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

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When penicillin is added to a suspension of spirochetes (*S. pallida*, Reiter), the number of viable organisms has been shown to fall off at a rate which is largely independent of the number of organisms, and which varies with the concentration of penicillin in the range 0.01 to 1 unit per cc. (1). If the initial number of organisms is increased, a larger proportion must be killed in order to prevent growth in subculture. It is clear that the penicillin must then be allowed to act for a longer period of time, or, the time factor remaining constant, the concentration of penicillin must be increased, within the range which affects the rate of its spirocheticidal action.

These elementary considerations probably apply to most organisms which are susceptible to penicillin *in vitro*. To a certain extent they are probably also valid *in vivo*. With acute bacterial infections, the longer one waits before administering penicillin, the more organisms must be killed in order to effect cure, and the more effectively must the penicillin be administered, either by giving more injections, or by increasing the size of the individual dose. By the same token, in experimental infections, if the size of the inoculum is progressively increased, more penicillin should be necessary for cure.

As will be shown in the present paper, precisely these relationships have been found to obtain in experimental syphilis. If rabbits are inoculated with varying numbers of organisms, there is a corresponding variation in the amount of penicillin necessary to abort the infection when administered 4 days after inoculation. Conversely, if the size of the inoculum is fixed, and if the animals are treated at varying intervals after their inoculation, there is a progressive increase in the amount of penicillin necessary to abort the infection, presumably because of the interim multiplication of spirochetes *in vivo*.

The conditions which most favor the drug are therefore a small inoculum and treatment during the incubation period, before the organisms have multiplied to a significant degree. Under such circumstances, extraordinarily small doses of penicillin suffice to abort syphilitic infection in rabbits. Even with inocula probably greater than those operative in the natural infection, the

abortive dose of penicillin is so small as to suggest that the drug can be used for the prophylaxis of syphilis in man.

EXPERIMENTAL

Methods and Materials

The Nichols strain of *S. pallida* was used throughout. The inocula were obtained by the emulsification of rabbit testicular chancres in 50 per cent rabbit serum (2–4), and the number of organisms per cubic centimeter determined by direct enumeration as described by Morgan and Vryonis ((5) *cf.* (6)). Dilutions in 50 per cent serum were prepared to contain concentrations of 10^6, 10^4, and 10^2 organisms per cc., and 0.2 cc. of the appropriate dilution was injected intracutaneously or intratesticularly. Penicillin treatment was given as a single intramuscular injection of a suspension in oil and beeswax (4.8 to 5.3 per cent, weight to volume), administered 4 hours, 4 days, or 2 weeks after inoculation. At each of these intervals, groups of four to eleven animals were treated with various doses, to determine that amount of penicillin which sufficed to prevent the development of syphilitic lesions This abortive dose was compared with that necessary to cure animals treated 6 weeks after inoculation, and after a darkfield-positive lesion had developed at the site of inoculation.

In order to detect asymptomatic infection in animals in which the disease had perhaps been merely suppressed and not aborted by penicillin, lymph node transfers were carried out 6 months after treatment in a large proportion of the surviving animals. As previously indicated (15) such asymptomatic infection proved uncommon (*cf.* page 431), no matter what the size of the inoculum, and no matter what the dosage of penicillin. In normal animals inoculated with *S. pallida*, the failure of a darkfield-positive lesion to develop could be taken as *prima facie* evidence that the animal had not been infected; and in animals inoculated with a certainly infective dose of spirochetes, and then treated with penicillin, the non-appearance of a lesion was a reliable indication that the disease had actually been aborted and not merely suppressed.

Relation of the Size of the Inoculum to the Curative (Abortive) Dose of Penicillin

Rabbits were inoculated intradermally into the skin of the back with 0.2 cc. of a spirochetal suspension diluted to contain 10^6, 10^4, or 10^2 organisms per cc., so that the inoculum consisted of 2×10^5, 2×10^3, or 2×10 organisms. 4 days later, the animals were treated with varying doses of penicillin, administered intramuscularly as a single injection of the suspension in oil and beeswax (7). The results are given in detail in Table I and are graphically summarized in Fig. 1.

In the control groups, not receiving penicillin, all of four rabbits inoculated with 200,000 spirochetes, twenty-one of twenty-three rabbits inoculated with 2,000 spirochetes, and seven of ten rabbits inoculated with twenty spirochetes developed darkfield-positive lesions at the site of inoculation within average periods of 18, 38, and 37 days, respectively. The administration of penicillin 4 days after inoculation effectively aborted the disease in all three groups, even those inoculated with 200,000 organisms; but the preventive dosage varied with the size of the inoculum. As the number of organisms inoculated was decreased in 100-fold steps from 200,000 to 2,000 to 20 spirochetes, the approximate amounts of penicillin necessary to abort the infection in half the animals

(PD_{50} in Fig. 1) fell from 3,500 to 500 to 200 units per kg., and the PD_{90} dosage, at which 90 per cent of the animals were protected, fell from 8,000 to 2,000 to 300 units per kg.

TABLE I

Relation of the Size of the Inoculum to the Curative (Abortive) Dose of Penicillin in Experimental Syphilis

Rabbits were inoculated intradermally and treated 4 days later with a single intramuscular injection of a suspension in peanut oil and beeswax.

