THE EFFECT OF THE SIZE OF THE INOCULUM AND THE AGE OF THE INFECTION ON THE CURATIVE DOSE OF PENICILLIN IN EXPERIMENTAL INFECTIONS WITH STREPTOCOCCI, PNEUMOCOCCI, AND TREPONEMA PALLIDUM

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Although the defense mechanisms of the host supplement the action of penicillin (1), the direct bactericidal effect of the drug is of major importance in its therapeutic action. It follows that factors which modify its bactericidal action *in vitro* may similarly effect its therapeutic action *in vivo*.

One such factor is the number of organisms to be killed. The individual bacteria in a given culture vary widely in the rate at which they are killed even by maximally effective concentrations of penicillin (2). The larger the initial number of organisms, the greater is the extreme of resistance encountered, and the longer it takes to sterilize the suspension. With the majority of the strains so far studied, it usually requires approximately 10 to 20 times as long an exposure to penicillin to sterilize a suspension containing a million organisms as it does to sterilize a suspension containing only a thousand (2). This presumably reflects the presence of more resistant organisms in the larger populations.

These considerations imply that the more bacteria present in the infected host, the longer will be the aggregate time for which penicillin must act in order to effect cure.[1] This has been found to be the case in the slowly developing syphilitic infection of rabbits (5), in which the incubation period is approximately 12 to 15 days even after infection with a large inoculum; and that same relationship is here confirmed in the case of the more acute pneumococcal and streptococcal infections of white mice and rabbits. Some implications with respect to the possible prophylactic use of penicillin are discussed in the text.

EXPERIMENTAL

1. Effect of the Number of Organisms Inoculated on the Curative Dose of Penicillin.—It had previously been shown (5) that when rabbits were inoculated

[1] This does not mean that the penicillin level must be *continuously* maintained. On the contrary, penicillin levels may fall below the effective levels for a number of hours without prejudicing the outcome of treatment. At least in part, this reflects the fact that some hours elapse before bacteria recover from the toxic effects of penicillin, and begin again to multiply (3, 4).

TABLE I

*Effect of the Size of the Inoculum on the Curative Dose of Penicillin G in Type I Pneumococcal Infections of White Mice**

Experiment No.	No. of organisms inoculated‡	Penicillin	Survived	Died	Dosage of penicillin which cured half of animals§
					CD_{50} ± standard error
		mg./kg.			*mg./kg.*
		512	9	7	
		256	4	12	
	23,800,000	128	2	14	470±127
		64	0	16	
		256	8	8	
	200,000	128	4	12	260±80
		64	1	15	
		128	11	1	
I		64	11	5	
	1,950	32	5	11	38±7.5
		16	2	14	
		0	0	10	
		8	12	4	
		4	7	9	
	15‖	2	4	12	4.8±1.2
		1	2	14	
		0‖	1	9	
		2,048	10	6	
		1,024	7	9	
	23,000,000	512	5	11	1,230±365
		256	2	14	
		128	0	16	
		1,024	15	1	
		512	12	4	
	220,000	256	6	10	280±50
		128	4	12	
		64	0	16	
II					
		256	16	0	
		128	8	8	
	1,680	64	7	9	94±21
		32	1	15	
		0	0	10	
		32	12	4	
		16	9	7	
		8	4	12	
	18‖	4	6	10	10.3±4.9
		2	3	13	
		0‖	1	9	

with 20, 2,000, or 200,000 *T. pallidum*, and treated 4 days later with a single injection of amorphous penicillin in oil and beeswax, the dosages necessary to cure 50 per cent of the animals (calculated by the method of Reed and Muench (6)), were 200, 500, and 3,500 units per kg., respectively. Recalculated from the same data by the method of Litchfield and Fertig (7), the CD_{50} values were found to be 150 ± 50, 570 ± 165, and 3,700 ± 1,370 units per kg.[2]

Subsequent to these experiments, when white mice were inoculated intraperitoneally with varying numbers of *Diplococcus pneumoniae* type I, and treated immediately by a single intramuscular injection of penicillin G in aqueous solution,[3] a similar correlation was found between the size of the inoculum and the dosage of penicillin necessary to abort the infection.

