIONS OF PENICILLIN AGAINST CERTAIN BACTERIA

A puzzling aspect of the present experiments was the demonstration that for many, but not all, strains of both α- and β-hemolytic streptococci, and for many strains of staphylococci, there was an optimal concentration of penicillin in excess of which the organisms were killed less rapidly than they were at the lower concentration (*cf.* (9)). This is evident in the curves of Figs. 4, 5, 6, 7, 11, and 12, in which the well defined minima represent the optimal concentrations of penicillin. With a Type I strain of pneumococcus this zone was observed only irregularly, and was not pronounced. With the other bacterial

species and strains here studied, there was no zonal effect; instead, the rate of bactericidal action reached a maximum at a concentration of penicillin characteristic of the particular strain, and which was not further affected even by a 2000- to 32,000-fold increase in penicillin concentration.

With the organisms which showed this zonal susceptibility, the maximally effective range was sometimes extraordinarily narrow. Thus, with the S strain of *Streptococcus fecalis* (page 110), 2 micrograms per cc. did not suffice to sterilize the culture, and 4 to 6 micrograms were maximally effective. When the penicillin concentration was increased to as little as 8 micrograms per cc., it was significantly less effective, and it became progressively less so the higher the concentration. Similarly, in the case of the Smith strain of *Staphylococcus aureus* (page 114), 0.032 microgram per cc. usually failed to sterilize the culture, 0.064 to 0.096 microgram per cc. were maximally effective, and an increase to as little as 0.128 to 0.25 microgram per cc. significantly retarded the rate of death of the organisms.

The zone phenomenon here described has not yet been satisfactorily explained. (*a*) It was not necessarily related to the production of free penicillinase. Although the M11 zonal strain of *Staphylococcus aureus* did inactivate penicillin in culture, the S strain of *Staphylococcus aureus*, the 5 strains of *Streptococcus fecalis*, and the M31 strain of β-hemolytic streptococci, all of which showed a definitely retarded bactericidal action at high concentrations of penicillin, had no effect on the drug when they were permitted to grow in solutions containing less than the inhibitory concentration (0.25 to 0.5 microgram per cc.) (*cf.* Table XII).

(*b*) It seems unlikely that the zones were due to the presence in penicillin of a trace of impurity which inhibited the action of the drug. Garrod (23) had noted the retarded action of penicillin at high concentrations against staphylococci, but ascribed this to the presence of impurities, and stated that this zonal effect was not obtained with penicillin of about 85 per cent purity (*cf.* also reference 24). However, the data here reported with crystalline penicillin G, some lots of which were, on the basis of counter-current distribution diagrams, reported to be more than 95 per cent pure, indicate that this zonal effect is actually a property of the drug and not due to associated impurities. It is further difficult on the latter basis to explain the fact that the inhibitory effect became evident at a level which differed so markedly among different bacterial strains, and corresponded so regularly to the range of effective concentrations. Further, different lots of crystalline penicillin G, from different manufacturers, behaved identically with respect to the zone phenomenon, and it was observed with penicillins K and X, as well as G.

A clue to the nature of this paradoxical zone may be provided by the observation that the degree to which the bactericidal action of penicillin was retarded at high concentrations varied not only with the concentration of the drug, but also with the number of bacteria. This point is under present study.

TABLE XII

Showing that the Zonal Susceptibility to Penicillin Is Not Necessarily Related to the Ability of the Particular Bacterial Strain to Destroy Pencillin

(Of 8 zone-sensitive organisms tested, only 1 was found to produce extracellular penicillinase)

Species	Strain No.	Minimal bactericidal concentration of penicillin, *micrograms per cc.*	Concentration of penicillin added to medium, *micrograms per cc.*	Inoculum, No. per cc. $\times 10^6$	Degree of bacterial multiplication after 6 hrs. at 37°C.	Degree of bacterial multiplication after 24 hrs. at 37°C.	Degree of bacterial multiplication after 48 hrs. at 37°C.	Residual penicillin activity after 6 hrs. at 37°C.	Residual penicillin activity after 24 hrs. at 37°C.	Residual penicillin activity after 48 hrs. at 37°C.	Conclusion
								per cent	*per cent*	*per cent*	
Group A hemolytic streptococcus	M31	4	0.25	6.0	44×			100			No significant inactivation of penicillin by growing organisms
			0.25	1.2	277×	736×		75	75		
Staphylococcus aureus	S	0.032-0.064	0.025	2.1	1,575×		2,560×	100		63	" "
	M11	4-16	0.25	1.1	14×			< 5			Essentially complete inactivation of penicillin in 6 hrs.
			0.1	0.8	95×			<13			
Streptococcus fecalis	H			4.7	40×	82×	76×	100	83	83	No inactivation of penicillin by growing organisms
			0.5	3.4	84×	148×	188×	100	100	50	
				1.3	233×	293×	—	75	100		
	M1		0.5	1.2	178×	342×		90	75		" "
	M3	3-4	0.5	0.6	7×	142×		71	75		" "
	M5		0.5	1.3	103×	156×		100	75		" "
	M6		0.5	1.3	153×	280×		100	100		" "
Controls in sterile broth			2					100			
			0.5					82	60		
			0.4					83	67		
			0.25					100	100	63	

