Relation of TPCF Antibody to Three Other Antibodies in Syphilitic Serum

Charlotte P. McLeod, Sc.D., and Warfield Garson, M.D., M.P.H.

INTERPRETATION of results of treponemal tests for syphilis in terms of the various antibodies measured has remained in many respects a perplexing problem. The experiments presented here are concerned with the relationship of the antibody, or antibody complex, measured by the TPCF test to the Reiter protein complement fixation (RPCF) antibody, the *Treponema pallidum* immobilizing (TPI) antibody, and the fluorescent treponemal antibody (FTA).

The three preceding papers have shown that TPCF antigen is a composite of reacting substances. Although the antigen is largely heat labile, it contains an antigenically distinct moiety which is stable to heating at 100° C. TPCF antigens are contaminated also with a substance which is reactive with reagin, but it has been demonstrated that there is no cross relationship between TPCF antibody and reagin.

It will be shown in the present study that TPCF antibody, or antibody complex, has no relationship to RPCF antibody; that TPCF antigens may react to some degree with TPI antibody; and that a definite relationship exists between TPCF antibody and a major part of the antibody complex which is measured by the FTA test. It is indicated by our results that the TPCF test and the FTA test measure to a large degree the same reactive substance in syphilitic serum.

Methods

Approved reactive TPCF antigen employed in TPCF tests was purchased from a commercial source. TPCF antigens of substandard reactivity were donated by Dr. Joseph Portnoy and by the Cappel and Difco Laboratories. The Portnoy antigen was used in TPCF tests after being heated at 100° C. for 60 minutes. The Cappel and Difco antigens were employed for absorption purposes.

Human syphilitic pool E has been described in the preceding papers. Anti-TPCF serum was pooled serum from five rabbits which were each injected with 14 cc. of unheated TPCF antigen during a period of 2 months. Anti-heated TPCF serum was pooled serum from five rabbits similarly injected with aliquots of antigen which had been heated at 100° C. for 60 minutes. Serum 8748 was obtained from a latent syphilitic rabbit infected 8 months previously.

As described in the second paper of this series, aliquots of serum pool E were absorbed with TPCF antigens of substandard reactivity, using unheated antigen C1, or antigen D1H which had been heated at 100° C. for 60 minutes. In TPCF tests with these antigens used undiluted, pool E gave a titer of 1:160 with the unheated antigen but was nonreactive with the heated antigen. In tests with the heated antigen concentrated four times by centrifugation, the titer of pool E was 1:10.

In the absorptions, 6 cc. of the 1:5 dilution of pool E was absorbed with the sediment from 12 cc. of antigen. The unheated antigen used for absorption had been concentrated 4 times by centrifugation and the heated antigen had been concentrated 16 times. The antigens were concentrated by spinning 2 cc. volumes in tubes of 15 cc. capacity for 5–6 hours in the cold in a Servel anglehead centrifuge at 13,000 rpm and 20,000 times G. The sediment was then resuspended in the supernatant to one-fourth the original volume, and the antigen was recentrifuged in volumes of 2 cc. One cubic centimeter of serum was then mixed with the sediment from 2 cc. of concentrated antigen. The mixtures were incubated for 3 hours in a water bath at 37° C., followed by 14 hours in a refrigerator at 5° C. Two cubic centimeter volumes of the mixtures were then centrifuged for 6 hours as described above in order to remove the antigen-antibody complex from the serum. Unabsorbed control serum was incubated and centrifuged along with the absorbed serum.

TPCF tests were run by the original method

of Portnoy and Magnuson (*9*). RPCF tests (*24*) were run in the laboratory of George Cannefax. Tpcf-50 tests (*19*) were run in Dr. Portnoy's laboratory. TPI and FTA tests were performed by the methods outlined in the 1959 Manual of Serologic Tests for Syphilis. The TPI tests were performed at the Venereal Disease Research Laboratory at Chamblee, Ga., and the results of the TPI tests were analyzed by Dr. Bernard Greenberg, professor of biostatistics at the University of North Carolina.

FTA tests were performed by Carrie C. Winter at the Venereal Disease Experimental Laboratory, Chapel Hill, N.C., with antigen supplied by Dr. Wilbur E. Deacon. The human syphilitic serums were tested with VDRL antigen lots 592, 593, and 595, and with VDRL fluorescein-labeled antihuman globulin, lot 117, diluted 1:40. The rabbit serums were tested with VDRL antigen lot 596, and with fluorescein-labeled antirabbit globulin purchased from Sylvana Laboratories (lot 729, diluted 1:5).