No. of spirochetes inoculated intradermally	Penicillin	No. rabbits	Developed syphilitic lesion	Result of lymph node transfer in animals which did not develop lesion		No. of animals protected	Animals protected*	Protective dose of penicillin	
				No. tested	Infectious			PD_{50} (50 per cent of animals)	PD_{90} (90 per cent of animals)
	units/kg.						*per cent*	*units/kg.*	*units/kg.*
	32,000	4	0	4	0	4	100		
	16,000	9	2	5	0	7	78 (90)		
	8,000	4	0	3	0	4	100 (82)		
200,000	4,000	4	1	3	0	3	75 (62)	3,500	8,000±
	2,000	6	5	1	0	1	17 (20)		
	1,000	6	5	—	—	1	17 (7)		
	0 (Control)	4	4	—	—	—	—		
	16,000	6	0	2	0	6	100		
	4,000	5	0	5	0	5	100		
	2,000	8	2	3	0	6	75 (90)		
2,000	1,000	7	2	5	0	5	71 (75)	500	2,000
	500	11	4	—	—	7	64 (47)		
	250	5	5	—	—	0	0		
	0 (Control)	23	21	—	—	—	—		
	16,000	6	0	2	0	6	100		
	2,000	6	0	1	0	6	100		
	1,000	4	0	4	0	4	100		
20	500	10	1	4	0	9	90 (93)	200±	500±
	250	4	0	3	0	4	100 (80)		
	125	3	3	—	1	0	0		
	0 (Control)	10	7	—	—	—	—		

* The numbers in parenthesis are the percentages of animals protected, recalculated after the method of Reed and Muench (8).

Relation of the Age of the Infection and the Site of Inoculation to the Curative (Abortive) Dose of Penicillin

In the experiments of the preceding section, it had been shown that the dosage of penicillin necessary to abort syphilitic infection in half the animals when

given 4 days after inoculation increased from 200 to 500 to 3,500 units per kg. as the intracutaneous inoculum was increased from 20 to 2,000 to 200,000 organisms. One could reasonably have anticipated that, in animals receiving a fixed inoculum, the curative dose would increase progressively, at least during the early stages of the infection, as the organisms multiplied *in vivo*.

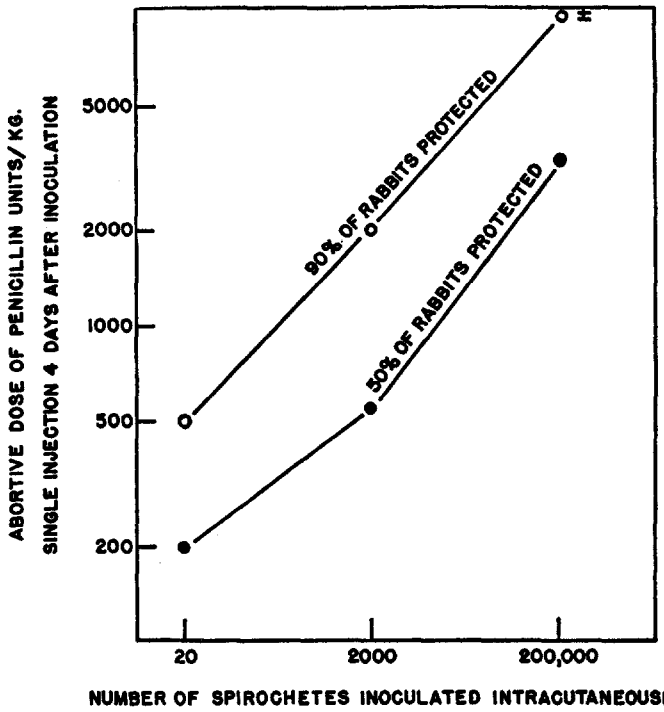

FIG. 1. The correlation between the number of spirochetes inoculated, and the amount of penicillin required to abort the infection. Rabbits were inoculated intracutaneously. Penicillin was administered 4 days later, as a single intramuscular injection in peanut oil and beeswax.

Two experimental groups were studied, one inoculated intracutaneously, in the middle of the back, and one intratesticularly. The inoculum was fixed at 2,000 organisms (0.2 cc. of a suspension containing 10^4 organisms). At varying periods after inoculation (4 hours, 4 days, 2 weeks, or 6 weeks), the animals were treated with penicillin, administered as a single injection of a suspension in oil and beeswax.

The data are given in Tables II and III, and are graphically summarized in Fig. 2. In the animals inoculated intracutaneously, and treated 4 hours later, 1000 units per kg. of penicillin aborted the infection in half the animals, and 2,500 units per kg. protected 90 per cent. 4 days later, the abortive doses were essentially unchanged (PD_{50} and PD_{90} levels approximately 500 and 2,000 units per kg.). However, when the penicillin was given 2 weeks after inocula-

TABLE II

Relation of the Age of the Infection to the Abortive (Curative) Dose of Penicillin in Experimental Syphilis

Rabbits were inoculated intradermally with 2,000 spirochetes. At varying intervals thereafter, penicillin was given as a single intramuscular injection of a suspension in peanut oil and beeswax.