[2] It is a pleasure to acknowledge the assistance of Mr. Nathan Mantel of the Office of the Statistical Coordinator, Division of Public Health Methods, U. S. Public Health Service, in calculating the CD_{50} values of the experimental data here reported, and their standard errors. The magnitude of some of these standard errors reflects the small number of animals used in some of the experiments, and their individual variability. Unless otherwise stated, "In calculating the probit relationships indicated in the footnotes to the tables, provisional estimates were made by Kärber's method or, when indicated, by an adaptation of Kärber's method after Cornfield and Mantel. (Graphic methods were used when this was not feasible.) These estimates were put through one or two cycles of computation of the maximum likelihood procedure, using the Cornfield-Mantel tables of weighted deviations and weighting coefficients. The standard errors indicated for the CD_{50} dosages have been increased to allow for significant values of chi square where indicated. Where there were natural survivors in the absence of treatment (as with small numbers of organisms injected intramuscularly in mice), the empirical responses were first adjusted by Abbott's formula for the assumed rate of natural survivors (5 to 10 per cent). In such cases, the Finney procedure and tables were used in the calculations." (Mantel.)

[3] The courtesy of the Squibb Laboratories in supplying the penicillin G used in these studies is gratefully acknowledged. The lot numbers used in the individual experiments are indicated in the tables.

Footnotes to Table I

The mice (CFW strain) were inoculated intraperitoneally with a dilution of a 6 hour culture on blood-broth, and treated immediately with a single intramuscular injection of penicillin G in aqueous solution.

* The penicillin G (lot V-31) used for these experiments was obtained through the courtesy of the Squibb laboratories.

‡ Determined by plate count on an appropriate dilution. This number represents the total number of clumps or chains, rather than discrete organisms. Although the average number per clump was not determined in this experiment, similar cultures have been largely discrete diplococci.

§ Calculated by the method of Litchfield and Fertig (7). The method of Wilson and Worcester (*cf.* reference 8) gave essentially similar CD_{50} values, and standard errors of the same order of magnitude. In 2 of the 8 experimental groups, the slope constant (increase in probits of survivors per 10-fold increase in dosage) was abnormally low (0.69±0.32 and 1.37±0.39). In the remaining 6, it varied from 1.7±0.47 to 2.6±0.54.

‖ In calculating the CD_{50} dosage with these small inocula, no attempt has been made to correct for the fact that they did not kill 100 per cent of the untreated, control mice, and that an occasional mouse survived without treatment.

TABLE II

*Effect of the Size of the Inoculum on the Curative Dose of Penicillin G in White Mice Infected with a Group B β-Hemolytic Streptococcus**

	No. of organisms inoculated‡	Penicillin	Survived	Died	Curative dose (CD_{50}) of penicillin G, ± standard error§, ‖
		mg./kg.			*mg./kg.*
		2,048	20	0	
		1,024	18	2	
Group 1	2,235,000	512	9	11	424±52
		256	7	13	
		128	1	19	
		0	0	10	
		1,024	20	0	
		512	14	6	
		256	5	15	
Group 2	180,000	128	0	20	339±45
		64	1	19	
		32	1	19	
		0	0	10	
		256	19	1	
		128	7	13	
Group 3	1,750	64	2	18	139±51**,§
	(estimated)	32	1	19	
		16	5	15	
		0	1	9	
		64	19	1	
		32	15	5	
		16	18	2	
Group 4	17¶	8	11	9	2.8±1.1
		4	14	6	
		2	11	9	
		1	7	13	
		0	1	9	

The mice (CFW strain) were inoculated intraperitoneally with an appropriate dilution of a 3 hour culture in blood-broth, and treated immediately with a single intramuscular injection of penicillin G in aqueous solution. The number of organisms indicated in the table is actually the number of bacterial clumps, determined by plate counts. The number of organisms per clump in the original culture averaged 2.0.

