AN ACCOUNT OF THE SO-CALLED REITER TREPONEME* (HISTORY, ISOLATION, CULTIVATION, SPECIFICITY, AND UTILIZATION)

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As I have been asked many times by colleagues in Germany and abroad for information concerning the origin of the so-called "Reiter strain" of *Treponema pallidum*, I should like to give the following details:

These investigations were first carried out by M. Ficker at the Kaiser-Wilhelm Institute of Experimental Therapy in Berlin and were continued and completed by myself after Ficker had left the Institute for South America in 1923. Ficker had been able to isolate a number of strains of *T. pallidum* and I continued his investigations and also succeeded in isolating some strains of *T. pallidum* from fresh cases of syphilis. The specificity of all these strains has been proved by inoculation of rabbits. There is no doubt that the so-called "Reiter strain" is a genuine *Treponema pallidum*. Altogether, nine to eleven strains were isolated during that time in the manner described below.

By these experiments some strains were found to behave differently when inoculated into rabbit testes. A second superinfection would "take" after a rather short interval and produce either orchitis or only some small nodules in the testicles which could hardly be discovered. Sometimes a second infection would not "take" at all. Several experiments were performed with the strain B.36, but unfortunately to-day I am not able to say definitely which strain I sent to Mulzer in 1927, because all protocols have been lost!

I should like to emphasize strongly the proved virulence of this so-called Reiter strain, quoting the following paragraph verbatim: "We inoculated one ml. of a pure fluid culture which was kindly provided by Prof. Reiter and which had been subcultured about 200 times in the usual manner into each testis of two rabbits. As the living, somewhat coarse-looking spirochaetes were rather poorly motile, we fully expected that these pallidum-like culture spirochaetes would have lost their pathogenicity for rabbits. On the contrary, after an incubation period which lasted about 50 days, both rabbits developed a bilateral orchitis containing *numerous typical and extraordinarily motile spirochaetes*. We have also

succeeded in transmitting this orchitis to other rabbits and cultivating a so-called "Culture spirochaete strain", and we were able to transmit this strain up to the sixth testis passage." (Mulzer and Nothaas, 1928).

Apparently Mulzer's paper has been completely forgotten; it was never mentioned in any of the articles dealing with the so-called Reiter spirochaete, until Ruge, Knothe, and Otten (1957) rediscovered this publication and stressed its importance.

In 1948, Gaetgens, who had been working for many years with this so-called Reiter strain (Gaetgens, 1929, 1937), told me that he was convinced that the Reiter treponeme was a genuine *Treponema pallidum*. He said that the strain was lost in Germany during the war, but was brought back afterwards from the U.S.A. where it had been maintained in the meantime.

At the beginning of my investigations, the excised primary sores or condylomata were carefully ground under aseptic conditions. The ground material was directly inoculated into the lower half of the fluid media described in the appendix. The surface was sealed with vaseline or agar. Material for subculture was taken after 4, 5, or 6 days. Apparently contaminated tubes were discarded. Positive results depended always on the nutritional requirements of the Pallida-strain in question.

The following technique proved much more reliable:

The excised chancres or condylomata from untreated patients were most carefully minced and suspended in a fluid medium. All pieces were examined for the presence of *T. pallidum*. Rabbits were injected *intravenously* with these treponeme-containing suspensions and kept under observation for 7 to 10 weeks. If the inoculum had "taken", small nodules developed in the testicles. From these nodules treponemata were easily demonstrated and rabbits could be infected intravenously or intratesticularly. This injection technique was valuable as the establishment of pure cultures required some experience; a positive new inoculation into a rabbit proved the successful isolation of a *T. pallidum* strain.

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Some of these strains were experimentally employed by Klopstock (1926a, b) and Gaetgens (1929), who were able to develop a specific complement-fixation test. The antigen giving rise to the positive Wassermann reaction comprised two components which were quite different from each other: the usual antigen, which was *not* made of treponemes, represented the *non-specific component* of the test, whereas the *treponemal antigen* was the *specific component*. These results were confirmed by Eagle and Hogan (1940). I should like to emphasize that *all these findings are based on the isolation and pure cultivation of a genuine strain of T. pallidum*.

After a considerable lapse of time, d'Alessandro, Oddo, Comes, and Dardanoni (1949), d'Alessandro, Oddo, and Dardanoni (1950), and various Norwegian, American, English, French, and German scientists greatly increased our knowledge in this field.

During the years 1929–1931, E. Müller obtained strongly positive results in syphilitic children using *T. pallidum* vaccine; controls using vaccine made from *Spirochaeta dentium* gave negative results. During 1925–1927, 67 cases of paralysis, so-called "lost cases", were inoculated intracutaneously by Dr. W. Sagel of Arnsdorf, with living *T. pallidum* vaccine. The general condition of some patients was markedly improved, the Wassermann reaction reverted to negative, the Argyll Robertson pupil changes diminished, and the blood picture changed; 23 of these patients recovered partly, 34 remained essentially unimproved, and twelve died.

