

STUDIES ON THE PHYSIOLOGICAL EFFECTS OF FEVER TEMPERATURES

III. THE THERMAL DEATH TIME OF *TREPONEMA PALLIDUM* IN VITRO WITH SPECIAL REFERENCE TO FEVER TEMPERATURES*

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Wagner-Jauregg's (1) classical work on the treatment of dementia paralytica by the injection of *Plasmodium malariae* has stimulated much research on the therapeutic use of fever and its relation to infectious syndromes. The cause of remissions in paretics following fever therapy is not well understood, but evidence is being accumulated to show that the increased heat of the fever is an important and fundamental factor which has not been emphasized in the literature. We believe that fever temperatures are injurious to many of the pathogenic organisms, and are submitting evidence indicating that the spirochete of syphilis is especially susceptible to temperatures above normal. We realize that this is not a new idea, but it has been lost sight of, to a great extent, in the teaching of modern medicine as indicated by the use of antipyretics in febrile diseases.

The data which we present are the first of a series of studies on the thermal death time of various microorganisms. These studies offer a practical basis for establishing the duration of the fever period and the height of the temperature of patients subjected to artificial hyperthermia (2, 3). Although the results of our *in vitro* studies on the longevity of *Treponema pallidum* at fever temperatures are very sug-

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gestive, a discussion of the clinical relations of the infection to the artificial therapeutic febrile reactions in the patient cannot be considered here. The clinical results of this method of treatment of paretics by others, as well as by us, have justified further investigation of fever as a therapeutic method of value in paresis. The data presented here suggest that the therapeutic fever may likewise be useful in the treatment of the earlier stages of syphilis.

Very little work has been reported on the thermal death time of *Treponema pallidum* *in vivo* or *in vitro*. This is no doubt due to the difficulty encountered in isolating and cultivating the organism, as well as to its failure to stain easily with dyes except under the complicated silver reduction technique. Another handicap is the uncertainty of animal inoculation in many instances and the long incubation period before the development of lesions. The majority of investigators consider that the thermal death time is short, and that because of its extreme parasitism it does not survive long in an environment slightly changed from its normal habitat.

Method

We undertook the following experiments to determine the length of time *T. pallidum* could live *in vitro* at temperatures ranging from 37.5–42°C., 41.5°C. being approximately the maximum temperature that can be safely withstood by man for from 5 to 7 hours (3).

A total of 126 healthy, adult male rabbits, with large, well developed and completely descended testes were used in these experiments. Blood Wassermann tests were made on all rabbits before inoculation with the spirochete of syphilis. The Zinsser-Hopkins and the Nichols strains of *T. pallidum* were employed for this investigation. The description of a typical experiment follows:

Three rabbits were inoculated with the Zinsser-Hopkins strain of *T. pallidum*. After they developed either the typical syphiloma or chancre of experimental syphilis, under ether anesthesia the testes of these rabbits were removed aseptically, placed in a sterile mortar, and cut with sterile scissors into bits 3 to 4 mm. square. 30 cc. of a sterile physiological salt solution were then added and the tissues were ground to a fairly fine suspension. Dark-field examinations showed from 5 to 15 actively motile spirochetes in each microscopic field. With a 30 cc. glass Luer syringe and a long needle, amounts of 1 cc. of the extract were introduced into sterile glass vials of approximately 1.5 cc. capacity. The vials were sealed, labelled, attached to dental film holders, and immersed in water baths at temperatures of

37.5°, 39°, 40°, 41°, 41.5°, and 42°C. The variation of the temperature in these baths is not greater than 0.002°C. (A description of the baths is given in an accompanying paper.) Two vials were left on a laboratory table at room temperature (approximately 25°C.). At hourly intervals a vial was removed from each water bath, the seal broken, and the contents removed by a hypodermic syringe. 0.5 cc. of the extract was then injected into each testis of a rabbit, one rabbit being used for the contents of each vial. The contents of one of the vials kept at room temperature was similarly injected into rabbits after the 1st and 6th hours.

Two similar experiments were completed on the Nichols strain of *T. pallidum*. However, in the first of these an extract was prepared from a single rabbit and subjected to only one temperature each day. This was necessary because at that time only one water bath was available. It was feared that the variation in the number of spirochetes in extracts prepared from different rabbits might introduce a serious error. We found that this was not a factor, inasmuch as a thermal death time was obtained which was similar to that observed when a mixed extract was subjected to the different temperatures simultaneously on the same day.

