

STUDIES ON THE PHYSIOLOGICAL EFFECTS OF FEVER TEMPERATURES

IV. THE HEALING OF EXPERIMENTAL SYPHILIS LESIONS IN RABBITS BY SHORT WAVE FEVERS*

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Wagner-Jauregg (1) was able to obtain clinical remissions in patients with dementia paralytica following a fever due to the injection of foreign proteins or to the tertian form of malaria. Believing that his results were produced chiefly by the effect of fever temperatures upon the spirochete of syphilis, we have studied experimentally the effect of fever on the healing of syphilitic lesions in rabbits. This can be done by placing the animal in a high frequency electrostatic field with the result that the temperature of the animal can be elevated and controlled at will, without introducing into the body any foreign material.

Since Bertarelli's (2) observation in 1906 that he could successfully produce syphilis in rabbits by the injection of spirochete-containing tissue from man, many studies on the treatment of experimental syphilis have been reported. A few workers have tried to influence the course of experimental syphilis in rabbits by the use of various methods to elevate the temperature of the animal. Weichbrodt and Jahnel (3) reported the healing of scrotal chancres in rabbits by placing the animals in an incubator at 41.0°C. for 1/2 hour twice daily over a period of from 3 to 5 weeks. Schamberg and Rule (4) were able to prevent the development of scrotal chancres by daily raising the body temperature of rabbits 2°C. with a total of eleven immersions in a hot water bath. The first bath was given on the 4th day after intratesticular injection of the spirochetes. Later, they (5) reported

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that they had cured two rabbits with scrotal chancres by submersing them on 15 successive days for 15 minutes in hot water baths at 45°C., which elevated their body temperature 5.2°F. and 5.8°F. (about 3°C.) respectively. Sublingual temperatures were recorded in their experiments.

In a previous report (6) we showed that short, repeated fevers prevented the development of syphilis in twenty-one of a series of twenty-five rabbits, when the treatments were begun 3 to 7 days after the intratesticular injection of extracts containing *Treponema pallidum*. In the absence of any obvious specific effects, we believe the elevation of temperature obtained when rabbits in the high frequency field produced by an oscillator (10,000 kilocycles) are exposed is due to heat from induced alternating currents and from the increased rate of vibration of the molecules of the cells produced by their alternate attraction to each of the plates in turn. As an accompanying paper shows, this type of fever is safe within the limits of the rabbit's body tolerance (42.5°C.).

The following experiments represent a continuation of the study of the effect of short multiple fevers on experimental syphilis in rabbits. Also, an attempt has been made to find the shortest number of treatments at a temperature of from 41–42°C. necessary to cure the animal of its infection. We have been able to demonstrate the efficacy of a prolonged single fever (41.5°C.). The influence of room temperature conditions (25–30°C.) on the testes must be guarded against when treating syphilitic lesions in the testes.

Method

A group of thirty-seven adult male rabbits with large, well developed testes were used for these experiments. No pure bred animals were used and the rabbits varied considerably, resembling the Belgian hare, the New Zealand Red, the Flemish Giant, and the Albino, respectively. Seven were inoculated with the Zinsser-Hopkins strain of *T. pallidum* and the remainder with the Nichols strain. The following technique always produced good syphilomata or chancres.

A rabbit with well developed chancres in each testis was chosen from a previously inoculated series. Under ether anesthesia the testes and popliteal lymph nodes were removed aseptically, cut up into small bits in a sterile mortar, and ground with 10 cc. of a sterile physiological salt solution. A dark-field microscopical examination of the extract revealed from 3 to 25 active spirochetes per field. From 0.1 to 0.5 cc. of this suspension was injected into each testis of the group to be studied. The rabbits were kept in individual cages and inspected at weekly intervals until the chancres or syphilomata were well developed.