* Penicillin G used in this experiment was lot F-20676, generously supplied by The Squibb Institute for Medical Research.

‡ *Cf.* footnote ‡, Table I.

§ "In the 2 groups receiving the largest inocula, preliminary estimates were made using Kärber's method (9). These were then put through one cycle of computation of the maximum likelihood probit analysis procedure after Garwood (*cf.* (15)). In the 2 groups receiving the smallest inocula, natural survival rates of 5 and 10 per cent, respectively, were arbitrarily assumed. Observed rates were adjusted for those natural survival rates, using Abbott's formula (*cf.* (15)). After obtaining preliminary estimates, maximum likelihood computations were made, using Finney's natural response adjusted weighting coefficients (15). In group 3, inoculated with 1,750 organisms, standard errors (indicated by **) have been adjusted upward because of the significant value of χ^2." (Mantel.)

‖ The slope constants (increase in probits of survivors per 10-fold increase in dosage) were 3.4±0.49, 2.78±0.44, 4.0±1.79, and 0.93±0.23 in groups 1, 2, 3, and 4, respectively.

¶ Of 10 control mice inoculated with a 1:10 dilution of this suspension, or an average of 1.7 bacterial clumps, 6 died and 4 survived. In view of the fact that, because of sample variation, some mice would have received no organisms, this means that an inoculum of 1.7 clumps almost regularly resulted in a fatal infection.

In Experiment I of Table I, with inocula of 15, 1,950, 200,000, and 23,800,000 bacterial clumps[4] the CD_{50} doses were 4.8, 38, 260, and 470 mg. per kg. The varying inocula in this experiment represented serial 100-fold dilutions of the same stock suspension.

When a type III strain of *Diplococcus pneumoniae* was inoculated intramuscularly in mice, the curative doses[5] (CD_{50}) after inocula of 100, 10,000, and 1,000,000 bacteria (not clumps) were 1.95 (± 0.27), 37 (± 5.5), and 106 (± 29) mg. per kg., respectively (Table III). With a group A β-hemolytic streptococcus (strain C-203), the curative doses in mice after intramuscular inocula of 100, 10,000, and 1,000,000 organisms were 0.35 (± 0.09), 22 (± 3.9), and 51 (± 12) mg. per kg. (*cf.* Table III). The details of these experiments are given in a following paper.

Similar experiments were carried out with a group B β-hemolytic streptococcus, highly virulent for mice. As shown in Table II, after the intraperitoneal inoculation of 17, 1,750, 180,000, and 2,235,000 bacterial chains, averaging 2.0 organisms per chain, the curative doses of penicillin G (again administered as a single intramuscular injection in aqueous solution) were 2.8, 139, 339, and 424 mg. per kg., respectively, with the standard errors indicated in Table II.

Fig. 1 and Table III summarize these effects of the size of the inoculum on the curative dose of penicillin in experimental infections with *T. pallidum*, *Diplococcus pneumoniae* types I and III, and β-hemolytic streptococci of groups A and B. The smaller doses required to abort infection after intramuscular as compared with intraperitoneal inoculation are particularly to be noted. The even smaller doses of penicillin which sufficed to cure the syphilitic infection are referable primarily to the fact that the penicillin used was a suspension in oil and beeswax rather than an aqueous solution.

