Brit. J. vener. Dis. (1970) **46**, 318

Fever after inoculation of rabbits with *Treponema pallidum*

H. GUDJÓNSSON AND E. SKOG

From the Department of Occupational Dermatology, Karolinska Sjukhuset, Stockholm, Sweden

In earlier publications we have reported the results of our investigations on humans during the Jarisch-Herxheimer reaction (J-H reaction) (Skog and Gudjónsson, 1966; Gudjónsson and Skog, 1968a). We have also tried to induce the J-H reaction in syphilitic rabbits with penicillin (Gudjónsson and Skog, 1968b), but in no case did such a reaction occur. In the literature we have found only one report of temperature changes after specific treatment of rabbits infected with syphilis (Putkonen and Helle, 1951), with the same absence of J-H reaction as in our study.

During our investigations, however, we observed that fever developed in the rabbits in close connection with the inoculation of *T. pallidum* without antisyphilitic treatment, and also that a large number of the rabbits died.

A preliminary report on this fever reaction (Gudjónsson and Skog, 1968b) also described our attempts to prevent the fever by administering prednisolone and by injecting serum and blood from fever-resistant rabbits (i.e. those which reacted to inoculation with fever but survived and did not develop fever again after further inoculations). This treatment did not, however, affect the fever.

The spontaneous death of rabbits inoculated with *T. pallidum* in laboratories working with *Treponema pallidum* immobilization (TPI) test has become a problem during the last decade, at least in Scandinavian countries. Thus, a report from the State Serum Institute in Copenhagen (Jørgensen, 1968) mentioned a mortality rate of about 20 per cent. At the National Bacteriological Laboratory in Stockholm similar observations have been made, with a mortality rate of about 50 per cent. (Hederstedt, 1968). Various attempts to elucidate the aetiology and pathogenesis of this phenomenon have been ineffective.

As far as we are aware, a fever reaction after the inoculation of rabbits with *T. pallidum* has not been previously described by other investigators. In this report we present our studies on various aspects of the reaction.

Received for publication September 29, 1969.

Material and methods

RABBITS

Male rabbits of different breeds (White Swedish country breed, Chinchilla, French Wädur, White Albino Angora, Black and White, and Big Silver), and weighing between 2·0 and 4·5 kg. were used, the average weight being 3 kg.

The animals were kept in separate cages, and were fed with either pellets or hay, and carrots, and had free access to water.

During the pre-experimental observation period of about one week, no symptoms of disease such as weight loss, diarrhoea, and sneezing were observed. The Wassermann reaction and TPI test were negative in all the animals before the experiments were started; these findings should exclude infection with *T. cuniculi* (Turner and Hollander, 1957).

Leucocyte and differential counts were made on blood samples from an ear vein in twenty animals; there were no pathological findings. All counts were within the normal limits (Schermer, 1958).

T. pallidum AND METHODS OF INOCULATION

The Nichols pathogenic strain (Nichols and Hough, 1913) of *T. pallidum* was used. The treponemes were obtained as a testicular suspension from the National Bacteriological Laboratory, Stockholm, and diluted with Nelson medium (Nelson and Diesendruck, 1951).

Number of organisms in the inoculations

These varied between $0{\cdot}012 \times 10^6$ and 144×10^6 and the amount of inoculum varied between 1 ml. in each testicle and 0·1 ml. in one testicle.

Site

The inoculations were mostly made intratesticularly, but, in a few cases, also intradermally and intrascrotally.

KILLED TREPONEMES

These were obtained by heating the testicular suspension twice to 56°C. for 40 and 30 minutes, with an interval of 8 hours.

GRADIENT CENTRIFUGED TREPONEMES

As pathogenic treponemes cannot be cultivated *in vitro* we used gradient centrifuged treponemes to obtain as pure a suspension of treponemes as possible. The Rathlev and Pfau (1965) method of preparation was applied, and the suspension was supplied by the National Bacteriological Laboratory, Stockholm. The treponemes are killed during this procedure.

FILTRATION OF TREPONEMES

A testicular suspension containing 54 $\times$ 10^6 treponemes per ml. was diluted with a sterile solution of saline to 21·6 $\times$ 10^6 treponemes per ml. This solution was then passed through a Seitz filter No. QEKS II, and 0·4 ml. of the clear filtrate was inoculated intratesticularly. Thorough investigation by dark-field microscopy did not disclose the presence of any treponemes in the filtrate.