The rabbits were then divided into two groups. Sixteen were kept as controls to study the length of time required for spontaneous healing of the chancres, as well as the duration of the infectivity of *T. pallidum* in the rabbit after inoculation. From 30 to 395 days after injection, the sixteen control rabbits were sacrificed and extracts made from their testes and popliteal lymph nodes, as previously described. The suspensions were then injected intratesticularly into an equal number of normal, male, adult rabbits, with well descended testes (see Table I). This reinoculated group of rabbits were then inspected at weekly intervals for evidence of the development of syphilis as indicated by gross lesions, blood Wassermanns, and dark-field examination.

The other group of twenty-one inoculated rabbits were subdivided and heated by irradiation in the short wave electrostatic field (Tables II and III). The fever treatments were started at various stages of development and healing of the testicular lesions. The number and length of the heating periods, as well as the period between the end of the treatment and reinoculation, were varied in order to study the effects of these different time intervals.

The heating was accomplished very simply by exposing the rabbits to high frequency waves (10,000 kilocycles) in a field between two aluminum plates of a high frequency oscillator (6). The animals were placed in an orange crate or a glass battery jar, and the field strength regulated to elevate the temperature of the rabbit within 20 to 30 minutes to about 41.5°C. The animals were removed from the field every 15 minutes for rectal temperature observations after which they were returned to the container and the heating continued if their temperature was to be elevated further. When it was to be maintained at a given level, the field strength was reduced accordingly or the current turned on and off at the proper intervals. After the treatment was completed the animal was placed on the cool cement floor or in a box while defervescence occurred. The period of cooling varied considerably with the room temperature and with the type of rabbit. Those with thick fur, such as the Angora rabbits, required an hour or more to return to the temperature which they had prior to the irradiation.

The first fourteen animals were heated from 41–42°, 5 or 6 times each week until the scrotal chancres had completely healed and the scabs had fallen off. This usually required 3 weeks, although in some instances 4 weeks of treatments were necessary. These daily treatments were used to imitate the paroxysms of malaria fever since this type of febrile reaction is used for the treatment of paresis. Later, rabbits were given longer and fewer treatments in order to determine the minimum number of fevers at the above temperatures necessary to kill the spirochete. As noted in Table III, only one 6 hour treatment was given to rabbits with well developed syphilis and found to be effective.

When the designated number of fevers were completed, the rabbits were sacrificed at intervals up to 214 days. Extracts of their testes and popliteal lymph nodes were injected into an equal number of normal, male, adult rabbits according to the procedure already described for the controls. Prior to injection, dark-field

examinations of the inoculated material were made in almost every case. Histological examinations were made on some of the tissues, but Levaditi stains for spirochetes and the usual hematoxylin and eosin stain were of little value in proving that the infection had been cured.

All of the rabbits irradiated possessed well developed syphilomata or chancres in the testes before treatment except No. 12-12. Clinically, the lesions in this case had healed spontaneously, but the animal was no doubt still infectious as shown by the findings in our control series. Rabbit 4-97 was overheated. His rectal temperature registered 46.6°C. during a treatment. Upon this finding, the testes and popliteal lymph nodes were immediately removed under ether anesthesia, an extract was prepared and injected into another rabbit.

RESULT

It will be noted from Table I that the inoculation of a group of normal male rabbits with extracts of testes and popliteal lymph nodes from the controls gave positive results in every instance. In some of the control rabbits, the extract from the testes was injected into one testis, while the extract from the popliteal lymph nodes was injected into the other testis. However, in most cases a bilateral intratesticular injection of 0.5 cc. of a single extract prepared from the testes and lymph nodes was made. Our experience has shown that in several cases extracts from the popliteal lymph nodes failed to infect, while the extract from the testes gave positive results. In no instance have we found evidence of *T. pallidum* by animal inoculation in the popliteal lymph nodes when the testicular extract failed to infect.

The finding that *T. pallidum* remained in the popliteal lymph nodes and in the testes for a long time after spontaneous clinical healing of the lesions confirms that of other observers. In the group of controls, viable spirochetes were found 395 days after injection. Good lesions developed in the reinoculated series after this comparatively long interval. No clinical evidence of generalized lesions or of extension of the infection beyond the testes, inguinal, and popliteal nodes occurred in the control group of rabbits. The average time required for the development and clinical healing of the chancres in the untreated rabbits was 4 months. Occasionally a small chancre would heal in 3 months when only some thickening of the scrotum, or perhaps small nodules in the epididymis or testis, remained. However, this material was still infectious.