2. Effect of the Age of the Infection on the Curative Dose of Penicillin.—It has been shown (5) that in syphilitic infection of rabbits, the curative dose of penicillin, administered as a single injection of the peanut oil-beeswax suspension, increases continuously during the incubation period of the disease. When rabbits were treated 4 hours, 4 days, 2 weeks, and 6 weeks after intradermal inoculation with 2,000 organisms, the curative doses (CD_{50}) were approximately 860, 570, 4,000, and 15,100 units per kg., respectively; and after intratesticular inoculation the corresponding values were 1,400, 2,400, 13,600, and 59,000 units per kg. (*cf.* Fig. 2). The progressive increase in the

[4] Although there were no measurements of the average number of organisms per chain in the suspensions used in thise experiment, similar cultures of the same strain have consisted largely of discrete diplococci. The number of such chains in each dilution was determined by plating out in blood agar.

[5] In the experiments with small numbers of *Diplococcus pneumoniae* type III and the group A hemolytic streptococcus, the empirical response to treatment was adjusted by Abbott's formula on the assumption of 10 per cent natural survivors (*cf.* footnote 2, page 597).

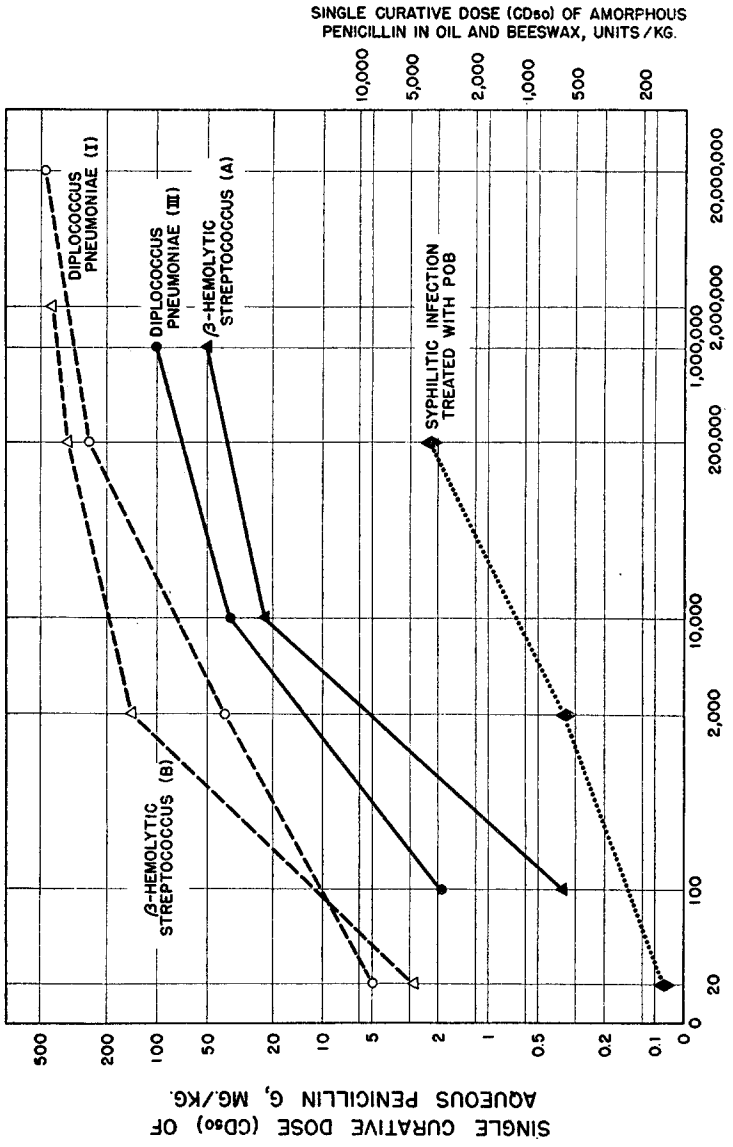

FIG. 1. The effect of the size of the inoculum on the curative dose of penicillin in a number of experimental infections.