TEMPERATURE MEASUREMENTS

The temperature was recorded rectally with an ordinary rectal mercury thermometer; this was inserted 3 cm. and the temperature recorded after 2 minutes. The measurements were made every second hour for 8 to 12 hours a day. In some of the experiments, in which penicillin had been injected before the inoculation, the temperature was recorded every second hour for 36 hours.

The temperature of all the rabbits was taken regularly twice to four times a day for from 2 to 7 days before inoculation with treponemes. The 'normal' temperature of the rabbits (number of animals 206) varied between 38·2° and 38·8°C. and the temperature in the laboratory between 21° and 25°C.

SEROLOGICAL TESTS

The TPI test was performed according to the method of Nelson and Mayer (1949) as modified by Laurell and Hederstedt (1958), and the Wassermann reaction according to the directions of Kabat and Mayer (1948) for complement fixation. Cardiolipin and a crude antigen (cholesterinized human heart extract) were used as antigens.

PENICILLIN

Procaine penicillin (Suspenin®, Kabi, Stockholm) was injected intramuscularly in a daily dose of 300,000 i.u.

ESTIMATION OF MORTALITY

In calculating the mortality, all rabbits that died within 14 days after inoculation were included.

Results

The following experiments were made in order to elucidate the aetiology of the fever reaction, and were especially intended to determine whether there was a connection between the reaction and the treponemes and to exclude the effect of any by-products.

EFFECT OF SIZE OF INOCULUM

The number of treponemes and the amount of solution inoculated varied as follows:

Group 1 Seven rabbits inoculated in one testicle with 0·012 $\times$ 10^6 treponemes in 0·1 ml. medium.

Group 2 Thirty rabbits inoculated in one testicle with 0·15–0·18 $\times$ 10^6 treponemes in 0·1 to 0·2 ml. medium.

Group 3 Twelve rabbits inoculated in both testicles or intrascrotally with a total of 90–142 $\times$ 10^6 treponemes in 1 to 2 ml. medium.

In all these groups almost the same type of fever occurred (Fig. 1). The mortality rate was highest when the largest amount of treponemes was used (75 per cent.).

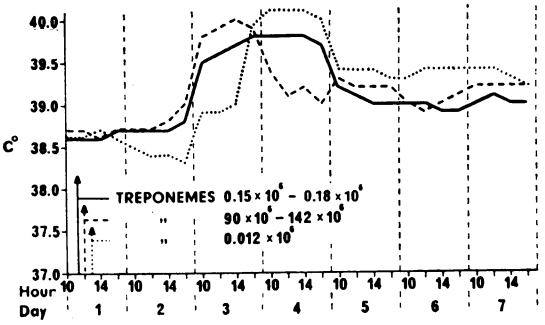

FIG. 1 *Mean temperature of thirty rabbits inoculated with 0·15–0·18 $\times$ 10^6 treponemes, twelve rabbits inoculated with 90–142 $\times$ 10^6, and seven rabbits inoculated with 0·012 $\times$ 10^6 treponemes*

Controls Seven rabbits were inoculated intratesticularly with 0·2 ml. Nelson medium and another seven with 0·2 ml. testicular suspension from uninfected animals. Eight rabbits were given a mixture of Nelson medium and testicular suspension, 0·2 ml. of the suspension being inoculated intratesticularly in each animal.

There were no changes in temperature or other symptoms and no deaths.

EFFECT OF KILLED *T. pallidum*

Seven rabbits were inoculated in both testicles with 90 $\times$ 10^6 heat-killed treponemes in 2 ml. medium, and twelve rabbits in one testicle with 0·18 $\times$ 10^6 heat-killed treponemes in 0·1 ml. medium. In Fig. 2 (overleaf) the temperature recordings from these animals are compared with the results obtained when

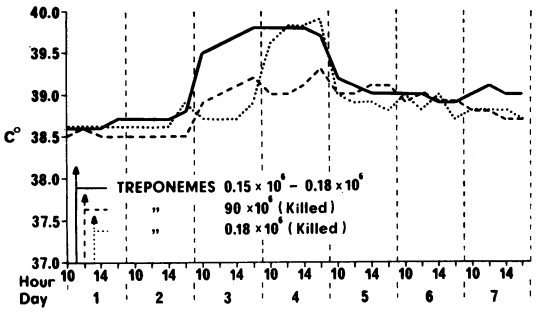

FIG. 2 *Mean temperature of thirty rabbits inoculated with 0·15–0·18 × 10⁶ treponemes, twelve rabbits inoculated with 0·18 × 10⁶, and seven inoculated with 90 × 10⁶ heat-killed treponemes*

using 0·15–0·18 × 10^6 living treponemes (in Group 2 above).