The results of the reinjections of extracts from the testes and popliteal lymph nodes of the treated group are tabulated in Tables II and III. It will be noted that with one exception (Rabbit 4-99), negative reinoculation results were obtained with the various fevers and intervals of irradiation used. We continued the fever of the malarial type (*i.e.* multiple short periods) until the chancre had completely

TABLE I
Control Rabbits

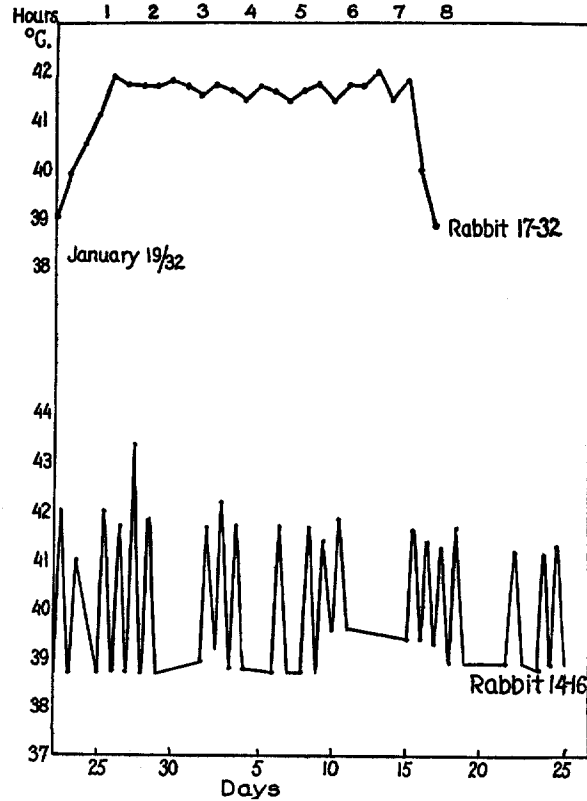
Rabbit No.	Date of injection	Strain	Amount injected		Dark-field examination	Date of reinoculation	No. of rabbit injected	Result of reinoculation
			cc.	days				
3-74	Sept. 18, 1929	Nichols	0.1	30	+	Nov. 18, 1929	4-53	Positive
13-41	Feb. 5, 1931	Nichols	0.5	43	+	Mar. 20, 1931	13-86	Positive
7-39	Apr. 1, 1930	Nichols	0.2	86	+	June 26, 1930	9-84	Positive
14-26	May 15, 1931	Zinsser-Hopkins	0.5	87	+	Aug. 11, 1931	15-87	Positive
16-29	Oct. 13, 1931	Zinsser-Hopkins	0.5	93	+	Jan. 14, 1931	17-31	Positive
7-34	Apr. 1, 1930	Nichols	0.2	115	+	July 25, 1930	10-57	Positive
13-40	Feb. 5, 1931	Nichols	0.5	123	+	June 8, 1931	14-58	Positive
13-86	Mar. 20, 1931	Nichols	0.5	131	+	July 29, 1931	15-80	Positive
13-71	Mar. 7, 1931	Nichols	0.5	144	+	July 29, 1931	15-78	Positive
13-76	Mar 7, 1931	Nichols	0.5	157	S	Aug. 11, 1931	15-84	Positive
13-75	Mar. 7, 1931	Nichols	0.5	172	—	Aug. 26, 1931	15-91	Positive
4-96	Jan. 14, 1930	Nichols	0.1	175	—	July 8, 1930	10-15	Positive
3-89	Sept. 18, 1929	Nichols	0.1	266	—	June 11, 1930	9-66	Positive
7-32	Apr. 1, 1930	Nichols	0.2	321	—	Feb. 16, 1931	13-51	Positive
2-51	July 22, 1929	Nichols	0.2	353	—	July 10, 1930	10-17	Positive
3-88	Sept. 18, 1929	Nichols	0.1	395	—	Nov. 18, 1930	4-54	Positive

healed. As a rule, an average of 20 fevers was required, although frequently more in the first experiments. This was found to be due to the very rapid cooling of the infected tissues and especially the testes, when the animals were removed from the oscillator and placed on a table at room temperature in order to obtain the rectal temperature. We (6) have previously demonstrated by the use of a thermocouple that the temperature of the testes is from 1-2°C. lower than