	Animal species	Route of inoculation
Diplococcus pneumoniae, type I	Mice	Intraperitoneal
Diplococcus pneumoniae, type III	Mice	Intramuscular
β-Hemolytic streptococcus (A)	Mice	Intramuscular
β-Hemolytic streptococcus (B)	Mice	Intraperitoneal

size of the curative dose during the first 2 weeks, and before the development of the primary lesion, presumably reflects the progressive multiplication of the organisms; and the slow rate of that increase reflects the slow rate at which *T. pallidum* multiplies *in vivo* (10, 11).

A similar experiment with a group B β-hemolytic streptococcus, also studied in rabbits, is summarized in Table IV and Fig. 2. Two organisms of this strain

TABLE III

Effect of the Size of the Inoculum on the Curative Dose of (Intramuscular) Penicillin in Experimental Infections

(Summary of all experiments)

Infecting organism		Route of inoculation	Approximate No. of organisms inoculated						
			20	100	2,000	10,000	200,000	1,000,000	2,000,000
			Single curative dose, CD_{50}, of penicillin G						
			mg./kg.	*mg./kg.*	*mg./kg.*	*mg./kg.*	*mg./kg.*	*mg./kg.*	*mg./kg.*
Diplococcus pneumoniae (mice)	Type I	Intraperitoneal	4.8		38		260		470
			10.3		94		280		1,230
	Type III	Intramuscular		1.95		37		106	
β-Hemolytic streptococcus (mice)	Group A	Intramuscular		0.35		22		51	
	Group B	Intraperitoneal	2.8		139		339		424
*T. pallidum** (rabbits)		Intracutaneous	0.09		0.34		2.2		

* The syphilitic rabbits were treated with an amorphous mixture of penicillins in oil and beeswax instead of aqueous penicillin G as in the other experiments of this table, and the much smaller curative doses are in large part a reflection of that fact. The curative doses in units per kg. have been expressed in mg. per kg. on the assumption of an activity of 1,667 units per mg. Since penicillin G is the most active of all the penicillins so far tested against *T. pallidum*, the curative doses given in the table are probably larger than they would have been with pure penicillin G. The CD_{50} values indicated in the table have been recalculated from previously published data (5) by the method of Litchfield and Fertig.

injected intramuscularly regularly caused a fatal infection. When rabbits were inoculated with 2,000 organisms, and treated after 0, 1½, and 6 hours with a single intramuscular injection of penicillin G in aqueous solution, the amount necessary to cure the infection in half the animals increased continuously from 0.30 to 0.57 to 38.6 mg. per kg., respectively. After 24 hours, the infection had progressed to the point that even in the largest doses tolerated by the rabbit,