Even the killed organisms gave rise to a fever reaction in the same manner as did the living organisms. The size of the inoculum did not alter the type of reaction, and the mortality rate was almost identical in the two groups, 43 per cent. in the group with the larger and 50 per cent. in that with the smaller inoculum.

EFFECT OF PENICILLIN TREATMENT

In order to study the effect of treponemes killed by some means other than heating, the rabbits were treated with penicillin.

Twenty rabbits were given daily intramuscular injections of 300,000 i.u. procaine penicillin for 2 to 3 days before the inoculation with living treponemes. The inoculation dose was 0·18 × 10^6 treponemes in 0·2 ml. medium injected intratesticularly. Simultaneously with the inoculation the rabbits were given the same dose of penicillin, and this treatment was continued until the fourth day after inoculation. The temperature was taken every second hour for 30 hours after inoculation.

At the same time another group of ten rabbits was inoculated with the same dose of living treponemes without penicillin treatment.

Fig. 3 shows the mean temperature of these two groups. In the penicillin-treated group the temperature rose slightly between 4 and 10 hours after the inoculation, and fell to normal during the second day. On the third day all the rabbits in both groups showed the same typical fever reaction as the other groups previously described.

The mortality rate of the penicillin-treated rabbits was 60 per cent. and that of the non-treated 40 per cent.

Controls

Four rabbits were given daily injections of 300,000 i.u. procaine penicillin for 10 days and no fever reaction or other symptoms developed.

EFFECT OF INOCULATION WITH DENSITY GRADIENT CENTRIFUGED *T. pallidum*

The suspension was diluted with sterile physiological saline and the inoculation dose was 0·2 ml. containing 0·18 × 10^6 treponemes inoculated intratesticularly.

Nine rabbits were inoculated; in Fig. 4 the mean temperature is again compared with the results of

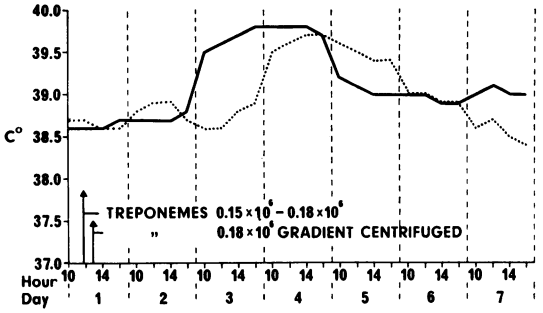

FIG. 4 *Mean temperature of thirty rabbits inoculated with 0·15–0·18 × 10^6 treponemes and nine rabbits inoculated with 0·18 × 10^6 gradient centrifuged treponemes*

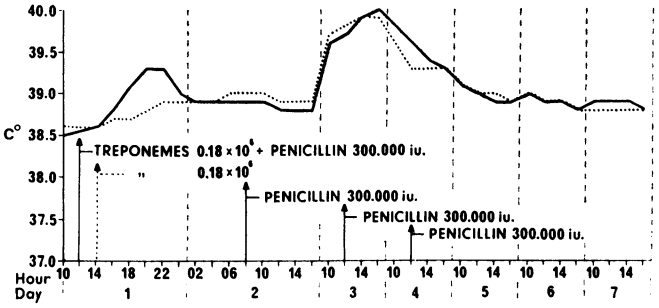

FIG. 3 *Mean temperature of twenty rabbits inoculated with 0·18 × 10^6 treponemes and treated with pencillin 2 to 3 days before and 4 days after inoculation; and of ten rabbits inoculated simultaneously with the same amount of treponemes but without penicillin treatment.*

the experiment in which inoculation was made with 0·15–0·18 × 10^6 living treponemes (Group 2) in ordinary testicular suspension diluted with Nelson medium. The fever reaction was much the same although the maximum rise in temperature occurred on the fourth instead of the third day.

The mortality rate was also almost identical with that in the other experiment, 44·4 per cent.

EFFECT OF INOCULATION WITH THE FILTRATE

In this experiment seven rabbits were inoculated intratesticularly with 0·4 ml. of the filtrate. In using this method we wished to obtain inocula containing no treponemes from previously infected testicular tissue. Since it is very difficult to be sure that the filtrate is completely free from treponemes by microscopical investigation alone, we checked the rabbits by means of the Wassermann reaction and the TPI test every third week for 6 months. None of the animals showed positive results. Of the seven inoculated rabbits one died spontaneously on the fifth day after inoculation. Fig. 5 shows that there was no typical temperature reaction comparable with that seen in the rabbits inoculated with 0·15–0·18 × 10^6 living treponemes (Group 2). No local reaction at the inoculation site was observed.

