REITER TREPONEME IN SYPHILIS SEROLOGY*

BY

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Origin

Attempts carried out about the year 1920 at the Kaiser-Wilhelm Institute of Experimental Therapy in Berlin to culture treponemes from eighty to ninety patients with primary syphilis, were successful in seven of these cases. One of the strains obtained, denoted as B 36, was said to have remained pathogenic for rabbits (Wassermann and Ficker, 1922). Reiter (1960), who continued the work of these authors, succeeded in isolating some additional strains. Investigating the nature of the treponemal antigens, Beck (1939) used the B 36 strain. Eagle and Germuth (1948) found this strain to be identical in its growth properties and serological specificity with a transplant of the same strain, obtained from Reiter in 1939. In Germany, the strain was lost during the war but regained from the U.S.A. in 1951 (Fühner and Gaetgens, 1954). Thus, all experiments with the Reiter treponeme since the second world war are likely to have been carried out with the B 36 strain. This fact is unquestionable with regard to the studies of antigenic structure by D'Alessandro and Dardanoni (1953), who obtained their strain from Eagle, and by De Bruijn (1956), who obtained his strain from Dardanoni. Pillot and Faure (1959) received their strain from Pautrizel, who only made reference to the work of Wassermann and Ficker (1922) (Pautrizel, Bonnardot, and Szersnovicz, 1957).

Use of Suspensions in Syphilis Serology

About 1930, flocculation tests for syphilis had been improved so much, that a higher level of sensitivity could be obtained by these techniques than by complement fixation (Gaetgens, 1930).

Whereas in the U.S.A. the deficiency in complement fixation was solved by the development of new techniques (Kolmer, 1929; Wadsworth, Maltaner, and Maltaner, 1931), in Germany, Gaetgens (1929) tried to obtain a better sensitivity by introducing a phenolized suspension of Reiter treponemes as an antigen in the Wassermann test. The commercial preparation "Palligen" (Sächsisches Serumwerk, Dresden) has been subjected to a great number of serological evaluations, enumerated by Erickson and Eagle (1940). These authors concluded that "Palligen" was superior both in sensitivity and in specificity

to beef heart antigen. The narrow range between the antigenic and the anticomplementary concentration, however, was considered to be an obvious drawback to its application in a routine test. In the present German preparation, "Pallida-Antigen" (Promonta, Hamburg), the antigenic activity of the suspension is enhanced by ultrasonic treatment (Fromm, 1954). A similar suspension, "Pallignost" (Istituto Sieroterapico Milanese Serafino Belfanti, Milano), has been evaluated by a number of investigators (Pautrizel, Szersnovicz, and Gimenez, 1953; Benazet, Brottes, Thivolet, and Sohier, 1954; Hardy, Bornand, and Durel, 1955; Mutermilch and Delaville, 1955; Wilkinson, 1957).

An agglutination test in which a formalinized suspension of Reiter treponemes (Behring-Werke, Marburg) is used, has been practised with cerebrospinal fluid (Roemer and Schlipköter, 1953) and serum (Roemer and Schlipköter, 1955).

A fluorescent antibody test employing fresh Reiter treponemes has been reported to compare favourably in sensitivity and specificity with the same test in which virulent *T. pallidum* was used (Covert, Kent, and Stevens, 1961).

Chemical Fractionation

Immunization of rabbits with suspensions (Klopstock, 1926; Hippius, 1954; Fromm, 1955) or with an alcoholic extract from Reiter treponemes (Fühner, 1957) caused the sera of these animals to become positive in the complement-fixation test with beef heart extract and cardiolipin antigen. On the other hand, alcoholic treponeme extracts were reactive as antigens in the complement-fixation test with syphilitic serum (Klopstock, 1926; Beck, 1939). Although the presence of ubiquitous lipid in the Reiter treponeme is evident, the serological activity of this fraction in treponeme suspensions is open to discussion. Absorption of syphilitic serum with lipid antigen did not reduce the titre of this serum in the complement-fixation test with a treponeme suspension (Beck, 1939; Eagle and Hogan, 1940; D'Alessandro and de Blasi, 1941) or reduced it only slightly (Gaetgens, 1932; Jeney, Csóka, and Biró, 1957). Absorption with treponemes removed the anti-lipid antibody from syphilitic serum partially (Gaetgens, 1932; Beck, 1939; Jeney, Csóka, and

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Biró, 1957) or completely (Eagle and Hogan, 1940; D'Alessandro and de Blasi, 1941). Generally, the ubiquitous lipid in the Reiter treponeme appeared to be present in a serologically less active form (Puccinelli, 1951).