Time when penicillin was administered after inoculation	Penicillin dosage	No. rabbits tested	Developed syphilitic lesion despite penicillin	Results of lymph node transfer on animals apparently protected		No. of animals protected	Animals protected*	Protective dose of penicillin	
				No. tested	Infectious			PD_{50} (50 per cent of animals protected)	PD_{90} (90 per cent of animals protected)
	units/kg.						*per cent*	*units/kg.*	*units/kg.*
	16,000	5	0	4	0	5	100		
	4,000	6	0	6	0	6	100		
4 hrs.	2,000	9	2	3	0	7	78 (85)	1,000	2,500
	1,000	6	2	3	0	4	67 (50)		
	500	7	7	—	—	0	0		
	16,000	6	0	2	0	6	100		
	4,000	5	0	5	0	5	100		
4 days	2,000	8	2	3	0	6	75 (90)		
	1,000	7	2	5	0	5	71 (75)	500	2,000
	500	11	4	—	—	7	64 (47)		
	250	5	5	—	—	0	0		
	64,000	6	0	4	0	6	100		
	32,000	6	1	5	0	5	83 (95)		
	16,000	6	1	4	0	5	83 (87)		
2 wks.	8,000	6	2	4	0	4	67 (67)	6,000	20,000
	4,000	6	3	3	0	3	50 (36)		
	2,000	5	5	—	—	0	0 (8)		
	1,000	5	4	1	0	1	20 (6)		
	320,000	4	0	4	0	4	100		
	160,000	6	0	5	0	6	100		
6 wks.‡	80,000	6	0	5	1	5	84 (94)		
	40,000	7	1	6	1	5	71 (80)	20,000	70,000
	20,000	7	1	6	3	3	43 (50)		
	10,000	7	1	5	2	4	57 (29)		

*Numbers in parenthesis indicate percentage of animals protected, recalculated after Reed and Muench (8).

‡ All but five of thirty-seven animals in this group had developed darkfield-positive lesions before injection of penicillin. This section therefore refers to the cure of syphilis by penicillin after the development of obvious syphilis, rather than the abortion of the disease in the incubation period.

TABLE III

Relation of the Age of the Infection to the Abortive (Curative) Dose of Penicillin in Experimental Syphilis

Rabbits were inoculated intratesticularly with 2,000 spirochetes. At varying intervals thereafter, penicillin was given as a single intramuscular injection of a suspension in peanut oil and beeswax.

Time when penicillin was administered after inoculation	Penicillin dosage	No. rabbits tested	Developed syphilitic lesion despite penicillin	Results of lymph node transfer on animals apparently protected		No. of animals protected	Animals protected*	Protective dose of penicillin	
				No. tested	Infectious			PD_{50} (50 per cent of animals protected)	PD_{90} (90 per cent of animals protected)
	units/kg.						*per cent*	*units/kg.*	*units/kg.*
	16,000	6	0	6	0	6	100		
	8,000	6	0	5	0	6	100		
4 hrs.	4,000	6	0	4	0	6	100	1,500	3,500
	2,000	6	2	2	0	4	67 (71)		
	1,000	4	3	1	0	3	25 (17)		
	16,000	5	0	4	0	5	100		
	8,000	5	0	5	0	5	100		
4 days	4,000	6	0	5	0	6	100	2,000	3,500
	2,000	5	3	2	0	2	40		
	1,000	4	4	—	—	0	0		
	64,000	6	0	6	0	6	100		
	32,000	6	3	3	0	3	50 (77)		
2 wks.	16,000	5	1	4	0	4	80 (64)	14,000	50,000
	8,000	6	4	2	0	2	33 (20)		
	4,000	5	4	1	1‡	0	0		
	160,000	5	1	3	0	4	80 (89)		
	80,000	5	0	5	1	4	80 (67)		
6 wks.§	40,000	5	1	4	4	0	0	65,000	160,000
	20,000	5	2	3	3	0	0		
	10,000	6	0	6	6	0	0		

* Numbers in parenthesis indicate percentage of animals protected, recalculated after Reed and Muench (8).

‡ This is the only instance in a total of 102 rabbits tested, in which an animal which failed to develop a lesion after abortive treatment with penicillin was found to have an asymptomatic infection. In all the others, lymph node transfers were negative. One must conclude that the infection had been actually aborted, and not merely suppressed.

§ All twenty-six animals in this group (and twenty-one of twenty-three untreated controls) had developed darkfield-positive lesions by the end of the 6th week. This section therefore refers to the *cure* of syphilis by penicillin after the disease had been established, rather than the abortion of the infection in the incubation period.

tion, the 50 per cent protective dose had increased to approximately 6,000 units per kg., and it required 16,000 units per kg. to protect nine-tenths of the