In the treatment of infections caused by these zone-sensitive organisms, the initial blood and tissue levels provided by large doses of penicillin may be far in excess of those which are optimally effective *in vitro*. Those high concentrations may be correspondingly less effective *in vivo*. Although the blood and tissue levels eventually fall to the optimal concentration, they remain in that

optimally effective range for a relatively short period of time, because of the rapid rate at which penicillin is excreted. In the treatment of infections caused by such zone-sensitive bacteria, a number of relatively small doses of penicillin repeated at appropriate intervals (22), or the slowly absorbed suspensions of penicillin in oil and beeswax, may therefore be more effective therapeutically than extremely large injections which maintain the tissue concentrations at excessively high levels. The ideal method of treatment in such infections may well be a continuous infusion at a rate designed to maintain, at the focus of infection, a concentration of penicillin approximating that which is maximally effective for the particular organism.

SUMMARY

1. The concentrations of penicillin G which (*a*) reduced the net rate of multiplication, (*b*) exerted a net bactericidal effect, and (*c*) killed the organisms at a maximal rate, have been defined for a total of 41 strains of α- and β-hemolytic streptococci, *Staphylococcus aureus* and *Staphylococcus albus*, *Diplococcus pneumoniae*, and the Reiter treponoma.

2. The concentration which killed the organisms at a maximal rate was 2 to 20 times the minimal effective level ("sensitivity" as ordinarily defined). With some organisms, even a 32,000-fold increase beyond this maximally effective level did not further increase the rate of its bactericidal effect. However, with approximately half the strains here studied (all 4 strains of group B β-hemolytic streptococci, 4 of 5 group C strains, 5 of 7 strains of *Streptococcus fecalis*, 2 of 4 other α-hemolytic streptococci, and 4 of 9 strains of staphylococci), when the concentration of penicillin was increased beyond that optimal level, the rate at which the organisms died was paradoxically reduced rather than increased, so that the maximal effect was obtained only within a relatively narrow optimal zone.

3. There were marked differences between bacterial species, and occasionally between different strains of the same species, not only with respect to the effective concentrations of penicillin, but also with respect to the maximal rate at which they could be killed by the drug in any concentration. Although there was a rough correlation between these two factors, there were many exceptions; individual strains affected only by high concentrations of penicillin might nevertheless be killed rapidly, while strains sensitive to minute concentrations might be killed only slowly.

4. Within the same bacterial suspension, individual organisms varied only to a minor degree with respect to the effective concentrations of penicillin. They varied strikingly, however, in their resistance to penicillin as measured by the times required to kill varying proportions of the cells.

BIBLIOGRAPHY

1. Hobby, G. L., Meyer, K., and Chaffee, E., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 277, 281.
2. Hobby, G. L., Meyer, K., Dawson, M. H., and Chaffee, E., *J. Bact.*, 1942, **43**, 11.

2*a*. Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Inv.*, 1943, **22**, 649.

3. Dawson, M. H., Hobby, G. L., and Lipman, M. O., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 101, 178.

3*a*. Rantz, L. A., and Kirby, W. M. M., *J. Immunol.*, 1944, **48**, 335.

4. Helmholz, H. F., and Ling, C., *Am. J. Dis. Child.*, 1944, **68**, 236.
5. Bigger, J. W., *Irish J. Med. Sc.*, 1944, 553, 585.
6. Eagle, H., and Musselman, A. D., *J. Exp. Med.*, 1944, **80**, 493.
7. Demerec, M., *Proc. Nat. Acad. Sc.*, 1945, **31**, 16.
8. Herrell, H. E., Nichols, D. R., and Heilman, D. H., *J. Am. Med. Assn.*, 1944, **125**, 1003.
9. Eagle, H., and Musselman, A. D., *Science*, 1948, **107**, 44.
10. Foster, J. W., *J. Biol. Chem.*, 1942, **144**, 285.
11. Foster, J. W., and Wilker, B. L., *J. Bact.*, 1943, **46**, 377.
12. Luria, S. E., *Bact. Rev.*, 1947, **11**, 1.
13. Robinson, H. J., *J. Pharmacol. and Exp. Therap.*, 1943, **77**, 70.
14. Meads, M., Ory, E. M., Wilcox, C., and Finland, M., *J. Lab. and Clin. Med.*, 1945, **30**, 725.
15. Hobby, G. H., and Dawson, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 178.
16. Parker, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**, 443.
17. Luria, S. E., *Proc. Soc. Exp. Biol. and Med.*, 1946, **61**, 46.
18. Eagle, H., Magnuson, H. J., and Fleischman, R., *J. Exp. Med.*, 1947, **85**, 423.
19. Eagle, H., and Musselman, A. D., data to be published.
20. Eagle, H., Musselman, A. D., and Fleischman, R., data to be published.
21. Parker, R. F., and Marsh H. C., *J. Bact.*, 1946, **51**, 181.
22. Tucker, H. A., and Eagle, H., *Am. J. Med.*, 1948, **4**, 343.
23. Garrod, L. P., *Brit. Med. J.*, 1945, **1**, 107.
24. Garrod, L. P., *Brit. Med. J.*, 1948, **1**, 382.