Besides the ubiquitous lipid, D'Alessandro, Oddo, Comes, and Dardanoni (1949) demonstrated the presence in the Reiter treponeme of a substance serologically related to brain lipid, thus confirming the observations of Witebsky (1929). In syphilitic serum, no antibody against this lipid exists (Puccinelli, 1951).

When dialysing a cryolysate of Reiter treponemes against distilled water, D'Alessandro, Oddo, Comes, and Dardanoni (1949) obtained a fraction which they concluded to be a protein on account of the thermolability and the destruction by papain of its serological activity with syphilitic serum. Subsequently, D'Alessandro and Dardanoni (1953) reported an improved method for preparing this so-called Reiter Protein (RP) antigen by precipitation with ammonium sulphate. The same procedure was practised by De Bruijn (1957), Cannefax and Garson (1957) and Wallace and Harris (1958). De Bruijn (1960) used ultrasonic disintegration instead of cryolysis and substituted a one-course salting-out for the original stepwise procedure. Pillot and Faure (1959) introduced a lipid extraction with butanol of the treponemes before ultrasonic treatment and precipitated the RP antigen by adding acetic acid to the lysate.

In the complement-fixation test with human syphilitic serum, RP antigen usually has an optimal activity in dilutions of 1:64 (D'Alessandro and Dardanoni, 1953), 1:100 to 1:150 (Pillot and Faure, 1959) or 1:80 (De Bruijn, 1957). Besides this optimum, De Bruijn (1958b) showed the presence of another one in the lowest antigen dilution; from absorption experiments he concluded that this optimum was due to the activity of a ubiquitous lipid component in RP antigen with "reagin" of the serum.

Heating RP antigen at 78°C. for 60 minutes destroyed its reactivity with human syphilitic serum and with anti-Reiter treponeme guinea-pig serum, whereas with anti-Reiter treponeme rabbit serum a slightly diminished reactivity remained, which has been ascribed to denatured protein (D'Alessandro and Dardanoni, 1953). De Bruijn (1959), observing the same persistent reactivity between heated RP antigen and anti-RP rabbit serum, assumed the presence in the antigen of a polysaccharide component, which subsequently could be isolated by ethanol precipitation after trypsin digestion of the

protein. The complexity of RP antigen has also been demonstrated in the gel precipitation test by Pillot, Dupouey, and Faure (1960). In comparison with anti-RP rabbit serum, anti-RP guinea-pig serum shows a much weaker reactivity with the polysaccharide component; with regard to the protein component the reverse is true (De Bruijn*). The presence in human syphilitic serum of an antibody against Reiter (lipo)polysaccharide is questionable. Discrepant results have been obtained with different preparations (Beck, 1939; D'Alessandro, Oddo, and Dardanoni, 1950; D'Alessandro and del Carpio, 1958; Pillot, Dupouey, and Faure, 1960). Recently, De Bruijn* found twelve out of 205 syphilitic and three out of 597 non-syphilitic sera to be positive in the complement-fixation test with Reiter polysaccharide antigen. The low incidence in syphilis, although twelve times higher than in non-syphilitic cases, makes it unlikely that the anti-Reiter polysaccharide antibody is provoked by the syphilitic infection.

Generally, the reactivity of RP antigen with syphilitic serum is only brought about by the protein component. By means of absorption experiments, a common protein antigen has been demonstrated in virulent *T. pallidum* and in the Reiter treponeme (Dardanoni and Censuales, 1957; Cannefax and Garson, 1959). Moreover, De Bruijn (1961) showed its presence in *T. zuelzeri*. This free-living organism, which has been isolated from mud by Veldkamp (1960), differed from the Reiter treponeme in the polysaccharide fraction.

D'Alessandro and Zaffiro (1961) observed a specific immobilization when motile Reiter treponemes were incubated together with anti-Reiter rabbit serum and complement. From the promptness of this phenomenon, the absence of a similar non-antigenic mucopolysaccharide which is supposed to coat virulent *T. pallidum* (Metzger, Hardy and Nell, 1961) may be concluded.

In addition to complement fixation, RP antigen has been applied in the Boyden (1951) hemagglutination technique by Lamedica and Robert (1957).

Application of RP Antigen in Syphilis Serology

Most of the serological evaluations have been carried out with antigen prepared according to the method of D'Alessandro and Dardanoni (1953). The results of those investigations, in which the sera were classified in a syphilitic and in a non-syphilitic group and in which every specimen was subjected to the tests indicated, are shown in Table I (overleaf).

* Investigations as yet unpublished.