TABLE II
Irradiated Rabbits
Multiple Short Fevers

Rabbit No.	Date of injection	Strain	Amount injected in each testis	Interval between injection and beginning of treatment	Lesions at beginning of treatment	Dark-field examination	No. of treatments	Duration of treatment	Maximum rectal temperature	Interval between beginning and end of treatment	Interval between end of treatment and reinoculation	Dark-field examination	Date of reinoculation	No. of rabbit injected	Result of reinoculation
			cc.	days				hrs.	°C.	days	days				
3-73	Sept. 18, 1929	Nichols	0.1	61	Chancre	+	18	17	41.9	36	214	—	July 21, 1930	10-44	Negative
3-90	Sept. 18, 1929	Nichols	0.1	85	Chancre	+	24	33½	42.0	60	171	—	July 31, 1930	10-72	Negative
4-97	Jan. 14, 1930	Nichols	0.1	41	Chancre	+	16	19	46.6	35	0	—	Mar. 31, 1930	7-25	Negative
4-99	Jan. 14, 1930	Nichols	0.1	93	Chancre	+	55	84	42.6	67	15	+	July 8, 1930	10-16	Positive
7-31	Apr. 1, 1930	Nichols	0.2	62	Chancre	+	34	43½	42.8	79	8	—	July 29, 1930	10-69	Negative
7-33	Apr. 1, 1930	Nichols	0.2	66	Chancre	+	30	36½	42.2	45	10	—	July 31, 1930	10-71	Negative
7-36	Apr. 1, 1930	Nichols	0.2	62	Chancre	+	37	47½	42.0	49	4	—	July 25, 1930	10-58	Negative
7-38	Apr. 1, 1930	Nichols	0.2	62	Chancre	+	25	34½	42.2	31	27	—	July 30, 1930	10-70	Negative
13-37	Feb. 5, 1931	Nichols	0.5	67	Chancre	+	20	69½	42.8	32	24	—	June 8, 1931	14-61	Negative
13-38	Feb. 5, 1931	Nichols	0.5	67	Chancre	+	19	64½	43.2	32	26	—	June 10, 1931	14-64	Negative
13-73	Mar. 7, 1931	Nichols	0.5	88	Syphiloma	+	20	63½	42.6	38	18	—	July 29, 1931	15-79	Negative
13-74	Mar. 7, 1931	Nichols	0.5	88	Syphiloma	+	20	64½	42.7	38	4	—	July 13, 1931	15-44	Negative
13-78	Mar. 7, 1931	Nichols	0.5	76	Chancre	+	20	70½	43.3	34	11	—	July 5, 1931	14-82	Negative
14-16	Mar. 20, 1931	Nichols	0.5	63	Chancre	+	20	66	43.3	34	12	—	July 6, 1931	14-83	Negative

that of the rectum. Recently using a similar method we studied the rapidity of defervescence in various parts of the body after removal of the rabbit from the short wave field. An example of the difference in the rates of cooling of the rectum and testes follows: In 25 minutes after discontinuing irradiation and removal to room temperature the



GRAPH 1. Types of fever produced in rabbits. Upper curve, one continuous fever of 6 hours. Lower curve, series of short unsustained fevers.

rectal temperature dropped $0.8^{\circ}\text{C}.$, while that of the testes fell $3.0^{\circ}\text{C}.$ It was likewise observed that the temperature of a chancre is lower by at least $1.0^{\circ}\text{C}.$ than that of a syphiloma embedded in the substance of the testes. The temperature gradients will be discussed in detail in another paper of this series. Therefore, the temperature in the syphilitic lesions remained at the high level of the rectal temperature