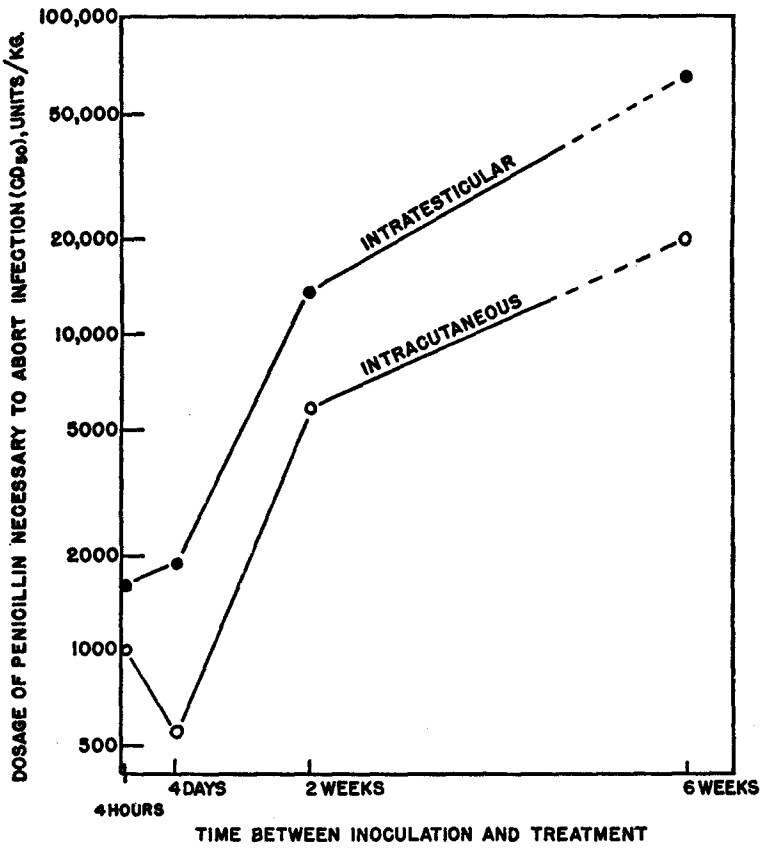

FIG. 2. Relation of the age of the infection to the curative (abortive) dose of penicillin. Rabbits were inoculated intracutaneously or intratesticularly with 2×10^3 organisms. At varying periods after inoculation, penicillin was administered as a single intramuscular injection of a suspension in peanut oil and beeswax. The dashed portion of the curves indicates that the animals had developed darkfield-positive lesions by the 6th week, and that treatment at that time was curative rather than abortive.

animals, doses five to eight times greater than those which sufficed 4 hours or 4 days after inoculation.

In all three of these groups, treated 4 hours, 4 days, or 2 weeks after inoculation, the animals were in the incubation period of the disease at the time of treatment. By the 6th week, however, thirty-two of the thirty-seven animals scheduled for treatment at that time period had developed darkfield-positive lesions; and at that time it required 20,000 units per kg. to cure half the animals,

and 70,000 units per kg. to cure 90 per cent, three to four times more than the doses which sufficed at 2 weeks, and thirty times more than the abortive dose at 4 days.

A similar result was obtained in the animals inoculated intratesticularly instead of intradermally. Again, the abortive dose of penicillin was essentially the same whether given 4 hours or 4 days after inoculation; by the end of the 2nd week it had risen sharply, the CD_{50} dose from 1,500 to 2,000 units per kg. to 14,000, and the CD_{90} dose from 3,500 units per kg. to 50,000, a seven- to fourteenfold increase in animals still asymptomatic. By the end of the 6th week the twenty-six animals scheduled for treatment had all developed darkfield-positive lesions. The curative dose at that time period in those

TABLE IV

The Abortive (Curative) Dose of Penicillin in Rabbit Syphilis in Relation to the Age of the Infection and the Route of Inoculation

Inoculum fixed at 2×10^8 organisms

Time between inoculation and treatment	Intracutaneous inoculation		Intratesticular inoculation	
	PD_{50} (Half of animals protected)	PD_{90} (90 per cent of animals protected)	PD_{50} (Half of animals protected)	PD_{90} (90 per cent of animals protected)
4 hrs.	1000	2500	1500	3500
4 days	500	2000	2000	3500
2 wks.	6000	20,000	14,000	50,000
6 wks.*	20,000	70,000	65,000	160,000

* Most of the animals in this group had already developed a darkfield-positive lesion: the doses given are those necessary to cure the established disease, rather than abort the infection in the incubation period.

animals was now 65,000 units per kg., and on the order of 160,000 units per kg. were necessary to cure 90 per cent of the animals, doses three to five times more than were necessary at 2 weeks, and approximately forty times more than sufficed at 4 days.

It is of interest that the abortive and curative doses of penicillin in animals inoculated intratesticularly were regularly two to four times greater than in animals simultaneously inoculated with the same suspension, but intracutaneously. This difference was observed in every group studied, was quantitatively of the same order of magnitude throughout, and is probably significant (*cf.* Table IV and Fig. 2).

This is perhaps related to the recent finding (6) that *S. pallida* is more infectious for rabbits when inoculated intratesticularly than it is when inoculated intradermally. As there reported, even two spirochetes regularly produced the disease when introduced into the testis; while the percentage of successful

inoculations with 2, 20, 200, and 2,000 organisms injected intradermally was 48, 73, 86, and 93 per cent, respectively.

