

EXPERIMENTS TO INDUCE FEBRILE JARISCH-HERXHEIMER REACTION IN SYPHILITIC RABBITS WITH PENICILLIN AND ERYTHROMYCIN

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Abstract. A total of 25 syphilitic rabbits at various stages of the disease were treated with antibiotics (penicillin and erythromycin). No febrile JHR or focal symptoms were observed following the treatment, such as are commonly found in humans.

The Jarisch-Herxheimer reaction (JHR) after anti-syphilitic treatment has been studied intensively since it was first described by Jarisch in 1895 (8). Until recent years the investigations were based mostly on clinical studies on humans.

When penicillin became the main therapeutic agent for syphilis, this stimulated a renewed interest in the JHR.

Reports on experiments to produce JHR in laboratory animals hardly existed until 1949, when Sheldon et al. (11) described histologic and morphologic changes in the primary and secondary lesions in syphilitic rabbits, after treatment with penicillin, similar to the changes they had described in humans. The most characteristic histologic changes were an inflammatory reaction with vasculitis and leucocyte migration through the vessel walls into the surrounding tissues. According to these authors histologic changes in the syphilitic skin lesions in humans, after treatment with penicillin, constituted a more reliable criteria of the JHR than either fever or leucocytosis (6).

In another paper, Sheldon et al. (13) describe a JHR-like reaction in rabbits with *Spirillum minus* infections on administration of penicillin or immune serum from rabbits with *Spirillum minus* infection. The same histologic changes in the lesions were observed.

These findings led the same authors (12) to use serum pooled from several rabbits with untreated syphilis of 8-10 weeks' duration, since such serum is known to contain immobilizing antibodies

against *T. pallidum* (9). Each of 6 rabbits with skin syphilomas of 2 to 7 days' duration was then given intravenously 70 ml of this pooled serum. Histologic examination of the syphilomas of 5 of the 6 animals which had received syphilitic serum showed reactions similar to those they had described in lesions from syphilitic patients after penicillin therapy (11). In none of these investigations were febrile reactions in the rabbits studied.

In an investigation of the JHR in humans, Skog et al. (14) were unable to verify the histologic changes described by Sheldon et al. (11) in the lesions of syphilitic patients.

As far as the author is aware, in only one experiment on rabbits infected with syphilis have observations been made on fever after treatment with penicillin (10). In 1968 Gudjónsson et al. (2) made preliminary experiments on rabbits inoculated with the Stockholm substrain of the Nichols pathogenic *T. pallidum* to induce the JHR after penicillin injection. The results soon proved to be unreliable as the rabbits developed high fever in close connection with the inoculation of *T. pallidum*, and about 50% of the animals died without antisiphilitic treatment.

In a series of papers Gudjónsson et al. (2, 3, 4, 5) described this fever reaction and mortality in detail, and how it was possible to separate the *T. pallidum* from the fever-producing and lethal agent, and to prepare a testicular suspension containing numerous pathogenic *T. pallida* without fever reaction or mortality in rabbits inoculated with this suspension. This made it possible to obtain syphilitic rabbits in various stages of the disease.

The main aim of these experiments was to ascertain whether syphilitic rabbits, treated with

Table I. Type and dosage of antibiotics used in the JHR tests at different intervals after inoculation with *T. Pallidum*

Group	No. of rabbits	Day of treatment after inoculation	Antibiotics	
			Type	Dosage per kg body weight
I	5	4	Penicillin	50 000 IU
II	5	14	Erythromycin	0.025 g
III	5	21	Penicillin	50 000 IU
IV	5	35	Penicillin	50 000 IU
V	5	102	Penicillin	50 000 IU

penicillin and erythromycin at different stages of the disease, develop a febrile JHR.

MATERIAL AND METHODS

Rabbits

White Swedish country breed male rabbits were used in all the experiments, weighing approximately 3 kg at commencement. The animals were kept in separate cages, were fed with antibiotic-free pellets and had free access to water.

During the pre-experimental period, 3-7 days, no symptoms of disease, such as weight loss, diarrhoea, or evidence of respiratory infection, were observed in the animals used in the experiments.

Serological tests, WR and TPI were also negative in all the rabbits used to exclude infection with *T. cuniculi*.

T. pallidum and methods of inoculation

The Stockholm substrain of the Nichols pathogenic *T. pallidum* was used. The treponemes were obtained as a testicular suspension from the National Bacteriological Laboratory, Stockholm. The rabbits were inoculated intratesticularly in each testicle with 0.5 ml of the suspension containing approximately 25×10^6 of the *T. pallidum*, i.e., 50×10^6 treponemes in each rabbit.

Temperature measurements

The temperature was recorded rectally with an ordinary rectal mercury thermometer, which was inserted 3 cm and the temperature recorded after 2 minutes.

Base-line temperatures were established by recording the temperature of each rabbit at least 3 days before inoculation with treponemes, and regularly every second hour (8 a.m.—5 p.m.) for at least 7 days after the inoculation. When antibiotics had been injected intramuscularly in infected animals, the temperature was recorded every second hour for 12 hours after the injection, and then 2 to 3 times a day for at least 1 week.