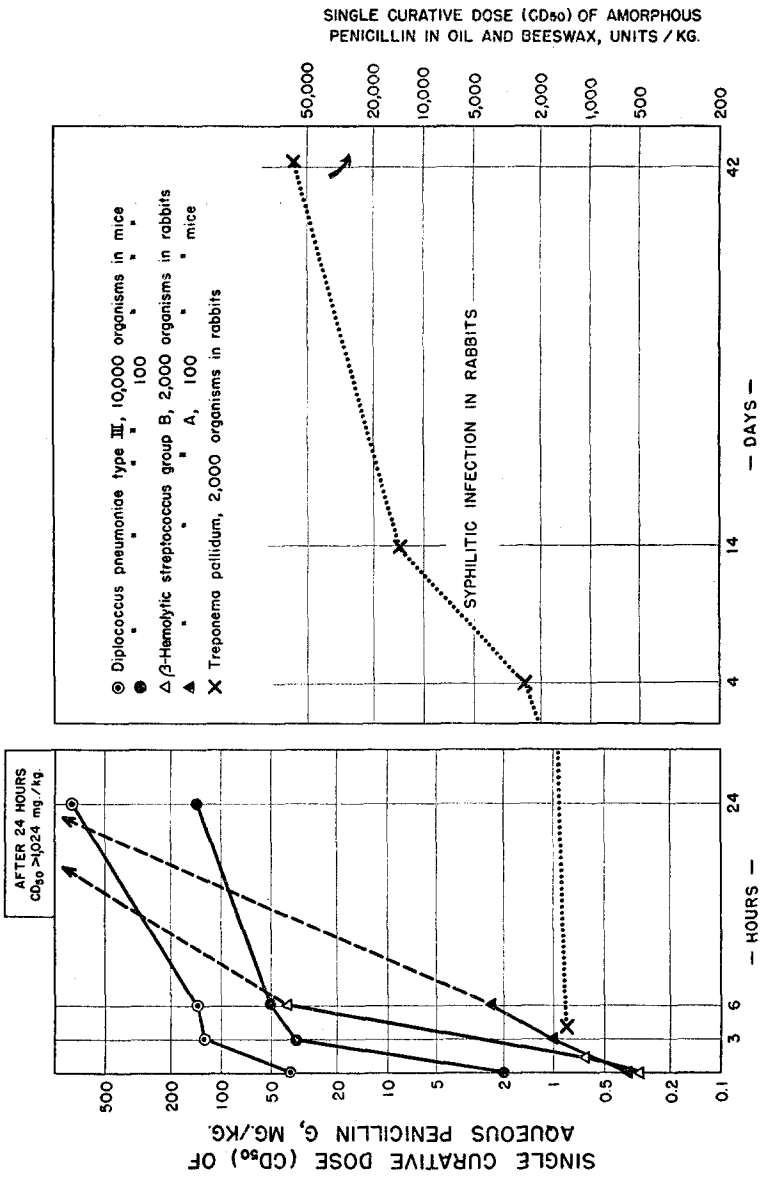

FIG. 2. The effect of the age of the infection on the curative dose of penicillin in a number of experimental infections. All the organisms were inoculated intramuscularly except *T. pallidum*, which was inoculated intratesticularly.

a single dose of penicillin G did not suffice to effect cure. With the C-203 strain of group A β-hemolytic streptococci inoculated intramuscularly in mice, the CD_{50} immediately after inoculation with 100 organisms was 0.35 ± 0.09

TABLE IV

*Effect of the Age of the Infection on the Curative Dose of Penicillin G in Rabbits Infected with Group B β-Hemolytic Streptococci**

Dosage of penicillin	Interval between inoculation and treatment			
	0 hr.	1½ hrs.	6 hrs.	24 hrs.
	Proportion of animals that died			
mg./kg.				
1,024				4/5
512				4/5
256			0/2	3/5
128			0/6	5/5
64			2/6	4/5
32			5/6	
16			4/6	
8			6/6	
4	0/2	0/5	5/5	
1	1/6	0/6		
1/2	0/6	4/6		
1/4	3/6	6/6		
1/8	6/6	5/5		
1/16	5/5			
Dose which cured half of animals, *mg./kg.* ± *standard error*‡....	0.30±0.06	0.57±0.13	38.6±7.6	>1,024

* 2,000 organisms inoculated intramuscularly; treatment also intramuscular, at a different site, with a single injection of aqueous penicillin G.

‡ The slope constants (increase in probits of survivors per 10-fold increase in dosage) in the animals treated at time zero and 6 hours after inoculation were 3.44±1.0 and 2.83±0.81. The common slope fitted to the first three groups of the table and used in the calculation of the standard error was 3.59±0.64.

mg. per kg.; but this had increased to 2.3 ± 0.37 mg. per kg. after 6 hours, and to >1,024 mg. per kg. after 24 hours[6] (Table VI).

In pneumococcal infections of white mice also, the dosage of penicillin necessary to terminate the infection increased rapidly with the duration of the

[6] The slope constants in this experiment were uniformly low. As the age of the infection increased from 0, 3, to 6 hours, these slope constants (increase in probits of survivors per 10-fold increase in dosage) increased progressively from 0.72 ± 0.1 to 1.3 ± 0.17, to 1.54 ± 0.21.