Thirty-four rabbits were used for tests with each strain. Examinations were made at hourly intervals of the six different temperatures above mentioned, with the exception of 42°C. in which case injections were made for the first 4 hours only. Each rabbit was kept in an individual cage. The rabbits were inspected weekly for 14 weeks, after which time a second blood Wassermann examination was made.

When the development of lesions occurred in the testes, dark-field examinations were made, and in every case typical *T. pallidum* was found in varying numbers. Rabbits showing no lesions at the end of 3 months were considered negative. This was confirmed by the outcome of the Wassermann reactions and by further animal inoculation. These were conducted in one of the two following ways: Extracts were prepared from the testes and popliteal nodes removed aseptically under ether anesthesia from some of the group, and these were injected into the testes of other adult rabbits. The animals were observed 3 months for evidence of syphilis. The remaining animals of the group were given an intratesticular injection with a known virulent strain of *T. pallidum*. Several investigators (4-6) have stated that rabbits infected with syphilis will not develop typical lesions on reinoculation. We have used this criterion as an indication of the absence of syphilis in these rabbits. The rabbits which developed no lesions and in which the outcome of the Wassermann test remained unaltered have developed typical extensive syphilomata in the usual time on reinoculation. On the other hand, rabbits with healed and quiescent lesions have not developed syphilomata on reinoculation.

RESULTS

The results of the examinations (as shown in Table I) are conclusive, and of great interest from several standpoints. The artificial conditions under which the spirochetes were kept apparently did not

injure them to any great extent, as determined by the injection of rabbits with the control extracts left standing in sealed vials at room temperature (25°C.) and in the water bath at 37.5°C. for 6 hours. In other experiments we have observed that even lower temperatures (22–23°C.) for 8 hours failed to injure the organism in such testicular extracts as evidenced by the fact that characteristic lesions of experimental syphilis were produced upon rabbit inoculation. There is some evidence (Duran-Reynals factor) (7) that a testicular extract is

TABLE I
The Thermal Death Time of Two Strains of Treponema pallidum at Fever Temperatures

Temperature	Hrs. in water bath											
	Zinsser-Hopkins						Nichols					
	1	2	3	4	5	6	1	2	3	4	5	6
°C.												
25.0	+					+						
37.5	+	+	+	+	+	+	+	+	+	+	+	+
39.0	+	+	+	–	–	+	+	+	+	+	–	–
40.0	+	–	–	–	–	–	+	+	–	–	–	–
41.0	–	–	–	–	–	–	+	–	–	–	–	–
41.5	–	–	–	–	–	–	–	–	–	–	–	–
42.0	–	–	–	–	–	–	–	–	–	–	–	–

+ indicates infection in rabbit following intratesticular injection of heated extract.

– indicates no infection in rabbit following intratesticular injection of heated extract.

a most favorable medium for suspending the spirochetes and for their injections, because of its increasing the susceptibility of the host.

The hydrogen ion concentration of the extracts during the time they were exposed to the fever temperatures remained practically constant. A change of from only 0.1 to 0.5 pH, as determined by the electrometric method, was noted at the highest temperature for the maximum heating period. The slight change observed was toward the acid side.

Any criticism of the technique can be met by the fact that typical

TABLE II

Results of Blood Wassermann Tests on Rabbits Injected Intratesticularly with Extracts Heated at Fever Temperatures

