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Dr. Lewis H. Weed, Chairman  
Division of Medical Sciences  
National Research Council  
2101 Constitution Avenue  
Washington 25, D. C.

Dear Doctor Weed:

Enclosed herewith is a copy of a report by Dr. Harry Engle on "The Effect of the Age of the Infection and the Site of Inoculation on the Curative (Abortive) Dose of Penicillin" in connection with syphilitic infection in rabbits. He reports that a very small dose of penicillin will cure (or abort) syphilis in rabbits if given in the first four days after inoculation. The implications of this work are that syphilis (and we hope, gonorrhoea) may be aborted in humans by relatively small doses of penicillin if given soon after exposure.

Since penicillin by mouth would be the only practicable method of using this on a large scale as a prophylactic we are very much interested in the possibility of trying this in the Navy on an experimental basis. In the light of present knowledge, an opinion is desired from the National Research Council as to the practicability of such a study. Suggestions for setting up the experiment would be appreciated if the Council considers that this can be accomplished without seriously jeopardizing the health of the men involved.

Sincerely yours,

C. A. SWANSON  
Rear Admiral (MC)  
Surgeon General, U. S. Navy

*Submitted to Subcommittee  
by Dr. Moore -  
Encl*

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The Effect of the Age of the Infection and the Site of Inoculation  
on 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.

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/kg. of penicillin aborted the infection in half of the animals, and 2,500 units/kg. protected 90 per cent. Four days later, the abortive doses were essentially unchanged ( $PD_{50}$  and  $PD_{90}$  levels approximately 500 and 2,000 units/kg.). However, when the penicillin was given 2 weeks after inoculation, the 50 per cent protective dose had increased to approximately 6,000 units/kg., and it required 16,000 units per kg. to protect nine-tenths of the animals, doses 5 to 8 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 and

clinically asymptomatic at the time of treatment. By the sixth week, however, 32 of 37 animals scheduled for treatment at that time period had developed darkfield positive lesions; and at that time it required 20,000 units/kg. to cure half the animals, and 70,000 units/kg. to cure 90 per cent, three to four time more than the doses which sufficed at 2 weeks, and thirty times more than the abortive dose at 4 days.

A qualitatively 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 second week it had risen sharply, the  $CD_{50}$  dose from 1,500 to 2,000 units/kg. to 14,000, and the  $CD_{90}$  dose from 3,500 units/kg. to 50,000 a 7- to 14-fold increase in animals still asymptomatic. By the end of the sixth week the 26 animals scheduled for treatment had all developed darkfield positive lesions. The curative dose at that time period in those animals was now 65,000 units per kg., and on the order of 160,000 units/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 40 times more than sufficed at 4 days.

It is of interest that the abortive and curative doses of penicillin of animals inoculated intratesticularly were regularly 2 to 4 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).

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 progressively with the size of the inoculum. To wipe out an intracutaneous

inoculum of 200,000 organisms with a single injection of penicillin in oil and beeswax required 15 to 50 times as much penicillin as was necessary with an inoculum of only 20 (cf. Fig. 1).

It has further been shown that, with a fixed inoculum of 2,000 spirochetes, the curative dose of penicillin varied 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 second week, however, it had increased five to fourteen-fold; and by the end of the sixth week, the curative dose, expressed either as the  $CD_{50}$  or  $CD_{99}$  level, averaged 30 to 40 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 for a period of at least four days after their intracutaneous or intratesticular inoculation, *S. pallida* multiplies so slowly as not to affect the amount of penicillin required for cure. Thereafter, however, the organisms multiply rapidly. The protective dose 2 weeks after the intracutaneous inoculation of 2,000 organisms was somewhat 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.), indicating that there had been approximately a 100-fold multiplication of organisms in the interim; and if one extrapolates the curve of Fig. 2, the observed curative dose ( $CD_{50}$ ) of 20,000 units per kg. 6 weeks after the intracutaneous inoculation of 2,000 spirochetes might imply the presence at that time of on the order of  $2 \times 10^6$  organisms, or 1,000 times the original inoculum.

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.  $2 \times 10^6$  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, the curative dose of penicillin might be greatest at that time, and subsequently fall off with the development of necrosis in the regressing lesion. It is of interest in this connection that Fleming (11) 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.

