unheated antigen and remained 1:20–1:80 with heated antigen. (The titer with unheated antigen was not a measurement of antibody to the heat stable substance since the unheated antigen was diluted 1:5 and the heated antigen was used undiluted.)

Discussion

It was demonstrated by absorption techniques that TPCF antigens contained an antigenically distinct substance or complex which was stable to heating at 100° C. for 60 minutes. Different antigens varied greatly in their content of this substance.

In experiments not reported here the antibody to heated TPCF antigen was demonstrated in the serum of rabbits infected with yaws, *Treponema cuniculi*, or bejel, as well with syphilis, and in syphilitic serum from which reagin had been removed by absorption with VDRL antigen. Also, its presence was demonstrated by the tpcf-50 test (*19*).

Of particular interest in the present study were the findings in regard to the persistence of this antibody in treated latent syphilitic rabbits. In TPCF tests 5 months after treatment, 8 of 22 rabbits showed a significant decline in titer with unheated antigen, but no decline in titer with heated antigen. In experiments described in the preceding paper rabbits were tested 2½ years after treatment, and each of 24 animals showed the same titer with unheated and heated antigen, both diluted 1:5. In the light of the present study, it seems probable that the TPCF tests on the earlier rabbits measured only the antibody to the heat stable portion of the antigen.

Although the present studies were curtailed because of lack of antigen, it would be of the greatest interest to compare the persistence of the antibody to heat stable TPCF antigen with the persistence of the TPI antibody in both untreated and treated latent syphilis. While certain results reported in the fourth paper of this series suggest that these two antibodies may be in some degree related, there is no evidence that they are identical.

Summary

The reactivity of TPCF antigens was reduced in direct proportion to the temperature at which they were heated.

Different lots of antigen showed marked variation in reactivity after heating at 100° C. for 60 minutes.

Absorption of human syphilitic serum with unheated TPCF antigen removed the reactivity with both unheated antigen and antigen which had been heated at 100° C. for 60 minutes.

Absorption of human syphilitic serum or anti-TPCF rabbit serum with heated antigen removed the reactivity of both serums with heated antigen but caused no decline in their titers with unheated antigen.

The antibody to heated TPCF antigen arose later in syphilitic infection than the antibody to unheated TPCF antigen.

Treated latent syphilitic rabbits which showed a significant decline in titer with unheated antigen showed no decline in titer with heated antigen.

Relationship Between TPCF Antibody and Reagin

Charlotte P. McLeod, Sc.D.

IN EXPERIMENTS reported in the first paper of this series it was demonstrated that injections of TPCF antigen caused the development of TPCF antibodies in normal rabbits, and this was accompanied by the development of reagin in a large proportion of animals. On the other hand, our unpublished experiments and also studies of Portnoy and Magnuson (*9*) have shown that the removal of reagin from numerous syphilitic serums by absorption with VDRL antigen caused no decline in their TPCF antibody titers.

These apparently conflicting findings suggested the possibility of a cross relationship between TPCF antibody and reagin, or that under some conditions the TPCF test might

be a measurement of reagin. These problems were the subject of the present study. It will be shown that TPCF antibody per se is not related to reagin, but that TPCF antigens were contaminated with a component which was reactive with reagin in both syphilitic and nonsyphilitic serum.

Also, it will be shown that the failure of VDRL absorbed syphilitic serum to decline in TPCF titer is not a valid criterion for the absence of reagin reactive material in the antigen. This contaminating component was present to some degree in all of 21 antigens available for study. The results suggest that some approved antigens were sufficiently contaminated to give false positive diagnostic TPCF tests.

Methods

The methods used were similar to those described in the two preceding papers. TPCF antigens of approved reactivity for the TPCF test were obtained from Dr. Joseph Portnoy or were purchased from commercial sources. Antigens of substandard reactivity were donated by Dr. Portnoy and by the Cappel and Difco Laboratories.