Temperature of bath	Length of time in bath	Rabbit No.	Blood Wassermann before inoculation			Blood Wassermann 3 mos. after inoculation			Dark-field examination	Lesions in testes
			Non-cholesterinized	Cholesterinized	Kahn	Non-cholesterinized	Cholesterinized	Kahn		
37.5 Control	1	16-27	-	-	-	+++	+++	++++	+	Chancres
	2	16-28	-	-	-	+++	+++	++++	+	Induration
	3									
	4	16-29	++	++	+++	++++	++++	++++	+	Chancres
	5									
	6	16-30	-	++	+++	+	++++	++++	+	Nodules
39.0	1	16-00	-	-	-	+++	+++	+++	+	Chancres
	2	16-05	-	-	-	++++	++++	++++	+	Nodules
	3	16-10	+	+++	+	++++	++++	++++	+	Nodules
	4	16-15	-	-	-	-	-	-	-	None
	5	16-19	+++	++++	++	+++	++++	++	-	None
	6	16-23	-	-	-	-	-	-	-	None
40.0	1	16-01	+	+	-	+	+++	+++	+	Induration
	2	16-06	-	-	-	-	-	-	-	None
	3	16-11	-	-	-	-	-	-	-	None
	4	16-16	-	++	-	-	-	-	-	None
	5	16-20	-	++	++	+-	+++	++	-	None
	6	16-24	-	+	-	-	-	+	-	None
41.0	1	16-02	-	++	+-	-	-	-	-	None
	2	16-07	-	+	++	-	+++	-	-	None
	3	16-12	-	-	-	-	-	-	-	None
	4	16-17	-	-	-	-	-	-	-	None
	5	16-21	-	-	-	-	-	+	-	None
	6	16-25	-	-	-	-	-	-	-	None
41.5	1	16-03	+	++	+++	-	++++	-	-	None
	2	16-08	+	+	-	-	-	-	-	None
	3	16-13	-	-	-	-	-	-	-	None
	4	16-18	-	-	-	+-	+-	-	-	None
	5	16-22	-	-	-	-	-	-	-	None
	6	16-26	-	++	++	++	++	+	-	None
42.0	1	16-04	+-	++	-	-	+-	-	-	None
	2	16-09	ac	ac	++	-	+-	+++	-	None
	3	16-14	+	++	++	+	++	++	-	None
	4	16-31	-	-	+	-	+	-	-	None
	5									
	6									

ac indicates anticomplementary.

experimental syphilis was produced by the injection of the control extracts maintained at 25° and 37.5°C. for 6 hours. It seems evident that the increased temperature was the fundamental factor in injuring or destroying the spirochetes. That this organism is so susceptible to slight changes in the temperature of its environment is remarkable.

The results of the blood Wassermann tests before and 3 months after inoculation on the series of rabbits injected with the Zinsser-Hopkins strain are given in Table II. In each instance the results with the non-cholesterinized and cholesterinized antigens are given, as well as those of the Kahn test. In several instances the blood of the uninjected rabbit showed some reaction that may be considered as non-specific or due to an infection with *Treponema cuniculi*. However, in no case was there a four plus reaction before injection with the three antigens used (see protocols). Our experience indicates that when typical syphilomas or chancres develop in rabbits' testes, the serum always shows a four plus reaction. We have noted no marked change in the Wassermann reaction during a 3 month period if infection was not established after injection. The results of our clinical findings and blood tests have been very consistent. Thomsen and Christensen (8) have reported that after rabbits were injected with *T. pallidum* extracts, a markedly positive Wassermann reaction developed regularly after from 5 to 6 weeks and continued for approximately 6 months, when it gradually disappeared regardless of the clinical course of the disease.

DISCUSSION

There are two general approaches to a study of fever temperature effects on *T. pallidum*: first, a consideration of the optimum temperature for its isolation and cultivation; and second, the resistance of the microorganism to temperatures higher than that normally observed in man.

The literature contains only a few reports on the optimum temperature for the isolation and cultivation of *T. pallidum*. It should be emphasized that some of the reported isolations of *T. pallidum* were evidently not those of the spirochete of syphilis, especially since these cultures failed to produce lesions when injected into experimental animals. In some of the standard texts on bacteriology a tem-

perature of 33.5°C. (Zinsser¹) is stated to be most satisfactory for obtaining growths of this organism, while in others 37°C. is given as the optimum temperature. A survey of the literature of those men who have reported studies on the cultivation of *T. pallidum* shows that the majority used a temperature of 37°C. for their work. Schereschewsky (9) used a temperature of 37°C. for his isolation and cultivation experiments. He states that *T. pallidum* will grow at 40°C. Ungermann (10) has reported extensive investigations on the cultivation of spirochetes, and has studied the effects of temperature on their growth. He grew *Leptospira icterohaemorrhagiae*, *Spironema recurrentis*, *Spironema gallinarum*, and *T. pallidum* at temperatures of 30° and 37°C. All of the above organisms grew well at both temperatures. At 30°C. the spirochetes lived longer and *Spironema gallinarum* multiplied as rapidly as at 37°C. The spirochete of Weil's disease showed no growth at 25°C., a slight growth at 28°C., but the best results were obtained when the fresh cultures were grown at 37°C. for a few days and later placed at 25°C. *Spironema recurrentis* grew somewhat more slowly at 30° than at 37°C. but lived longer at the lower temperature. Ungermann presented fewer data on *T. pallidum*, but he states that as good growths were obtained at 30° as at 37°C. and that the spirochete of syphilis lived longer at the lower temperature. The similar experience of Inada, Ido, Hoki, Kaneko, and Ito (11) and of Noguchi (12) with the cultivation of *Leptospira icterohaemorrhagiae* of Weil's disease is likewise of interest. They demonstrated that the spirochete of Weil's disease grows between temperatures of 10° and 37°C., that it lives longer at temperatures from 25–30°C., and grows more slowly than at 37°C. Noguchi failed to get growth at 42°C. Although we realize that microorganisms from several genera in one family may vary considerably in their biological activities, it is obvious that these species of spirochetes pathogenic for man may be expected to have a similar optimum temperature for artificial cultivation. With the exception of the above citations, we have been unable to find any work to support the idea that a temperature lower than normal human body temperature is best for the isolation of this spirochete.