Asymptomatic Infection and Asymptomatic Relapse

It is to be emphasized that in those animals which failed to develop darkfield-positive lesions because of a single injection of penicillin given 4 hours, 4 days, or 2 weeks after their inoculation, the disease had been actually aborted, and not merely suppressed. Of the total of 132 rabbits so protected in the experiments of Tables II and III (fifty-seven inoculated intratesticularly, and seventy-five intradermally, all with 2×10^8 spirochetes), it was possible to carry out lymph node transfers 6 months later in 102. Forty-six of these came from dosage groups at which other animals, similarly treated, had nevertheless developed darkfield-positive lesions. Particularly in those forty-six animals, one might have anticipated a high proportion of asymptomatic infections, in which penicillin had prevented the appearance of the lesion, but not the development of a systemic infection. Instead, in the entire series of 102 rabbits, there was only one instance of an asymptomatic infection (footnote 3 of Table III); the remaining 101 lymph node transfers were negative. This corroborates the findings in two other studies from this laboratory (6, 15). Provided rabbits are examined carefully and repeatedly for the necessary period of 10 to 15 weeks after inoculation, asymptomatic infection is so uncommon, even in those given subcurative doses of penicillin during the incubation period, that it can safely be ignored as a complicating factor.

However, the foregoing discussion applies only to animals receiving penicillin during the incubation period of the disease, before it had become apparent. A quite different picture was observed in animals similarly inoculated, but treated with penicillin 6 weeks later, after the development of a darkfield-positive lesion. Of the sixty-three animals so treated, seven were obvious treatment failures, in that motile spirochetes either did not disappear from the lesion, or reappeared days, weeks, or even months after treatment. In the remaining fifty-six rabbits, the testicular or skin lesion healed, and there were no obvious signs of relapse during the 6 months of observation. Nevertheless, when fifty-two of these animals were tested by lymph node transfer 6 months after treatment, no less than twenty-one were found to harbor spirochetes in the lymph nodes, evidenced by the appearance of darkfield-positive lesions in the transfer animals.

Previous experience (2) has shown that the greater portion of these twenty-one failures of treatment would have been apparently cured if tested by lymph node transfer 6 weeks instead of 6 months after treatment. They may therefore be considered examples of asymptomatic relapse occurring after inadequate treatment. It follows from the preceding discussion that although asymptomatic *infection* is uncommon in rabbits given subcurative doses of penicillin during

the incubation period (4 hours to 2 weeks after inoculation), asymptomatic *relapse* is the usual type of failure in animals treated 6 weeks after inoculation, and after the development of a primary lesion.

The data of Table V emphasize this striking difference in the evidences of treatment failure in animals given penicillin at these two time periods. Of the sixty-five failures observed in animals treated in the incubation period (4 hours, 4 days, or 2 weeks after inoculation), sixty-four were obvious failures, evidenced by the development of a darkfield-positive lesion, and only one was an asymptomatic infection, demonstrated by lymph node transfer. In contrast,

TABLE V

The Marked Difference in the Incidence of Asymptomatic Treatment Failures in Rabbits Treated (a) during the Incubation Period (4 Hours to 2 Weeks after Inoculation) and (b) after the Development of Lesions (6 Weeks after Inoculation)

	Obvious infection (or relapse)	Asymptomatic infection (or relapse)	Total failures
Animals treated during incubation period*.....	64	1	65
Animals treated after development of lesions* ...	7	21	28

* As indicated in the following tabulation, the two groups had received comparable doses of penicillin. The widely discrepant incidence of asymptomatic treatment failure in the two series cannot be related to differences in the dosage level:

Time of treatment	Fractions of the CD_{50} dosage					
	½	½–1	½–1	1–2×	2–4×	4×
	No. of animals developing treatment failure					
4 hrs., 4 days, or 2 wks. after inoculation.....	4	14	25	12	8	1
6 wks. after inoculation.....	6	5	8	5	3	1

of the twenty-eight failures observed in rabbits treated 6 weeks after inoculation, and after the development of a primary lesion, only seven were obvious relapses, and twenty-one were asymptomatic, demonstrable only by lymph node transfer. As shown in the footnote to Table V, this difference could not be related to differences in the dosages of penicillin used in the two series (for obviously, small doses would be expected to predispose to symptomatic relapse, and large doses to asymptomatic relapse). Expressed as fractions of the CO_{50} level, there was a comparable distribution of penicillin dosages in the two series.

The significance of the experimental results is discussed on page 437.

DISCUSSION

The Curative (Abortive) Dose of Penicillin in Relation to the Age of the Infection and the Number of Organisms Inoculated

It has here been shown that the dosage of penicillin necessary to abort syphilitic infection in rabbits when given 4 days after inoculation increases pro-

gressively with the size of the inoculum. After an intramuscular inoculum of 200,000 spirochetes it required 15 to 50 times as much penicillin to abort the disease as was necessary with an inoculum of only twenty organisms (*cf.* Fig. 1). The converse has already been demonstrated (15): when a fixed dosage of penicillin was administered 3 days after inoculation, no infections were observed with inocula of up to 2,000 organisms; but a significant proportion of animals developed syphilitic infection after inoculation with 20,000 or 200,000 organisms.

It has further been shown in the present experiments that, with a fixed inoculum of 2,000 spirochetes, the preventive or curative dose of penicillin varies with the age of the infection. For a period of at least 4 days after inoculation it remained essentially unchanged. By the end of the 2nd week, however, it had increased five- to fourteenfold; and by the end of the 6th week, the curative dose, expressed either as the CD_{50} or CD_{90} level, averaged thirty to forty times that which would have sufficed to abort the infection if given within the first few days.