TABLE I
COMPARISON OF RPCF TEST, STS, AND TPI TEST IN CLASSIFIED SERA

Authors	Date	RPCF Technique	(Presumably) Syphilitic Sera				(Presumably) Non-syphilitic Sera			
			Total	RPCF +	STS +	TPI +	Total	RPCF +	STS +	TPI +
Cannefax and Garson	1957	1/5-Volume Kolmer test with 2 "exact units" of complement	765	632	—	577	615	7	—	0
De Bruijn and Bekker	1957	Own technique (De Bruijn, 1958a)	143	140	130	133	155	7	29	0
DeGroat and Miyao	1958	1/5-Volume Kolmer test with 2 "exact units" of complement	148	112	133	—	44	1	10	—
Miller, Boak, and Carpenter	1958	1/5-Volume Kolmer test with 1½ "exact units" of complement	189	180	165	177	180	2	92	0
Bekker	1959	1/2-Volume Kolmer test with 2 "full units" of complement	368	347	—	350	377	8	—	2
		1/2-Volume Kolmer test with 1½ "exact units" of complement	187	185	—	179	168	4	—	0
Kjellander, Sievers, and Vogelsang	1959	1/5-Volume Kolmer test with 2 "exact units" of complement	306	253	—	261	175	57	—	48
Sequeira	1959	Modified Whitechapel technique	89	62	74	79	—	—	—	—
Wilkinson and Johnston	1959	Modified Whitechapel technique	133	98	—	117	—	—	—	—
Bauer and Pinke	1960	1/5-Volume Kolmer test with 2 "exact units" of complement	195	141	189	—	90	9	61	—
Berner, King, and Reich	1960	1/5-Volume Kolmer test with 2 "exact units" of complement	91	69	75	—	40	11	32	—
Kiraly	1960	Own technique	119	99	106	112	81	2	24	0
Peterson, DeHaven, Wright, and Miller	1960	1/5-Volume Kolmer test with 1½ "exact units" of complement	27	17	—	15	15	4	—	6
Patton and Gaurie	1961	1/5-Volume Kolmer test with 2 "exact units" of complement	424	343	424	—	99	16	99	—

Of course, criteria for including sera in the non-syphilitic group differ from author to author. The great number of positive TPI (*Treponema pallidum* immobilization) test results obtained in this group by Kjellander, Sievers, and Vogelsang (1959) seems rather sensational. The high incidence of "biologic false positive" (BFP) reactions, *i.e.* positive STS (Standard Tests for Syphilis) results in non-syphilitic cases, suggests that investigations have been carried out with preselected sera. In a random population, this incidence is much lower; of 10,000 sera forwarded for routine serological testing to the Regional Laboratory of Public Health in Utrecht, 125 were reactive in the STS, and ten of them were BFP (Bekker, 1960). The results obtained with syphilitic sera show the Reiter protein complement-fixation (RPCF) test to equal the TPI test in *sensitivity*, provided that an adequate complement-fixation technique is used. The results obtained with non-

syphilitic sera show the *specificity* of the RPCF test to be only slightly less than that of the TPI test, and much better than that of the STS. This is also evident from the results obtained with the RPCF test in diseases which are frequently associated with BFP reactions (Table II).

Other investigations, which have not been included in Tables I and II or referred to in the text, are recorded in Table III; generally, their results agree with the conclusions mentioned above. Miller, Carpenter, Boak, Peterson, Pait, Lawrence, Heidebreder, Linscott, and Fazzan (1961) found a considerable number of specimens showing discrepant RPCF test results upon retesting. Bissett, Browne, Coffey, and Michelbacher (1961) found this lack of *reproducibility* to be connected with the great number of weakly-positive reactions obtained in the RPCF test. Bekker (1962) stressed that, whereas specificity in serological tests is determined by the

TABLE II
COMPARISON OF RPCF TEST, STS, AND TPI TEST IN DISEASES FREQUENTLY ASSOCIATED WITH BFP REACTIONS

Authors	Date	Clinical Entity	Total	RPCF +	STS +	TPI +
D'Alessandro and Dardanoni	1953	Leprosy	19	4	10	—
Rein, Kelcec, D'Alessandro, and De Bruijn	1957	Lupus erythematosus	15	0	—	—
Cannefax, Ross, and Bancroft	1959	Leprosy	248	8	94	12
Wilkinson and Johnston	1959	Leprosy	86	7	—	11
Lynch, Kimball, and Kernan	1960	Smallpox vaccination	212	1	57	—

nature of the antigen, sensitivity and reproducibility entirely depend on the technique.