#### The Feasibility of the Prophylaxis of Human Syphilis with Penicillin

When rabbits were inoculated intracutaneously with 20 organisms, the intramuscular injection 4 days later of 200 units/kg. of penicillin prevented syphilitic infection in half the animals, and 500 units/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 penicillin may be used prophylactically in man to abort probable infection, by treatment during the incubation period. 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 intracutaneously or intratesticularly has been found to cause infection in a significant proportion of animals; and 20 organisms have been found to be almost regularly infectious (6). The apparently well-documented instances in which only a small proportion of men sexually exposed to the same infectious source have developed syphilis suggests that the number of organisms responsible for

most of the natural infections in man may be of that same small order of magnitude, barely greater than the minimal infective dose.

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 is maximal (13), the blood level falls at essentially the same rate, and there are comparable rates of absorption from an intramuscular depot. Equal dosages per kg. in the 2 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 10,000 to 30,000 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 10 times that amount (100,000 to 300,000 units) should be effective in most cases. Such administration is to be considered in the occasional instance in which there has been recent exposure to a known infectious source.

However, the most challenging possibility suggested by the present experiments is in the large-scale epidemiologic control of syphilis and gonorrhoea. 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 post-war increase in the venereal disease rate in such groups, in some areas to levels in excess of 500 per 1000 per annum, is further evidence for the inadequacy of such measures. Whether the cases of venereal disease 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 debatable point for which no conclusive evidence has yet been offered.

The present data suggest a new approach to the problem of preventing syphilis and gonorrhoea. 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 so small that even tablets given by mouth might prove effective. Moreover, if the organisms multiply as slowly in man as they 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 would serve to establish the dosages necessary. Due allowance must be made for the fact that the blood levels afforded by peroral penicillin are 1/3rd to 15th those attained on injection; and the cost of an effectively prophylactic dose of peroral penicillin may preclude its large-scale use. There is nevertheless the possibility that e.g. 100,000 to 200,000 units, given at one time or in divided doses, and taken any time within 2 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, any 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 clinically apparent infection. Studies to test the feasibility of such peroral prophylaxis are now in progress.

### Plan for Suggested Study

To simplify the administrative procedures, it is suggested that all men at the station be given tablets perorally as they check in from leave, and at the following dosage levels;

- a) 400,000 units
- b) 200,000 units
- c) 100,000 units
- d) placebo, containing no penicillin

The tablets would be administered to the four groups of men without regard to the time since exposure, the duration of their leave, or other prophylactic procedures used: Nor would any attempt be made to modify any other prophylactic procedure they might wish to use, chemical or mechanical. It would, however, be essential to keep a record of all of these points. In a sufficiently large series, the distribution with respect to these variables would equalize in all four experimental groups, and the only variable would be the amount of penicillin received.

If it were feasible, it would be highly desirable to have another group of subjects given e.g. a 100,000 unit tablet twice instead of once, with a 12-hour interval between the two administrations.

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  $2 \times 10^3$  organisms)

Time between inoculation and treatment	Intracutaneous inoculations PD <sub>50</sub> , units/kg. PD <sub>90</sub> , units/kg. (Half of animals protected) (90% of animals protected)	Intratesticular inoculation PD <sub>50</sub> , units/kg. PD <sub>90</sub> , units/kg. (Half of animals protected) (90% of animals protected)	Intracutaneous inoculation PD <sub>50</sub> , units/kg. PD <sub>90</sub> , units/kg. (Half of animals protected) (90% of animals protected)
4 hours	1000	2500	3500
4 days	500	2000	3500
2 weeks	6000	20,000	50,000
6 weeks*	20,000	70,000	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.

The Effect of the Size of the Inoculum on 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, units/kg.	No. rabbits	No. of animals protected	Protective dose of penicillin, units/kg.	
				PD <sub>50</sub> (50% of animals)	PD <sub>90</sub> (90% of animals)
200,000	32,000	4	4	3,500	8,000 †
	16,000	9	7		
	8,000	4	4		
	4,000	4	3		
	2,000	6	1		
	1,000	6	1		
	0 (Control)	4	-		
2,000	16,000	6	6	500	2,000
	4,000	5	5		
	2,000	8	6		
	1,000	7	5		
	500	11	7		
	250	5	0		
0 (Control)	23	-			
20	16,000	6	6	200 ‡	500 ††
	2,000	6	6		
	1,000	4	4		
	500	10	9		
	250	4	4		
	120	3	0		
	0 (Control)	10	-		