This progressive increase in the sterilizing dose of penicillin was not related to the development of lesions, since the largest percentage increase occurred between 4 days and 2 weeks after inoculation, and before lesions had developed. It is a reasonable surmise instead that it reflects the multiplication of organisms. If this interpretation is correct, then at least within the first 4 days after its intracutaneous or intratesticular inoculation, *S. pallida* has multiplied to so small an extent as not to affect the amount of penicillin required for cure. This may reflect an initial lag period, or it may indicate that, in general, *S. pallida* multiplies only slowly *in vivo*. The fact that the protective dose 2 weeks after the intracutaneous inoculation of 2,000 organisms was greater than that required to abort a 4 hour infection with 200,000 organisms (PD_{50} values 6,000 and 3,500 units per kg.), would indicate that there had been somewhat more than a 100-fold multiplication of organisms in the interim.

The rate of multiplication of *S. pallida in vivo*, considered in the light of these and other data, will be discussed in detail in a following paper (6), in which it is suggested that the division time for *S. pallida in vivo* may average approximately 30 hours in rabbits.

The curves of Fig. 2, relating the curative dose to the age of the infection, show no evidence that the peak had been reached at 6 weeks in animals inoculated with 2,000 spirochetes. However, in animals inoculated with *e.g.* 2,000,000 organisms instead of 2,000, in which the primary lesion is already manifest in 10 to 12 days, and reaches its peak in 3 to 4 weeks, one might find the curative dose of penicillin to be greatest at that time, and to fall off subsequently with the development of necrosis in the regressing lesion. It is of interest in this connection that Fleming (10) has found the curative dose of penicillin in rabbit syphilis treated 6 months after inoculation to be significantly lower than in animals similarly treated 6 weeks after inoculation.

Implications with Respect to Chemotherapeutic Assay in Experimental Syphilis: Age of Infection, Route of Infection, and Size of Inoculum

It is a necessary inference from the data here reported, and from the preceding discussion, that in assaying the chemotherapeutic activity of *e.g.* penicillin in experimental syphilis (*cf.* (9)), one must rigorously control both the age of the infection at the time of treatment, the size of the inoculum, and the route of infection.

The necessity for controlling the time factor requires no discussion, given the 30- to 40-fold difference in curative dose as between 4 days and 6 weeks, and the 3- to 5-fold difference as between 2 weeks and 6 weeks.

The importance of controlling the route of infection follows from the finding that in every experimental group here studied, whether treated at 4 hours, 4 days, 2 weeks, or 6 weeks after inoculation, before or after the appearance of the primary lesion, the curative dose of penicillin was greater in the animals inoculated intratesticularly than in those inoculated intracutaneously. In any one experiment, the differences would be within the degree of experimental error; but when the data are considered in their entirety, the consistent two- to three- fold difference in curative dose becomes significant, and introduces yet another variable which must be controlled in chemotherapeutic assays.

This difference probably reflects the fact that in the testis the organisms find a more favorable environment for survival and multiplication than they do in the skin. As will be discussed in a following paper (6), infection regularly results from the injection of two spirochetes into the testis; but 2, 20, 200, and even 2,000 organisms infected only 48, 73, 86, and 93 per cent of the animals on intradermal inoculation. Indeed, the paradoxical decrease in the curative dose when penicillin was given 4 hours and 4 days after intracutaneous inoculation, and which was ignored in the preceding discussion as being perhaps within the limit of experimental error, may have been a real difference, and may have reflected an initial decrease in the number of organisms surviving 4 days after intracutaneous inoculation.

The desirability of controlling the size of the inoculum rests on the possibility that with a large inoculum the number of organisms to be killed, and thus, the curative dose of penicillin, may reach its peak sooner than with a small inoculum, and the maximum value attained may also be greater. Under such circumstances, different curative doses might be observed if all the animals were treated at the same time; *e.g.*, 6 weeks after inoculation. A possible alternative would be to treat all the animals so long after their inoculation that the number of surviving organisms would have stablized at a level perhaps independent of the size of the original inoculum.

(It is not relevant to this discussion, but of interest, that the curative dose of mapharsen in rabbit syphilis does not vary with the number of organisms to the same degree as does that of penicillin. One of us has found the curative

dose of mapharsen, injected 1 hour after the inoculation of rabbits with 1,000 spirochetes, to be 2 mg. per kg., while the curative dose 6 weeks after inoculation with approximately 5 million organisms was 6 mg. per kg. This threefold difference in the curative dose of mapharsen, despite a 5,000-fold difference in inoculum, and despite a 1 hour to 6 week difference in the time of treatment, is to be contrasted with the 30- to 40- fold difference in the curative dose of penicillin caused by the time factor alone, the inoculum remaining constant.)

The Feasibility of a Rapid Assay of Antisyphilitic Agents Based on the Dosage Required to Abort the Infection during Its Incubation Period

The method in general use for the assay of antisyphilitic agents requires approximately 9 to 12 months for the completion of the preliminary orienting assay:—

(*a*) Approximately 6 weeks are allowed between the time of inoculation and treatment.

(*b*) Symptomatic cure is a wholly misleading criterion of therapeutic efficacy, and it is necessary to carry out lymph node transfers (*cf.* page 431). Such lymph node transfers give falsely negative results if carried out *e.g.*, 6 weeks after treatment (2), and it is customary to wait for 4 to 6 months.

(*c*) The animals inoculated with the lymph node emulsion must then be observed for a period of 2 to 3 months before being adjudged negative. The range of activity having thus been determined, another 9 to 12 month experiment is necessary for the final precise interpolations.