TPCF tests were run by the original method of Portnoy and Magnuson (*9*). Serums were diluted 1:5 and then in serial twofold dilution. VDRL tests were run by the method of Harris, Rosenberg, and Del Vecchio (*13*) although in one instance tests were performed on serums diluted in Kolmer saline (table 3). Tpcf-50 tests (*19*) were run in Dr. Portnoy's laboratory. Anti-VDRL serum, or nonsyphilitic serum containing reagin, was produced by the method of Eagle (*21*) in normal rabbits whose serum was initially negative in VDRL and TPCF tests. The animals were injected with lipoidal antigen-antibody precipitate obtained by absorbing 65 cc. of human syphilitic serum (VDRL titer 1:64) with the sediment from 920 cc. of VDRL antigen which had been prepared as for testing. The pooled washed precipitate was diluted in saline so that each dose was contained in 5 cc. Rabbits were inoculated intraperitoneally with nine doses each during a period of 20 days, and were bled 2 days following the last injection.

Positive control serum pool E was human syphilitic serum obtained from the Public Health Service Rapid Treatment Center in Durham, N.C. Human syphilitic serum pool X and presumably nonsyphilitic leprosy serum were supplied by George Cannefax. The leprosy serums were obtained from patients at the Public Health Service Hospital at Carville, La., and had been extensively studied by Cannefax, Ross, and Bancroft (*22*).

Results

Reactivity of TPCF antigens with artificially prepared reagin serum (anti-VDRL rabbit serum). Three antiserums with VDRL titers of 1:256, 1:32, or 1:4 were used. Twenty-one

Table 1. TPCF titers of anti-VDRL reagin serums in tests with approved reactive antigens

TPCF antigen	Approved dilution	Dilution tested	TPCF titers		
			Control pool E (VDRL 1:32)	Reagin serum 5437 (VDRL 1:256)	Reagin serum 5330 (VDRL 1:32)
S25AB	1:5	1:5	1:160	1:80	Negative.
Pool X67	1:5	1:5	1:160	1:160	Do.
Pool Z3	1:5	1:5	1:160	1:80	
Pool Z5	1:5	1:5	1:160	1:160	
Pool Z6	1:5	1:5	1:160	1:80	1:10
132	1:5	1:5	1:160	1:20	
S22AB	1:10	1:10	1:160	1:40	Negative.
196A	1:10	1:10	1:160	Negative.[1]	
196A	1:10	Undiluted	----------	1:320	
Pool Q	1:10	1:10	1:160	Negative.[1]	
Pool Q	1:10	Undiluted	----------	1:160	

[1] In 1:10 dilution (1:5 was anticomplementary).

Table 2. TPCF titers of anti-VDRL reagin serums in tests with antigens of substandard reactivity

TPCF antigens	TPCF titers with undiluted antigen			
	Syphilitic pool E (VDRL 1:32)	Reagin serum 5437 (VDRL 1:256)	Reagin serum 5330 (VDRL 1:32)	Reagin serum 5345 (VDRL 1:4)
C1	1:160	1:320	1:40	1:20
C11	1:80	1:40	1:10	Negative.
C12	1:80	1:80	1:20	Do.
C15	1:160	1:320	1:40	1:20
D2A	1:160	1:160	1:20	Negative.
D2B	1:10	1:320	1:20	1:10
D2B heated	Negative	1:40	Negative	Negative.

TPCF antigens were tested. Nine antigens were of approved reactivity for diagnostic tests in dilutions of 1:5 or 1:10, and 12 antigens were of substandard reactivity. The different antigens varied widely in TPCF reactivity as shown by testing human syphilitic control serum pool E. They also varied in reagin reactivity, but all were reactive to some degree with the reagin serum showing the highest VDRL titer.

Table 1 shows the results of testing two of the reagin serums (VDRL titers 1:256 and 1:32) and control pool E (VDRL titer 1:32) with nine approved reactive TPCF antigens. Each antigen was tested in the approved dilution, and two pools (196A and Q) were also tested undiluted. The TPCF titer of pool E was 1:160 with all the diluted antigens. Reagin serum 5437 (VDRL titer 1:256) showed positive TPCF tests with seven of the diluted antigens with titers of 1:20–1:160, and negative TPCF tests with the remaining two diluted antigens. However, positive tests with high titers were obtained with these antigens when they were tested undiluted.