TABLE V

Effect of the Age of the Infection on the Curative Dose of Penicillin G in Mice Inoculated with Diplococcus pneumoniae, Type III

	Dosage of penicillin	Interval between inoculation and treatment			
		0 hr.	3 hrs.	6 hrs.	24 hrs.
		Proportion of animals that died*			
	mg./kg.				
	1,024				11/42
	512			0/15	13/60
	256			0/15	11/45
Experiment I	128		0/15	1/15	26/45
	64		2/15	6/15	20/45
(100 organisms inoculated)	32	0/15	7/15	10/15	29/45
	16	1/15	12/15	12/15	22/30
	8	1/30	10/15	13/15	8/15
	4	7/30	14/15		
	2	12/30			
	1	20/30			
	½	26/30			
Curative dose (CD_{50}) of penicillin, *mg./kg.* ± *standard error*		1.95±0.27	35±6	50±8.5	140±29
	4,096				14/19
	2,048				9/30
	1,024				20/50
	512	1/15	1/15	1/15	25/45
	256	2/30	3/15	5/45	20/35
Experiment II	128	5/45	16/30	12/30	20/30
	64	14/30	9/15	40/45	5/5
(10,000 organisms inoculated)	32	13/30	13/15	11/15	
	16	22/35	10/15	5/5	
	8	22/30			
	4	18/20			
Curative dose (CD_{50}) of penicillin, *mg./kg.* ± *standard error*		37±6	127±29	135±16	777±300

Organisms inoculated intramuscularly; treatment also intramuscular, at a different site, with a single injection of aqueous penicillin. The data here summarized represent a composite of 9 experiments carried out at different times.

* In control mice inoculated with 1,000,000, 10,000, 1,000, 100, 10, and 1 bacteria (determined by direct microscopic count and appropriate dilution), the mortality was 28/28, 64/75, 30/40, 102/117, 96/120, and 58/115, respectively. The plate counts on these inocula averaged 900,000, 10,100, 1,090, 98, 10, and 1.3, respectively, with approximately a 100 per cent variation around these means. In the experiments with 100 and 10,000 organisms, "the empirical response to treatment was adjusted by Abbott's formula on the arbitrary assumption of 10 per cent natural survivors (*cf.* footnote 2, page 597).

infection. With a type III organism, the CD_{50} dosages at 0, 3, 6, and 24 hours after intramuscular inoculation with 100 organisms were 1.95, 35, 50, and 140 mg. per kg. (Table V and Fig. 2). After similar inoculation with 10,000 organisms, the curative doses at 0, 3, 6, and 24 hours were 37, 127, 135, and 777 mg. per kg., the latter value approaching the maximum tolerated dose of penicillin G in white mice.

TABLE VI

Effect of the Age of the Infection on the Curative Dose of Penicillin G

(Summary of all experiments)

Infecting organism*	Animal species	No. of organisms inoculated	Interval between inoculation and treatment							
			0	1½ hrs.	3 hrs.	6 hrs.	24 hrs.	4 days	14 days	42 days
			Single curative dose of penicillin							
			mg./kg.	mg./kg.	mg./kg.	mg./kg.	mg./kg.	mg./kg.	mg./kg.	mg./kg.
Diplococcus pneumoniae, type III	Mice	100	1.95		35	50	140			
		10,000	37		127	135	777			
Streptococcus pyogenes Group A (C-203)	Mice	100	0.35		1.0	2.3	>1,024			
Group B	Rabbits	2,000	0.30	0.57	—	38.6	>1,024			
					4 hrs.					
T. pallidum‡ Intratesticular	Rabbits	2,000			0.85			1.44	8.2	35
Intracutaneous		2,000			0.52			0.34	2.4	9

* All inoculated intramuscularly except *T. pallidum*.

