

genic Reiter treponemes did not belong to a species of *T. pallidum*. Király (1960a) compared Reiter treponeme to *T. pallidum*, but did not consider the two identical, even though they had common antigens. Dupouey (1963c) stated that the paucity of common

bonds between Reiter treponeme and *T. pallidum*, plus the morphological differences and the absence of pathogenicity of the Reiter treponeme, weakens the hypothesis of Reiter treponeme being a strain of *T. pallidum*.

2. HISTORICAL BACKGROUND

ORIGIN

Wassermann & Ficker (1922) reported success in cultivating 7 strains of *T. pallidum* from 80 to 90 syphilitic chancres, of which one strain, B-36, had produced typical syphilis in rabbits. The strain was passed from animal to animal, and Ficker succeeded in obtaining pure cultures of this treponeme from the rabbits.

Reiter (1926a, 1926b) described a medium containing horse serum, normal saline, and rabbit or guinea-pig kidney, or liver for the cultivation of *T. pallidum* and *S. dentium*. He mentioned that his colleague, Klopstock, had used Reiter's cultures of *T. pallidum* for the preparation of an antigen for the complement-fixation test in human and rabbit syphilis. The Berlin correspondent for *Lancet* (1926) stated that Dr Reiter reported to the Microbiological Society that he had succeeded in cultivating *T. pallidum* in a fluid nutrient, and from the pure culture had produced a vaccine which gave a typical reaction on being injected into children suffering from florid syphilis and showing a reactive Wassermann test. Reiter (1929) tested blood samples from 588 syphilitic cases with his cultured treponemes as antigen and with the Wassermann test, and had agreement in 498 cases. He attributed Wassermann-reactive and treponemal-antigen-non-reactive results in 30 sera to an organic inability or a weak ability on the part of the patient to form the specific antibody.

Recently, at the request of some of his colleagues, Dr Reiter (1960a, 1960b) published information regarding the origin of the Reiter strain of treponemes. After Ficker left the Kaiser-Wilhelm Institute of Experimental Therapy in 1923, Dr Reiter continued investigations and succeeded in isolating from 9 to 11 strains of treponemes from fresh cases of syphilis and proved the specificity by inoculation of rabbits. He stated: "There is no doubt that the so-called 'Reiter strain' is a genuine *T. pallidum*". Although several experiments were performed with "strain B.36", he reported: "Unfortunately, today I am not able to say definitely which strain I sent to

Mulzer in 1927, because all protocols have been lost!" Mulzer & Nothhaas (1928) inoculated two rabbits with a pure culture of treponemes, obtained from Reiter, which had undergone 200 passages in the culture medium. They expected these treponemes to be non-pathogenic, but, after 50 days, both rabbits developed syphilitic orchitis, and Mulzer & Nothhaas were able to infect other rabbits intratesticularly.

USE AND DISTRIBUTION OF THE STRAIN

Alcoholic extracts of treponemes were prepared by Klopstock (1926, 1927) from pure cultures of *T. pallidum*, isolated years before by Ficker, for use in complement-fixation tests. These cultures, continuously cultivated on Ficker's ascitic agar or Reiter's serum-saline medium, had become completely avirulent for rabbits. Reiter also furnished cultures of *S. dentium* and the spirochete of Weil's disease for comparative investigations with the *T. pallidum*.

Hoeltzer & Popoff (1928) obtained a Reiter treponemal culture from Klopstock. Witebsky (1929) obtained alcoholic treponemal extracts from Klopstock and inoculated those with a protein *Schlepper* (i.e., conveyor) into rabbits. The antisera from the rabbits reacted both with alcoholic treponemal extracts and with alcoholic extracts and aqueous suspension of brain tissue. Antibrain sera reacted with the treponemal extracts.

Fortner (1928) obtained from Dr Reiter strain 36—*der Pallida-Stamm Nr. 36 (Reiter)*—which had been continuously cultivated in liver bouillon for several years. He cultured this strain in an airtight Petri dish on rabbit blood agar partially inoculated with *Bacillus prodigiosus* (= *Serratia marcescens*) to consume the oxygen in the air space. The formation of visible, characteristic surface colonies took about one week.