Reagin serum 5330 (VDRL titer 1:32) was reactive in the 1:10 dilution with one of the four diluted TPCF antigens tested.

The three reagin serums and control pool E were tested with 12 subreactive TPCF antigens. The antigens were used undiluted since they were most reactive in this concentration. Aliquots of some of the lots were tested after they had been heated at 100° C. for 60 minutes. The results obtained with six antigens were representative of those obtained with the entire group and are shown in table 2.

Pool E and the two most potent reagin serums gave positive TPCF tests with all of the unheated antigens, and reagin serum 5345 (VDRL 1:4) was positive with three of the six antigens. A comparison of the TPCF titers of pool E (VDRL 1:32) and reagin serum 5330 (VDRL 1:32) suggests that the reagin titer of pool E was masked by the TPCF titer in tests with five of the six antigens.

The remaining antigen, D2B, showed reagin reactivity as high as that of any antigen tested, but extremely low TPCF activity. The titer of pool E (1:10) represented TPCF antibody, not reagin alone, since an aliquot of pool E absorbed with VDRL antigen and negative in VDRL tests also gave a titer of 1:10 with this TPCF antigen. The reagin reactivity of antigen D2B persisted but was markedly reduced after the antigen was heated at 100° C.

Reactivity of TPCF antigens with presumably nonsyphilitic leprosy serums. Studies were made on 21 presumably nonsyphilitic leprosy serums earlier shown by Cannefax, Ross, and Bancroft (*22*) to be positive in Kahn and VDRL tests and negative in TPI, RPCF, and TPCF tests. In the present experiments the serums were first run in TPCF tests using antigen D2A, which had shown a substantial degree of reactivity with both TPCF antibody and reagin (table 2). With this antigen 19 of the 21 leprosy serums gave positive TPCF tests with titers of 1:5–1:40.

The eight serums with the highest titers were then retested using antigen D2A and also antigen D2B, which had shown extremely low TPCF reactivity but high reagin reactivity (table 2). In addition two leprosy serums were

tested with pool Q, an approved antigen which showed no reagin reactivity when used in the dilution designated for diagnostic testing (table 1). Control serums were syphilitic pool E and reagin serum 5437. Because of scarcity of serum, VDRL tests were performed on the same dilutions used for the TPCF tests.

Table 3 shows that positive TPCF tests with similar titers of 1:40 or greater were obtained on the eight leprosy serums with antigens D2A and D2B. Both of these antigens showed high reagin reactivity, but only D2A showed appreciable TPCF reactivity. Negative TPCF tests were obtained on the two leprosy serums tested with pool Q, which had standard TPCF reactivity but no reagin reactivity. VDRL titers on the eight leprosy serums ranged from negative, in the 1:5 dilution, to greater than 1:40.

Reactivity of TPCF antigen with reagin in syphilitic serum. An antigen-antibody reaction between TPCF antigen and syphilitic reagin was demonstrated by testing a syphilitic serum, which had been absorbed with VDRL antigen, with a TPCF antigen which showed high reagin reactivity and low TPCF reactivity.

Human syphilitic serum pool X (TPCF 1:1,280, VDRL 1:2,048) was absorbed twice with VDRL antigen, and the VDRL titer was reduced to 1:32. Unabsorbed and absorbed samples of pool X, together with two control serums, syphilitic pool E, and anti-VDRL reagin serum 5437 were then tested with three TPCF antigens. The antigens were pool Q, D2A, and D2B, which had been shown (tables

Table 3. TPCF titers on presumed nonsyphilitic leprosy serums with antigens which measured TPCF antibody or reagin, or both

Serum	TPCF titers			VDRL titers
	Antigen D2A (undiluted)	Antigen D2B (undiluted)	Antigen pool Q (1:10)	
Controls:				
Syphilitic pool E	1:160	1:10	1:160	1:40
Reagin serum 5437	1:160	1:320	Negative [1]	1:320
Leprosy serum No.:				
746	1:40	1:40	Negative	Negative.[2]
1026	1:40	1:40	Negative	1:40
1332	1:40	1:40	----------	1:5
1361	1:40	1:40	----------	1:5
1779	1:40	1:40	----------	>1:40
1790	>1:40	>1:40	----------	>1:40
2070	1:40	>1:40	----------	1:40
2073	1:40	1:40	----------	Negative.[2]

[1] In 1:10 dilution (1:5 was anticomplementary).
[2] In 1:5 dilution.