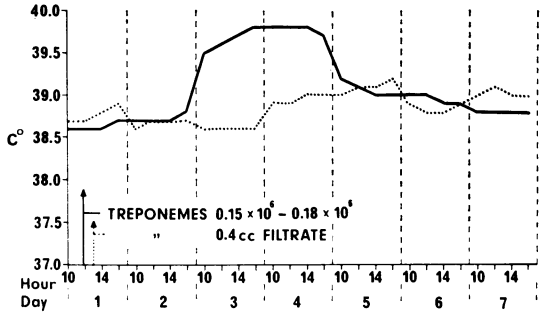

FIG. 5 *Mean temperature of thirty rabbits inoculated with 0·15–0·18 × 10^6 treponemes and seven rabbits inoculated with 0·4 ml testicular filtrate from previously infected rabbits*

EFFECT OF REPEATED INOCULATIONS

To eight rabbits that reacted with fever after the first inoculation with 0·18 × 10^6 treponemes, repeated inoculations were given at different intervals varying from 14 to 64 days after the first inoculation. After the first inoculation orchitis developed in all the animals after 2 to 3 weeks, and crusted erosions at the inoculation site after 3 to 4 weeks. The same number of treponemes and the same size of inoculum were used for the second as for the first inoculation. In all the rabbits except one (the one re-inoculated after 14 days) chancres had developed at the time of the second inoculation.

Fig. 6 shows the mean temperature of these rabbits after the second inoculation. In no case was a rise in temperature recorded.

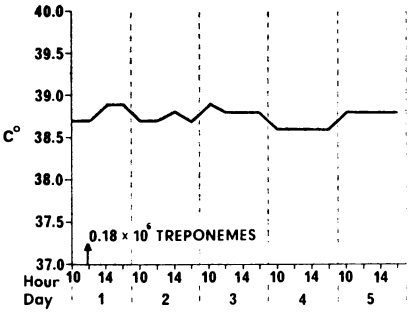

FIG. 6 *Effect of re-inoculation with 0·18 × 10^6 treponemes. Mean temperature of eight rabbits.*

MORTALITY

The Table (overleaf) summarizes the mortality rate in the different groups. The rate was highest when the largest inocula were used.

The symptoms, except for the fever reaction, agreed on the whole with those described by Jørgensen (1968), *i.e.* loss of appetite, atony, and tachypnoea. The microscopical investigation carried out on four animals also showed the same principal lesions as those described by Jørgensen: acute stasis and lung oedema, with hyaline thrombi in the capillaries of the heart with surrounding necrotic fibrils and eosinophilic cells.

Discussion

This remarkable reaction, which occurs in rabbits after inoculation with Nichols strain of *T. pallidum* and is characterized above all by fever after 2 to 3 days which persists for 5 to 10 days in the animals that survive, has not been previously described. The reaction entails a high mortality rate; death usually occurs 3 to 7 days after inoculation but in a few cases may be delayed until between the 12th and 14th day. It seems to be a new reaction pattern that has developed during recent years. The reaction is not restricted to any particular breed of rabbits since it took place without exception in more than 200 rabbits including several breeds found in Scandinavia. Nor did feeding (pellets or in some cases only grass, Swedish turnips, and carrots) influence the reaction. Age and weight, which varied within wide limits, did not appear to have any effect.

The mechanism is difficult to explain. The reaction is released only if the inoculum contains treponemes. Experiments with testicular tissue only,

TABLE *Mortality rates in the different experimental groups*

Group	*No. of rabbits*	*Mortality*		*Day of death*	
		No.	%	*3rd to 7th*	*8th to 14th*
0·15–0·18 × 10^6 treponemes	30	12	40	7	5
0·012 × 10^6 treponemes	7	2	28·5	1	1
90–142 × 10^6 treponemes	12	9	75	7	2
90 × 10^6 killed treponemes	7	3	42·9	0	3
0·18 × 10^6 killed treponemes	12	6	50	3	3
0·18 × 10^6 treponemes + pretreatment with penicillin	20	11	55	7	4
0·18 × 10^6 gradient centrifuged treponemes	9	4	44·4	1	3
Inoculation with filtrate	7	1	14·3	1	0
Reinfection with 0·18 × 10^6 treponemes	8	0	0	0	0

Nelson medium, a mixture of these components, or treponemal-free filtrate from previously infected testicular tissue produced no reaction. The reaction occurs whether the treponemes are dead or alive, as is evident from the experiments in which the treponemes were killed by heating, by penicillin treatment, or by gradient centrifugation.