Kroó & Schultze (1929) had two strains of treponemes—K22 cultivated by Kroó and R36 by Reiter—both verified as causative agents of syphilis. By using immune sera and skin tests they demonstrated

that the two strains apparently possessed different antigenic properties. Plaut & Kassowitz (1930) found that strain R32, furnished by Reiter, reacted exactly as strain R36, and confirmed the results of Kroó with strain K22. Virulence tests in rabbits showed all three strains to be avirulent. They concluded that K22 was a variant of *T. pallidum* and that R32 and R36 were of the general pallida type.

Gaetgens (1929b) was one of the first scientists to use the Reiter strain of treponeme as antigen in the serodiagnosis of syphilis. The antigen was an aqueous phenolized suspension of Reiter treponemes, and the complement-fixation test was called the Pallida-reaction. Fühner (1952) and Fühner & Gaetgens (1954) prepared a new Palligen antigen, consisting of an aqueous suspension of Reiter treponemes fragmented by ultrasonic waves.

D'Alessandro et al. (1949) demonstrated the presence of four antigens in the Reiter treponeme, of which only one had properties of a complete antigen, the others being considered as haptenes. Three of these antigens had corresponding antibodies in syphilitic serum and were: (a) specific treponemal thermolabile antigen with protein characteristics, *treponemico cotto labile* (TCL), a complete antigen; (b) thermostable treponemal antigen, polysaccharide in nature, *treponemico cotto stabile* (TCS); and (c) ubiquitous lipid antigen (L), exhibiting serological behaviour identical with alcoholic extracts of guinea-pig or beef-heart tissue. The fourth antigen was lipid in nature, similar to the organ-specific cerebral antigen isolated by Witebsky in 1927, and did not have a demonstrable antibody in syphilitic serum. D'Alessandro & Dardanoni (1953) described the extraction, purification, and serological behaviour of

the group treponeme specific protein antigen (TCL) in syphilis and called it ATPS (soluble protein antigen of the treponemata).

Gaetgens's old strain of treponemes from the Saxony Serum Works in Dresden was lost during the Second World War, and Fühner & Gaetgens (1954) obtained a new strain from Magnuson and Thayer in 1951, as did Cannefax (personal communication, 1961). Thayer (personal communication, 1961) stated that this strain was from Eagle. Oddo & Dardanoni (1947a) also obtained a new strain from Eagle for the preparation of Palligen after their strain was lost during the war. Roemer & Schlipkoter (1953) got cultures of Reiter treponeme from Eagle and from the Behring Company, Marburg, for their agglutination test. Eagle & Germuth (1948) stated that they had received living cultures of Reiter treponemes from Dr Reiter and Dr Beck. Beck (1939) used *S. [Spirocheta] pallidum* strain Reiter 36, but did not state the source. Rathlev & Pfau¹ used Reiter treponemes, B-36 type strain, from de Bruijn. Pucinelli (1951) mentioned that he had been using a strain from Gelperin that proved to be void of ubiquitous lipid antigen. Wallace & Harris (1958) used a strain from the Sylvania Chemical Company and Hardy & Nell (1961a) obtained a Reiter treponemal culture from D'Alessandro. Dupouey (1963b) obtained Reiter treponemes in 1954 at the Antigens Laboratory of the Pasteur Institute from Pautrizel of the Faculty of Medicine, Bordeaux. Nielsen² stated that the WHO Serological Reference Centre for Treponematoses, Copenhagen, distributed Reiter treponeme cultures to workers in several countries, but did not give the origin of this strain.

3. MORPHOLOGY

MOTILITY

Eagle & Germuth (1948), who studied 5 cultured strains of supposed *T. pallidum*, including the Reiter treponeme and 2 strains of mouth treponemes, stated that the morphology of cultured treponemes had varied among strains and within strains during a period of a few days, and the treponemes had shown varying motility. The cultured treponemes were significantly coarser than *T. pallidum*, but some cultures would have been difficult to differentiate from *T. pallidum*. Wilson & Miles (1955) stated that the group *Treponema* had no obvious axial fibre or

crista; the presence of metachromatic granules was doubtful; there were a number of primary spirals closely or loosely wound, and secondary turns sometimes developed during movement. Meinicke (1956b) observed the nature of the Reiter treponeme movement to be different from that of *T. pallidum*, but was not able to observe in Reiter treponemes the rotational and bucking movements or the preformed rigidity of the convolutions of the *T. pallidum*.

¹ Unpublished working document WHO/VDT/RES/40 of 25 July 1963.

² Unpublished working document WHO/VDT/RES/45 of 9 December 1963.