The most valuable outcome of these investigations is certainly the employment of the "Reiter strain" of *Treponema pallidum* for the *serological diagnosis of syphilis*. This antigen is much more specific than the classical reaction of Wassermann, and is already being used as an antigen in many countries. In 1958 and 1959 more than thirty authors published papers describing its use (see Bibliography).

Examination of the following questions about the biological properties of *T. pallidum* is recommended:

Are there differences of virulence among individual strains of *T. pallidum* and if so, what is the reason? Also, is the virulence of the strain a stable property?

Have the various strains of *T. pallidum* different nutritive requirements? Are these requirements different in human beings and experimental animals such as monkeys or rabbits?

Are certain strains of *T. pallidum* associated with general paralysis? Have such strains special nutritive requirements or is some feature of cholesterol metabolism

responsible for its development? Is it perhaps possible in this connexion to prevent general paralysis by developing an immunobiological therapy based on the cultivation of strains of *T. pallidum* found in the brains of paralytics?

Sometimes it is profitable to look backwards in scientific questions; this often avoids repeating work already done and leads deeper into the centre of scientific problems than letting slide the streams of time!

Summary

After some historical introduction a concise description is given of the technique of isolation and maintenance of *Treponema pallidum* (Reiter strain), its proved virulence and specificity, and its utilization for sero-diagnostic purposes. The non-specific and specific components of the Wassermann reaction were discovered as early as 1926 by scientists working with the "Reiter strain" of *Treponema pallidum*.

Earlier experiments using the "Reiter strain" of *T. pallidum* for therapeutic purposes are mentioned and some questions in connexion with this strain in diagnosis and therapy are posed.

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Exposé de l'ainsi nommé tréponème de Reiter

Résumé

Après un préambule historique, on présente une description concise du procédé d'isolation et de maintien du *Treponema pallidum* (souche Reiter), de sa virulence et spécificité prouvées et de son utilisation séro-diagnostique. Les composants spécifiques et non-spécifiques de la réaction de Wassermann furent découverts déjà en 1926 par des hommes de science utilisant la "souche Reiter" du *Treponema pallidum*.

On mentionne les premières expériences utilisant la "souche Reiter" du *T. pallidum* pour des fins thérapeutiques et on pose quelques questions à propos de cette souche dans le diagnostic et traitement.

APPENDIX

Cultural Requirements of *T. pallidum*

Solid Media

Mix equal parts of horse serum and 1 per cent. normal saline; place on the bottom of the tubes sterile pieces of rabbit liver or kidney. Heat to 56°C. on two consecutive days. On the 3rd day heat to 70–80°C. for coagulation. Each tube should contain 8 to 10 ml. medium.

Inoculation should be performed with glass capillaries in the lower half of the tube, just above the pieces of liver. *T. pallidum* produces neither liquefaction nor splitting of the medium and there is no odour. For subculture, the tube has to be opened aseptically, cutting it off with a glass file in the lower third. Subculture is performed after microscopical examination for treponemata. They grow generally most abundantly just above the pieces of tissue.

Fluid Media

(1) Mix equal parts of sterile rabbit serum with 1 per cent. normal saline. Add small pieces of rabbit or guinea-pig brain. Heat for 24 hrs at 37°C. Place for another 24 hrs at 37°C. to check sterility. Inoculation is done as described before. Seal with sterile

paraffin or white vaseline. Incubate at 37°C. Optimal growth is obtained after 4 to 5 days. The cultures remain almost completely transparent and there is no odour. Subculture after 7 days.

(2) *Autolysate-saline-Ascitic Fluid*.—Place beef liver (without gall-bladder) aseptically at 37°C. for 24 hrs. Filter the expelled liquid. Examine for sterility after 24 hrs at 37°C. Mix equal parts of 10 per cent. autolysate in equal parts of 1 per cent. normal saline and ascitic fluid.

Incubation and sealing as in (1). Optimal growth after 2 days. Microscopically no changes of the medium are observed and there is no odour. Subculture after 4 days.

(3) To 250 g. beef liver (gall free) add 800 ml. tap water, autoclave for one hr, add 3 per cent. peptone and 0.5 per cent. NaCl. Boil for 30 min. Add 2 per cent. dextrose, boil for 30 min. Cut boiled liver to small pieces 1 to 2 cm. in length and sterilize for 60 min. Place 3 to 5 pieces of liver in the tubes containing 8 ml. liver bouillon. Sterilize in the autoclave.