Table 4. TPCF titers of syphilitic serum absorbed with VDRL antigen in tests with TPCF antigens which measured TPCF antibody or reagin, or both

Serums	VDRL titers	TPCF titers		
		Antigen pool Q (1:10)	Antigen D2A (undiluted)	Antigen D2B (undiluted)
Syphilitic pool E	1:32	1:160	1:160	1:10
Reagin 5437	1:256	Negative	1:160	1:320
Pool X unabsorbed	1:2, 048	1:1, 280	1:1, 280	1:1, 280
Pool X absorbed with VDRL antigen	1:32	1:1, 280	1:1, 280	1:40

1 and 2) to vary greatly in their reactivity with both TPCF antibody and reagin.

As shown in table 4 serum pool X absorbed with VDRL antigen showed no decline in TPCF titer with either antigen pool Q, which was nonreactive with reagin, or with antigen D2A, which was highly reactive with both TPCF antibody and reagin. However, a marked decline in TPCF titer (from 1:1,280 to 1:40) occurred with antigen D2B, which was highly reactive with reagin but showed little reactivity with TPCF antibody. In tests with this antigen there was no high titer of TPCF antibody to obscure the reduction in reagin titer.

In another experiment it was shown that a reduction in VDRL titer occurred when syphilitic serum was absorbed with TPCF antigen which showed high reactivity with reagin (antigen C1 in table 2). Serum pool E was absorbed as described in detail in the following paper, and the TPCF titer was reduced from 1:160 to 1:10. For VDRL tests the absorbed serum and the unabsorbed control were diluted 1:10 and then in increments of 10 out to 1:60. The VDRL titer of the control serum was 1:50 and that of the absorbed serum was 1:30. This difference, although small, was obtained in repeated tests.

Discussion

The results presented here show that TPCF antigens were contaminated with varying amounts of a component reactive with reagin and provide additional evidence that the antigen is a complex of reacting substances.

It was demonstrated in the preceding paper that TPCF antigen, although largely heat labile, contained an antigenically distinct heat stable substance, and that the antibody to this substance was masked by the antibody to the heat labile portion of the antigen. The results of the present study show that the antibody to the component reactive with reagin also may be masked by the TPCF antibody titer.

The reagin reactivity was obscured by the greater TPCF reactivity of most of the antigens tested. For this reason VDRL absorbed syphilitic serum failed to show a decline in TPCF titer even though reagin reactive material was present in the antigen. On the other hand, with *T. pallidum* agglutination antigens in which reagin reactivity was predominant, VDRL absorbed serum did show a decline in titer which unmasked the presence of another less active agglutinating antibody (*23*).

The presence of reagin reactivity in TPCF antigens was demonstrated also by the tpcf-50 test, but a comparison of its reactivity in the two tests was not made. Although the less potent approved antigens here examined showed the presence of the contaminating substance in the concentration designated for testing, the substance is probably diluted out in the majority of highly reactive preparations. In the present study it was not demonstrated in two of three antigens tested in the 1:10 dilution.

Summary

No cross relationship was found between TPCF antibody and reagin, but TPCF antigens were contaminated with a substance which reacted with reagin.

The contaminating component was present to varying degrees in all of 21 antigens tested, both approved reactive and subreactive, and was partially resistant to heating at 100° C.

This component was reactive with artificially prepared reagin serum, with presumably biologic false positive leprosy serum, and with reagin in syphilitic serum.

The failure of VDRL absorbed syphilitic serum to show a decline in TPCF titer is an unsatisfactory criterion for the absence of reagin reactive material in the antigen.