Rake and Dunham (11) have recently suggested that, instead of treating rabbits after the disease has been established, in which case lymph node transfers must be used as the criterion of cure, the animals be treated during the incubation period of the disease, and the suppression of the lesion used as the criterion of therapeutic activity. Such assays could be completed within a period of 2 to 3 months. The data here reported, and those described in a previous paper (15) show that the suppression of the lesion in the great majority of cases signifies actual sterilization of the host and cure. Asymptomatic infection is so uncommon that it can be disregarded as a complicating factor, and it is unnecessary to carry out lymph node transfers. The assay method of Rake and Dunham has the further advantage, at least with penicillin, of requiring far less drug, since the dosage necessary to abort the disease is one-twentieth to one-fortieth that necessary to cure the established infection.

One major reservation is, however, essential in considering the significance of assays based on the abortion of the disease rather than its cure. The possibility must be borne in mind that in the incubation period, and before the development of an inflammatory lesion, the treatment of the host may be analogous to a test tube experiment, and measure primarily the direct activity of the drug on the spirochete. In the established infection, however, the vulner-

ability of the organisms may have been modified in at least two major and opposing respects. The inflammatory reaction in the lesion, and the often almost cartilaginous nature of the tissue in which the spirochetes are embedded, may prevent the free diffusion of the drug, and protect the organisms; while the developing immune reaction in the host may contribute to their eradication. Since there is no assurance that different drugs would be identically affected by these two complicating factors, the relative abortive activity of a group of compounds administered during the incubation period may not necessarily be the same as their relative therapeutic activity in the established infection. Moreover, one cannot assume that the relative activities of two drugs in sterilizing the host of *e.g.* 2,000 organisms is necessarily the same as their relative activity in sterilizing an infection with *e.g.* 2,000,000 organisms.

TABLE VI

Differences in the Apparent Therapeutic Activity of Mapharsen and Penicillin as Measured at Different Stages of the Infection and by Different Methods of Treatment

Size of inoculum	Time of treatment	Schedule of administration	Curative doses (CD_{50}) of		Apparent relative activity of penicillin: mapharsen
			Mapharsen	Penicillin	
			mg./kg.	*mg./kg.*	
1,000	1 hr.	Single massive dose	2	1	2:1
10,000,000	6 wks.	Single massive dose	6	30	1:5
		Multiple injections over 8 day period	6	0.4	15:1

Differences in the mechanism and kinetics of the bactericidal process may give wholly discrepant results in the two types of assay. The example cited of mapharsen and penicillin is a case in point (*cf.* Table VI). Tested by a single injection 1 hour after inoculation with 1,000 to 2,000 organisms, penicillin was perhaps twice as effective as mapharsen (abortive doses of approximately 1 and 2 mg. per kg., respectively); but similarly tested in the established infection, 6 weeks after inoculation with a large number of organisms, mapharsen was approximately five times as effective as penicillin.

The chief value of the suppressive method of assay appears to be in the comparison of closely related compounds, similar in their pharmacological behavior and mode of action. Even with these, however, the validity of the assay should perhaps be confirmed in assays which involve the treatment of the established infection.

The Rôle of Developing Immunity in Modifying the Response to Inadequate Treatment

When rabbits were treated with subcurative doses of penicillin during the incubation period (4 hours to 2 weeks after inoculation, and before the ap-

pearance of a lesion), asymptomatic infection was rare, and the almost invariable manifestation of failure was the development of a darkfield-positive lesion. However, if treatment was given 6 weeks after inoculation, and after the development of the primary lesion, 75 per cent of the treatment failures were then asymptomatic, demonstrable only by lymph node transfer, and obvious relapse was observed in only seven of twenty-eight failures. A possible explanation for this striking difference in the type of treatment failure observed in animals treated 2 weeks and 6 weeks after inoculation may be the developing immunity in the older animals.

As discussed by Chesney (16), a certain measure of acquired resistance may have developed, not sufficient to eradicate the infection, but sufficient to suppress the multiplication of small numbers of organisms and prevent the development of an inflammatory lesion. Recent studies (13, 14) indicate that such partial "immunity" may be apparent as early as the 6th week after the original inoculation. Animals treated at that time and then reinoculated with small numbers of organisms often develop asymptomatic infection, demonstrable only by lymph node transfer, and without the appearance of a primary lesion at the site of inoculation.

The asymptomatic relapse commonly observed in rabbits treated inadequately at the 6th week of infection has its analogy in man in (*a*) cases of early syphilis who develop serologic relapse without other manifestations of treatment failure, and perhaps also in (*b*) so called seroresistant cases of early syphilis in whom the serologic tests for syphilis remain positive for months and years after treatment. It has been a moot question whether the latter patients are treatment failures, or whether there is here involved merely the persistence of reagin in cases actually cured. Such persistence of reagin is the rule in cases of late or late latent syphilis, and one could assume the occasional seroresistant case of early syphilis to be analogous to that group. The present experiments suggest the possibility that these sero-resistant cases may be analogous to the asymptomatic relapse commonly observed in rabbits.