TABLE III
Irradiated Rabbits
Prolonged Fevers

Rabbit No.	Date of injection	Strain	Amount injected in each testis	Interval between injection and beginning of treatment	Lesion at beginning of treatment	Dark-field examination before treatment	No. of treatments	Duration of treatment	Maximum rectal temperature	Interval between beginning and end of treatment	Interval between end of treatment and reinoculation	Dark-field examination after treatment	Date of reinoculation	No. of rabbit injected	Result of reinoculation	Duration of observation
14-27	May 15, 1931	Zinsser-Hopkins	0.5	90	Syphiloma	+	3	18½	41.9	18	9	—	Sept. 9, 1931	15-94	Negative	120
14-59	June 8, 1931	Zinsser-Hopkins	0.5	67	Syphiloma	+	2	12	41.9	9	4	—	Oct. 26, 1931	15-90	Negative	120
12-12	Dec. 9, 1930	Nichols	0.5	116	Syphiloma	+	1	9½	43.7	1	1	—	Apr. 8, 1930	14-20	Negative	120
16-01	Oct. 13, 1931	Zinsser-Hopkins	0.5	98	Syphiloma	+	1	6	41.7	0	3	—	Jan. 22, 1932	17-57	Negative	120
16-05	Oct. 13, 1931	Zinsser-Hopkins	0.5	98	Syphiloma	+	1	6	41.8	0	3	—	Jan. 22, 1932	17-55	Negative	120
16-28	Oct. 13, 1931	Zinsser-Hopkins	0.5	98	Syphiloma	+	1	6	43.0	0	3	—	Jan. 22, 1932	17-58	Negative	120
16-32	Oct. 13, 1931	Zinsser-Hopkins	0.5	98	Chancere	+	1	6	41.9	0	3	—	Jan. 22, 1932	17-56	Negative	120

only while the animals were in the oscillator, which, as a rule, was not more than 2 hours in the case of the short multiple exposures. These findings may explain the failure to kill the spirochetes in Rabbit 4-99, despite the large number (55) of short treatments. This rabbit had very extensive chancres on both scrotal walls, practically the entire scrotum being involved. These chancres healed very slowly and much scar tissue remained after healing. It was difficult, for some unknown reason, to establish a fever in this rabbit and in many treatments the rectal temperature did not reach more than 40.5°C. In later experiments this condition was rectified by treating the animals in a room with a temperature of from 35–37°C.

DISCUSSION

The important observation in the treated series (Table III) is the fact that a fever at 41–41.5°C., sustained for 6 hours was as effective in injuring or destroying *T. pallidum* in the rabbit as was a series of from 20 to 30 short pyrexias. In the case of those animals exposed to short, repeated fevers, there was a total maximum of from 17 to 70 hours in the high frequency field during which time their body temperature was elevated above normal. It is interesting to find that one treatment of 6 hours in the field is just as effective as the greater number of hours of irradiation from the many treatments. This was true, regardless of the size or type of lesion in the testes. Of course, complete healing of the syphilitic lesion could not occur in the short interval of from 3 to 9 days after one sustained fever treatment, at which time the rabbit was sacrificed and extracts from the testes and lymph nodes reinjected. Nevertheless, a complete change in the character of the lesion was noted in this period. The syphilomas in the testes became softer and less extensive and the same was true of the indurated testes. The chancres showed evidence of rapid healing, were dry, decreased in size, became markedly umbilicated, while the periphery of the scabs was elevated and free from the tissue healing beneath them.

We have included in Tables II and III the maximum temperature reached during the fever treatment, which in some cases was higher than we desired to produce. Although rectal temperatures were taken every 15 minutes, it was difficult to avoid a sudden rapid eleva-

tion of temperature, especially on summer days when the treating room was warm and had a high humidity. Because of the lower temperature level of the testes, it is evident that at no time, with the exception of Rabbit 4-97 that was killed by overheating, was the temperature of the testes above that of 41.5°, and in most instances for only a brief interval at this point.