The base-line temperature of the rabbits in the present series varied between 38.2° and 39.2°C before the inoculation of treponemes. The temperature in the laboratory varied between 21° and 25°C.

Infectivity tests

Testicular material obtained by puncture from all the rabbits was examined for motile treponemes by darkfield microscopy 4-14 days after inoculation. Serological tests, WR and TPI, were made 3 weeks after inoculation.

The JHR tests

These were made by injecting antibiotics at different intervals; 4 days, 2 weeks, 3 weeks, 5 weeks and 15 weeks respectively after inoculation with the testicular suspension of *T. pallidum*. To do this the rabbits were divided into five groups, each group consisting of 5 rabbits (Table I).

Inspection of focal symptoms, such as increased swelling, macroscopic changes in the lesions and palpation of regional lymph nodes was made regularly after the antibiotic treatment.

Antibiotics

Procaine penicillin (Suspenin, benzyl-procaine penicillin; Kabi, Stockholm, Sweden) was used in all groups except one, where erythromycin was used. The penicillin was injected intramuscularly in a dose of 50 000 IU per kg body weight in each rabbit. The erythromycin was also injected i.m., in a dose of 0.025 g per kg body weight in each rabbit of this group (Table I).

Determination of the concentration of penicillin and erythromycin in the plasma of the rabbits

The concentrations of penicillin and erythromycin in plasma were determined by a paper disc micromethod described by Jalling et al. (7). Capillary blood samples were taken from all rabbits in groups I, II and IV, 1, 3 and 5 hours after the injection. The tests were performed at the Department of Clinical Microbiology, Karolinska Hospital, Stockholm.

Controls

Five healthy rabbits were treated with penicillin and erythromycin respectively, and the temperature was recorded in the same way as in the test animals.

RESULTS

Temperature measurements after inoculation of *T. pallidum*

In no rabbit in any of the 5 groups was a rise in temperature observed after the inoculation, compared with the base-line temperature before inoculation. The observation period was 7-14 days, or until the antibiotic treatment was started.

Results of the inoculations

In every rabbit in all the groups, live *T. pallidum* was observed by darkfield microscopy after testicular puncture before treatment with penicillin or erythromycin. In those groups which were not treated until 3 to 15 weeks after inoculation, sero-

positivity (WR and TPI) was also confirmed. In all rabbits which were not treated until 14 days after the inoculation, orchitis had developed; and in those treated still later, primary ulcers were observed after 3 weeks.

Thus, it was possible to establish that all the rabbits had been infected with syphilis before antibiotic treatment was given.

Temperature measurements after antibiotic treatment

No rise in temperature, compared with the baseline temperature before inoculation, was recorded after penicillin or erythromycin therapy, i.e., the temperature varied only within the range of healthy rabbits (38.2–39.2°C) one of the most reliable signs of the JHR in humans. Those rabbits that had focal symptoms, such as orchitis and/or primary ulcers at the time of antibiotic treatment, were inspected regularly during the first 12 hours after treatment, but no definite changes were observed, which were signs of JHR. There was no difference between penicillin or erythromycin. No biopsies were taken for histologic studies.

Controls

No rise in temperature was observed in the healthy rabbits after treatment with either penicillin or erythromycin.

Antibiotic activity in blood plasma after injection of penicillin and erythromycin

The concentration of penicillin and erythromycin in the plasma of the rabbits was of the same magnitude as the concentration obtained in humans treated for syphilis (1). The average concentration of penicillin in the plasma was, after 1 hour, 3.4 µg/ml; after 3 hours, 2.9 µg/ml; and after 5 hours, 2.3 µg/ml. The corresponding values for erythromycin were 1.0, 0.6, and 0.3 µg/ml respectively.

DISCUSSION

The results of the experiments to induce febrile JHR in syphilitic rabbits at various stages of the disease cannot be compared with earlier experiments described by Sheldon et al. (11, 12, 13), who based their observations not on the fever reaction, but only on histologic changes in the

syphilomas. To develop this reaction they used serum from syphilitic rabbits, and when they describe the JHR after penicillin therapy, the animals had been infected with *Spirillum minus*, but not with *T. pallidum*.

The only study the author has found in the literature comparable with the experiments reported in this paper is that by Putkonen & Helle (10). Their findings are in agreement with those of the present investigation, i.e., that syphilitic rabbits do not react with fever after treatment with penicillin.

The reason for measuring the plasma concentration of the antibiotics used in the experiments was to verify that they were absorbed by the rabbits in sufficient quantities, compared with humans treated for syphilis (1). Erythromycin was used in one series of experiments to test another antibiotic used in the treatment of syphilis.

The reason why it is not possible, as in humans, to induce a febrile JHR in syphilitic rabbits after treatment with antibiotics, can only be a matter of speculation. The difference between man and rabbit may be due to the possibility that fever-producing substances are not activated by antibiotics in rabbits in the same way as in humans. Rabbits are known to be very sensitive to pyrogens. Therefore, it is unlikely that the JHR is caused by the liberation of common pyrogens.

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Received April 10, 1972

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