From the divergence of results obtained in serological evaluations, it is evident that the RPCF test, STS, and TPI test detect different antibodies. For a correct interpretation of test results, knowledge of the antibody pattern in syphilitic infection is important. In experimental rabbit syphilis, Bekker and Onvlee (1959) found the STS to become positive 7 to 8 days after inoculation, followed shortly afterwards by the RPCF and TPI tests. The same sequence has been noticed in human primary syphilis by Pariser (1960). Brown and Bunch (1959), however, found the RPCF test to become reactive after infection almost as rapidly as the STS. Upon treatment, in both experimental and human syphilis, TPI and RP antibodies took longer to disappear than "reagin". In treated late stages of treponemal infection, TPI and RPCF tests remain almost invariably positive in spite of therapy. Consequently, the greatest value of the RPCF test lies in its use as

an aid to diagnosis rather than in its use as a test of cure.

Summary

(1) The origin of the Reiter treponeme and the application of suspensions of the organisms as antigens in complement-fixation, agglutination, and fluorescent antibody tests for the serological diagnosis of syphilis are reviewed.

(2) Work on the chemical fractionation of the Reiter treponeme into lipid, protein, and polysaccharide or lipopolysaccharide components is described. Although Reiter protein antigen contains some lipid, this latter is not reactive when the antigen is used at its optimal titre, reactivity with syphilitic sera being due to the protein component. A common protein antigen has been demonstrated in virulent *T. pallidum*, the Reiter treponeme, and *T. zuelzeri*, a free-living organism isolated from mud.

(3) The results of serological evaluations of Reiter protein antigen reported in the literature are presented. The RPCF test is thought to equal the TPI test in sensitivity provided that an adequate complement-fixation technique is used. The specificity of the RPCF test is slightly less than that of the TPI test but much better than that of tests using lipoidal antigens. The RPCF test is more useful as an aid to diagnosis than as a test of cure.

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TABLE III
VARIOUS COMPARATIVE INVESTIGATIONS

Authors	Date	Scope
Bossak, Falcone, Duncan, and Harris ..	1958	Comparison between RPCF and TPI tests with 1,567 selected sera
Daguet, Pillot, and Faure	1958	Comparison of different antigen preparations in the RPCF test with STS and TPI test on 184 syphilitic, 25 BFP, and 94 normal sera
Foster, Nicol, and Stone	1958	Comparison between RPCF test and STS in 1,000 unselected cases
Kostant and Kelcec ..	1958	Comparison between RPCF and other treponemal tests with 235 syphilitic and 167 BFP sera
Foster, Nicol, and Stone	1959	TPI test results of 151 sera showing discrepancies in STS and RPCF test
Kostant, Landy, Miller, and Kelcec	1959	Comparison between RPCF test, STS, and TPI test with 91 syphilitic and 96 non-syphilitic spinal fluids
Berner, Reich, and King	1960	Comparison between RPCF test and STS with 3,122 sera
Brown, Price, and Simpson	1960	Comparison between RPCF and other treponemal tests in SERA and Macon County serological evaluations
Lynch and Binder ..	1960	Study of RPCF test with 86 syphilitic, sixteen BFP, and 44 problem sera
Simpson, Harris, Garson, and Bunch ..	1960	Comparison between RPCF, other treponemal tests, and STS with 209 syphilitic sera in the Macon County serological evaluation
Vogelsang, Pillot, and Faure	1961	Comparison of two antigen preparations in the RPCF test with STS on 1,078 sera

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Le tréponème de Reiter dans la sérologie de la syphilis RÉSUMÉ

(1) L'auteur passe en revue l'origine du tréponème de Reiter et l'emploi des suspensions de cet organisme comme antigène dans la réaction de fixation du complément, l'agglutination et la réaction d'immunofluorescence dans le sérodiagnostic de la syphilis.

(2) Le fractionnement chimique du tréponème de Reiter en composées de lipide, de protéine et de polysaccharide ou de lipopolysaccharide est décrit. Quoique l'antigène protéique de Reiter contienne des lipides, ceux-ci ne sont pas sérologiquement actifs, pourvu que l'antigène soit employé en dilution optimum. Dans ce cas l'activité sérologique vis à vis du sérum syphilitique est provoquée uniquement par le composant protéique. Un antigène protéique commun a été démontré dans le *T. pallidum* virulent, le tréponème de Reiter et le *T. zuelzeræ*, un organisme qui a été isolé de la boue.

(3) Les résultats de l'évaluation sérologique de l'antigène protéique de Reiter décrits dans la littérature sont passés en revue. La sensibilité de la réaction de fixation du complément avec l'antigène protéique de Reiter est presque analogue à celle de la réaction TPI, si du moins une technique appropriée de fixation est employée. La spécificité est un peu inférieure à celle du test TPI, mais elle est supérieure à celle des réactions employant des antigènes lipidiques. Le test RPCF est plus indiqué pour le diagnostic que pour le contrôle des résultats de traitement.