The dark-field examinations of extracts from the site of the lesions, or the testes after the lesions had disappeared in the case of the fever-treated group, were always negative. Occasionally suspicious bodies were observed, but we could not in any case see definite typical motile spirochetes. The microscopic bodies seen may have been dead spirochetes. The histological sections of those lesions observed after staining with Levaditi's method failed to reveal any spirochetes, although this is not as good evidence as the reinoculation test (see tables).

The results are in accord with our earlier studies (6), in which we prevented lesions of experimental syphilis from developing by producing in rabbits a series of about 20 short wave fevers, beginning from 3 to 7 days after intratesticular injection of *T. pallidum*. The data show that single fevers are just as effective in healing the lesions and in destroying the spirochete in the body of the host. It is evident from an accompanying paper (7) that the increased heat of the fever provides an unfavorable environment for the spirochetes that either destroys or injures them so that they lose their infectivity. We do not know whether in syphilis the elevated temperature also stimulates or activates those factors in the body that are concerned with its protection against infection. However, in studies on gonorrhea we have observed increased phagocytosis during artificially induced fever. This leads us to believe that such factors may play a prominent part in syphilis. Two of the rabbits, Nos. 3-73 and 3-90, were allowed to live 214 and 171 days, respectively, after treatment. Since their testicular and nodal extracts failed to produce syphilis on injection into other rabbits, it seems plain that the spirochetes must have been killed and not injured only temporarily.

The fertility of some of the rabbits was tested after treatment by breeding to normal females. Some of the males were sterile temporarily, while normal litters were obtained in other cases. One male

failed to cause conception in a female known to be fertile when bred to normal males. The extent of the injury caused by the lesions of syphilis no doubt had much to do with the production of sterility. We have noted in untreated syphilitic males that an extensive lesion causing severe injury to both testes resulted in sterility.

Some of the rabbits lost weight during the frequent heatings, but it was recovered rapidly after irradiation ceased. In general, we failed to see any injury to the general health of the rabbits, except in the case of Rabbit 4-97 which was overheated.

The thermolability of *T. pallidum* is again emphasized. The findings suggest the practicality of fever therapy in the treatment of acute as well as chronic syphilis in man.

SUMMARY AND CONCLUSION

1. Multiple, unsustained fevers (41–42°C.) produced by irradiation in a high frequency electrostatic field (10,000 kilocycles) destroyed *T. pallidum* in rabbits with active syphilitic lesions as determined by the injection into normal rabbits of extracts prepared from their testes and popliteal lymph nodes.

2. One febrile period of 6 hours at a temperature of 41.5–42°C. was likewise found to be sufficient to destroy *T. pallidum*.

3. Infection with *T. pallidum* persisted in a control series of untreated rabbits for as long as 395 days after inoculation, but clinical healing occurred in from 3 to 4 months after injection.

4. The time interval between inoculation and fever treatment, or between the end of the fever treatment and reinoculation, did not affect the results.

5. The fever treatment was effective at any stage of experimental syphilis in rabbits.

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BIBLIOGRAPHY

1. Wagner-Jauregg, J., *Psychiat.-neurolog. Woch.*, 1918, **20**, 132.
2. Betarelli, E., *Centr. Bakt., 1. Abt., Orig.*, 1906, **41**, 320.

3. Weichbrodt, R., and Jahnel, F., *Deutsch. med. Woch.*, 1919, **45**, 483.
4. Schamberg, J. F., and Rule, A., *Arch. Dermatol. and Syphilol.*, 1926, **14**, 243.
5. Schamberg, J. F., and Rule, A., *J. Am. Med. Assn.*, 1927, **88**, 1217.
6. Carpenter, C. M., and Boak, R. A., *Am. J. Syph.*, 1930, **14**, 346.
7. Boak, R. A., Carpenter, C. M., and Warren, S. L., *J. Exp. Med.*, 1932, **56**, 741.