The Feasibility of the Prophylaxis of Human Syphilis and Gonorrhea with Penicillin

When rabbits were inoculated intracutaneously with twenty organisms, the intramuscular injection 4 days later of 200 units per kg. of penicillin prevented syphilitic infection in half the animals, and 500 units per kg. protected almost all the animals tested. Even with an inoculum of 2,000 spirochetes, 500 units per kg. protected half the animals.

Under such circumstances, the possibility suggests itself that in man also, small doses of penicillin administered during the incubation period may suffice to abort the disease. Properly to evaluate that possibility, one should know the number of organisms which ordinarily pass the epithelial barrier to cause the natural infection in man. In rabbits, even one organism injected intra-

cutaneously or intratesticularly has been found to cause infection in a significant proportion of animals; and twenty organisms have been found to be almost regularly infectious (6).

There is no reason to believe that penicillin administered soon after inoculation would behave differently in man than it does in rabbits. In both species, the renal clearance of penicillins F, G, and X (12) is maximal, the blood level falls at essentially the same rate, and there are comparable rates of absorption from an intramuscular depot. Equal dosages per kilogram in the two species should therefore have similar effects on the spirochete. If one assumes this to be the case, then a total of 200 to 500 units per kg., equivalent to a total of 15,000 to 50,000 units in the average adult, given as a single intramuscular injection in peanut oil and beeswax, might suffice to abort some early infections if given within *e.g.* 4 days after exposure; and a single injection of ten times that amount (*e.g.* 150,000 to 600,000 units) might be effective as late as 1 to 2 weeks after the original exposure. Such administration is to be considered in persons recently exposed to a known infectious source. Studies are now in progress to determine whether a single injection of penicillin will prevent syphilitic infection in the recent contacts of known primary and secondary cases.

In the armed forces local chemical prophylaxis with soap and calomel has proved insufficient to reduce the incidence of venereal disease below a level of 30 to 40 per 1000 per year, this despite an intensive educational program. The striking postwar increase in the incidence of venereal disease in such groups, in some areas to levels in excess of 300 cases per 1000 men per annum is further evidence for the inadequacy of such measures. Whether the infections occur because of failure to use the material provided, failure to use it properly, failure to use it in time, or indeed, because such local prophylaxis with soap and calomel ointment is ineffective, is a point on which no conclusive evidence has yet been offered.

The present data suggest a new approach to the prophylaxis of syphilis and gonorrhea. If the natural disease in man involves the penetration of the skin or mucous membranes by small numbers of organisms, then the infection may be susceptible to abortion by doses of penicillin so small that a single injection of a suspension in oil and beeswax, or perhaps even tablets given by mouth, might prove effective. Moreover, if the organisms multiply as slowly in man as they apparently do in rabbits, then in marked contrast to chemicals applied locally, such peroral penicillin might be effective even if given days after exposure, rather than hours.

Only the actual test in a controlled series would serve to establish the dosages of penicillin necessary. Due allowance must be made for the fact that the blood levels afforded by peroral penicillin are one-third to one-fifth those attained on injection; and the cost of an effectively prophylactic peroral dose of penicillin may preclude its large scale use. There is nevertheless the possi-

bility that *e.g.* 100,000 to 300,000 units, administered at one time or in several divided doses, and any time within 1 to 4 days after exposure, might effectively abort a large proportion of the cases of early syphilis. Further, given the far greater vulnerability of the gonococcus to penicillin, a dose adequate to abort syphilitic infection would probably be equally effective in aborting gonococcal infection, provided the organisms had not already multiplied to the extent of producing a manifest infection.

SUMMARY

1. A relatively small amount of penicillin sufficed to abort syphilitic infection in rabbits when administered during the incubation period of the disease.

2. The abortive dose, given as a single intramuscular injection in oil and beeswax, varied with the age of the infection.

(*a*) With a fixed intratesticular inoculum, the amount of penicillin necessary to prevent infection in half the animals remained at a constant level for 4 days. By the end of the 2nd week more than seven times this dosage was needed; and by the 6th week, after the chancre had appeared, more than thirty times the amount was needed to obtain the same result. The progressive increase in the abortive dose of penicillin with the passage of time probably reflects the interim multiplication of organisms.

(*b*) Qualitatively similar results were obtained in rabbits inoculated intracutaneously.

3. The abortive dose varied also with the size of the inoculum. In animals inoculated intracutaneously with 20, 2,000, and 200,000 spirochetes, and treated 4 days later, it required 200, 500, and 3,500 units per kg., respectively, to protect half the animals, and the corresponding PD_{90} dosages were 500, 2,000, and 16,000 units per kg.

4. The present observations, indicating the ease of aborting experimental rabbit syphilis during the incubation period by a single injection of penicillin, are perhaps applicable to the prevention of the disease in man.

5. Asymptomatic infections were rare in animals receiving inadequate doses of penicillin during the incubation period. Sixty-four of sixty-five such animals developed darkfield-positive lesions at the inoculated site. In animals treated 6 weeks after inoculation, however, after the development of lesions, inadequate treatment was usually manifested by an asymptomatic redissemination of organisms demonstrable only by lymph node transfer. The difference in the two groups probably reflects the beginning development of immunity in the rabbits treated 6 weeks after inoculation.

6. As suggested by Rake and Dunham (11), the treatment of animals during the incubation period permits a rapid assay of antisyphilitic agents, and one which requires only small amounts of material. Possible limitations of the method are discussed in the text.

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