The onset of fever 2 to 4 days after inoculation does not indicate that pyrogenic substances were the cause. The reaction occurs to the same high degree whether the dose is large or small. This may correspond with an immunological (allergic) release mechanism, the antigen being some component of the treponemes. However, the time lapse does not tally. Sensitization cannot reasonably be assumed to occur in the course of 2 to 4 days. Neither can there be previous sensitization with *T. cuniculi*, since all the animals were WR and TPI negative at the start of the experiment. If antibodies common to *T. pallidum* and *T. cuniculi* had developed, then the reaction should have occurred much more rapidly. The reaction cannot be repeated in the same animal, and it is not influenced by corticosteroids or by serum or blood from fever-resistant rabbits. These facts do not fit in with an allergic reaction.

Hence it seems that the treponeme is not the sole cause but starts a reaction of a hitherto unknown type. The characteristics of this reaction are a fever, with symptoms of lung oedema and circulatory insufficiency, that kills a high percentage of the animals.

Moreover, the microscopical investigation of different organs has given no indication of its aetiology. Further investigations are in progress.

Summary

Different breeds of rabbits inoculated with the Nichols strain of *T. pallidum* reacted with fever 3 to 5 days after inoculation.

The fever reaction was released by both dead and living treponemes and was of the same order of magnitude even if the inoculum varied in size. Gradient centrifuged treponemes gave rise to the same fever curve. On the other hand, the fever could not be repeated in the same rabbit. Nor did filtrate of the treponemal suspension cause fever. From 30 to 50 per cent. of the rabbits died between 3 and 14 days after inoculation.

References

GUDJÓNSSON, H., and SKOG, E. (1968a). *Acta derm.-venereol. (Stockh.)*, **48,** 15.

—— —— (1968b). 'Proceedings of 18th Meeting of Scandinavian Association of Dermatology, Turku, Finland'.

HEDERSTEDT, B. (1968). Personal communication.

JØRGENSEN, B. B. (1968). *Z. Versuchstierk.*, **10,** 46.

KABAT, E. A., and MAYER, M. M. (1948). 'Experimental Immuno-chemistry'. Thomas, Springfield, Ill.

LAURELL, A.-B., and HEDERSTEDT, B. (1958). *Acta path. microbiol. scand.*, **44,** 88,

NELSON, R. A., JR., and DIESENDRUCK, J. A. (1951). *J. Immunol.*, **66,** 667.

NELSON, R. A., and MAYER, M. M. (1949). *J. exp. Med.*, **89,** 369.

NICHOLS, H. J., and HOUGH, W. H. (1913). *J. Amer. med. Ass.*, **60,** 108.

PUTKONEN, T., and HELLE, J. (1951). *Ann. Med. exp. Fenn*, **29,** 137.

RATHLEV, T., and PFAU, C. J. (1965). *Scand. J. clin. Lab. Invest.*, **17,** 130.

SCHERMER, S. (1958). 'Die Blutmorphologie der Laboratoriumstiere', 2nd ed. Barth, Leipzig.

SKOG, E., and GUDJÓNSSON, H. (1966). *Acta derm.-venereol. (Stockh.)*, **46,** 136.

TURNER, T. B., and HOLLANDER, D. H. (1957). 'Biology of the Treponematoses'. WHO Monograph Series, No. 35. Geneva.

La fièvre après inoculation de *Tréponema pallidum* au lapin

SOMMAIRE

Différents lignées de lapins inoculés avec la souche Nichols de *T. pallidum* réagirent fébrilement 3 à 5 jours après l'inoculation.

La réaction fébrile apparut aussi bien avec des tréponèmes morts qu'avec des organismes vivants et fut d'une intensité du même ordre, même pour des quantités différentes d'inoculum. Des quantités croissantes de centrifugat de tréponèmes détermina la même courbe fébrile. D'autre part, la réaction fébrile ne put pas être répétée chez le même lapin. Le filtrat de la suspension tréponémique n'entraîna pas de fièvre. 30 à 50 % des lapins moururent entre 3 et 14 jours après l'inoculation.