‡ The CD_{50} values have been recalculated from previously published data (5) by the method of Litchfield and Fertig. The curative doses of penicillin administered as a single injection in oil and beeswax are here expressed as mg. per kg. on the assumption of 1,667 units per mg. (*cf.* footnote * of Table III).

The foregoing data demonstrating the effect of the age of the infection on the curative dose of penicillin are summarized in Table VI and Fig. 2.

DISCUSSION

It has been shown in a variety of experimental infections, and in both mice and rabbits, that the curative dose of penicillin increases with the size of the inoculum, and increases also with the age of the infection. In both cases, and primarily because of the larger number of bacteria in the infected animal,

penicillin must remain at effective levels for a longer aggregate time[1] in order to effect cure. This point is discussed in greater detail in a following paper.

As would be expected, those bacterial species which multiply rapidly showed a correspondingly rapid increase in the curative dose of penicillin with the duration of infection. Thus, for the strains of pneumococci and streptococci here studied, the generation time *in vitro* varied between 23 and 30 minutes. In mice inoculated intramuscularly with 100 type III pneumococci, the curative doses of penicillin injected 0, 3, 6, and 24 hours after inoculation were 1.95, 35, 50, and 140 mg. per kg., respectively. A similarly rapid increase in the curative dose was observed in rabbits inoculated with a group B β-hemolytic streptococcus, and a somewhat slower increase in mice inoculated with a group A streptococcus. In both of the latter infections, within 24 hours after inoculation with a relatively small number of organisms, no single dose of penicillin, up to the toxic level of the drug, sufficed to effect cure.

In contrast to this group of organisms, the generation time of pathogenic *T. pallidum in vivo* has been estimated from two different experimental approaches to be on the order of 30 hours (10, 11). Corresponding to that slow rate of multiplication, there was no significant difference in the curative dose when penicillin was injected 4 hours or 4 days after incubation, and only a 7-fold increase after 2 weeks.

The Feasibility of the Prophylactic Use of Penicillin.—The present experiments suggest the possibility that penicillin may have an important application in the prevention as well as in the treatment of bacterial infections in man. With known or suspected exposure to an infection involving an organism susceptible to penicillin, it may be possible early in the incubation period to abort the contact case with a single relatively small dose of penicillin. In confirmation of that possibility, it has been shown that a single tablet of penicillin (100,000 to 250,000 units) taken by mouth within several hours after exposure suffices to prevent gonococcal infection in men, aborting most of the infections which otherwise developed in a control group similarly exposed (12).

It has also been found that if the contacts of cases with early infectious syphilis are given a single 600,000 unit dose of penicillin in oil and beeswax, the proportion who develop syphilitic infection is greatly reduced (13), this despite the fact that several weeks had usually elapsed since the original exposure. There is as yet no information as to the amount of penicillin which would suffice to abort syphilitic infection if the drug were injected or taken by mouth within 12 to 24 hours after possible exposure.

In epidemic outbreaks of acute infections occurring in closely confined groups and caused by organisms susceptible to penicillin, it may prove possible to halt the spread of the infection by the daily peroral administration to the entire susceptible exposed group of one or two tablets of penicillin. As in the prevention of gonococcal infection, the success of the procedure in such groups

would depend on the ability of a small dose of penicillin, which provides effectively bactericidal levels for only a brief period, to destroy the relatively small number of organisms which presumably serve as the initial inoculum. The possible dangers of such general prophylactic use of penicillin are considered elsewhere (14).

SUMMARY

The amount of penicillin which suffices to abort pneumococcal and streptococcal infections in white mice and rabbits, as well as syphilitic infection in rabbits, has been shown to increase markedly with the number of organisms inoculated. The curative dose increases also with the age of the infection, presumably owing to the interim increase in the number of organisms in the nfected host.

The necessity for the larger doses of penicillin is attributed to the longer itime for which effectively bactericidal concentrations of penicillin must then be provided in order to kill the larger number of organisms.

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