Few investigations of the resistance of *T. pallidum* to fever temperatures or to those above 45°C. have been made. In 1912 Arnheim (23) reported that spirochetes which he believed to be *T. pallidum* were not killed by incubation for several days at 45°C., nor by heating at 56°C. for 10 minutes. Nevertheless, all of his pathogenicity tests were negative. Bronfenbrenner and Noguchi (24) placed pure cultures of *T. pallidum* in physiological salt solution and exposed them to water bath temperatures of 37°, 40°, and 45°C. Control tubes were left at room temperature. After exposure for various lengths of time, sterile rabbit tissue and

¹ Zinsser has stated in a personal communication that he believes the use of a temperature of 33.5°C. for the isolation of *T. pallidum* evolved from observations that lesions of experimental syphilis developed best in the rabbit's testes, where the temperature is usually at that level.

ascitic agar were added to each tube, incubated at 37°C. for 10 days, and examined. They observed that their controls were viable for 12 hours at room temperature. The other cultures were viable for 6 hours at 37°, 1 hour at 40°, and 7 minutes at 45°C. No growth occurred after 10 minutes exposure at 45°C. They state that 37°C. for 6 hours injured the spirochetes because much less growth occurred in the tubes exposed at this temperature than in those at room temperature. However, they say that the organism would survive many hours at 45°C. if kept under strictly anaerobic conditions with properly balanced saline constituents and other nutrient substances. Zinsser and Hopkins (25) determined the resistance of cultures of *T. pallidum* mixed with cocci and bacilli, and placed on bits of cloth in diffuse light at room temperature varying from 21.5–25°C. The spirochetes were viable 11½ hours under these conditions, as were their controls in tubes in the same environment. When the same mixture of spirochetes and other microorganisms was placed on glass slides and allowed to dry under the above conditions, *T. pallidum* was dead after 1½ hours. Schamberg and Rule (26) heated an extract made from a rabbit's testis for 1 hour at 40°C. and found that it failed to infect on inoculation. They state that the thermal death time outside the body is 41°C. for 6 hours. Bessemans, Vergoullie, and Hacquaert (27) have been quoted on the effects of hot baths on human chancres. They observed that the healing of local lesions in rabbits and man occurred rapidly when exposed for 1 or 2 hours from 40–42°C. They conclude that exposure for 2 hours at 40°C., or for 1 hour at 42°C., will kill the spirochete of syphilis but will not injure the tissue cells. That *T. pallidum* has the same thermal death time *in vivo* as *in vitro* is much more difficult to determine. Wakerlin (28) has recently reported a case of syphilis due to an accidental laboratory infection with the Nichols (29) strain of *T. pallidum*. Such evidence indicates that this strain of spirochete has not lost its pathogenicity for man during many years of animal passage.

These observations suggest that the thermal death time of human strains is probably not longer than that of the strains of experimental syphilis used by us in these experiments. This will be discussed in another paper of this series.

SUMMARY AND CONCLUSIONS

1. The thermal death time of *Treponema pallidum* in extracts from lesions in rabbits' testes was determined *in vitro* at fever temperatures using the Zinsser-Hopkins and Nichols strains.
2. The criteria to determine the persistence of infectivity of the heated extract were the following: the development of lesions on inoculation into rabbits, dark-field examination of tissue from the lesions, the outcome of blood Wassermann tests, and of reinoculation tests.

3. The thermal death time of the two strains of spirochetes was approximately the same, although the Nichols strain was somewhat the more resistant. In the case of the latter 5 hours at 39°C., 3 hours at 40°C., 2 hours at 41°C., and 1 hour at 41.5°C., were required to render infective extracts innocuous to other rabbits.

4. The thermal death time of *T. pallidum* in testicular extracts *in vitro* at fever temperatures is so short as to suggest that induced fever may be useful therapeutically in human syphilis.

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