Results

Results of TPCF tests on syphilitic serum after absorption with unheated or heated TPCF antigen. Aliquot samples of the 1:5 dilution of human syphilitic serum pool E were absorbed as described under "Methods" with unheated TPCF antigen or antigen which had been heated at 100° C. for 60 minutes. The two absorbed serums and the unabsorbed control were then tested with both unheated and heated antigen. The serums were diluted twofold, and in tests with heated antigen the 1:30 dilution was also tested. The unheated antigen was employed in the 1:5 dilution as approved for diagnostic testing. The heated antigen was used undiluted since it was most reactive in this concentration.

The results, shown in table 1, were similar to those reported in the second paper of this series. The TPCF titers of the control serum were 1:160 with unheated antigen and 1:40 with heated antigen. In the serum absorbed with unheated antigen the titers were reduced to 1:10 with both unheated and heated antigen. In the serum absorbed with heated antigen there was no change in titer with unheated antigen, but in tests with heated antigen the titer was reduced to 1:10. Identical results were obtained on these serums in the tpcf–50 test.

Table 1. Results of TPCF tests on syphilitic serum absorbed with unheated or heated TPCF antigen

Syphilitic serum human pool E (diluted 1:5)	TPCF titers	
	Unheated antigen (diluted 1:5)	Heated antigen[1] (undiluted)
Control—unabsorbed	1:160	1:40
Absorbed with—		
TPCF	1:10	1:10
Heated TPCF	1:160	1:10

[1] In tests with heated TPCF antigen the 1:30 dilution of each serum was tested in addition to the twofold dilutions.

Results of RPCF tests on syphilitic serum absorbed with TPCF antigen. Serum pool E absorbed with unheated TPCF antigen and the unabsorbed control serum were diluted 1:20, 1:40, and then in increments of 10 through 1:80; and RPCF tests were run on the dilutions. As shown in table 2, the RPCF titer of both serums was 1:70, and no decline in titer occurred in the serum absorbed with TPCF antigen.

To further investigate the relationship between TPCF and RPCF antibody, RPCF tests were run on serums obtained from normal rabbits before and after inoculations with unheated or heated TPCF antigen. None of the five rabbits in either group showed an increase in RPCF titer as a result of the injections.

Results of TPI tests on syphilitic serum absorbed with unheated or heated TPCF antigen. TPI tests were performed on pool E, which had been absorbed with unheated or heated TPCF antigen, and on the control unabsorbed serum. Fifteen dilutions, ranging from 1:5 to 1:130, were made of each absorbed serum. The serums were diluted 1:5, 1:10, 1:15, and 1:20 and were then further diluted in increments of 10, which gave dilutions of 1:30, 1:40, 1:50, 1:60, and so on, through 1:130. The control serum was similarly treated, beginning with the 1:15 dilution. In reading the TPI tests, motility counts were made on both tubes of every dilution of each of the three serums. The motility

of treponemes in the control tubes ranged from 80 to 92 percent.

Table 3 shows the 50 percent immobilizing titer of each serum calculated by the method of Karber (*25*) and expressed in actual dilutions of serum. The titer of the control serum was 1:74; the titer of the serum absorbed with TPCF antigen was 1:57; and the titer of the serum absorbed with heated TPCF antigen was 1:58. Although the immobilizing activity of the two absorbed serums was almost identical, there was a significant difference between their activity and that of the control serum. This difference was significant at the 1 percent level.

Pools of serum from rabbits injected with unheated or heated TPCF antigen which were positive in TPCF tests gave nonreactive TPI tests in this same experiment.

Results of FTA tests on syphilitic serum absorbed with unheated or heated TPCF antigen. Three FTA tests using three different lots of antigen were performed on serum pool E, which had been absorbed with unheated or heated TPCF antigen and on the unabsorbed control serum. The serums were first tested in twofold dilutions which ranged from 1:5 through 1:320. The FTA titers of the control serum and of the serum absorbed with heated antigen were 1:160, while the titer of the serum absorbed with unheated antigen was reduced to 1:40. These same dilutions were retested with another lot of FTA antigen with identical results.

New dilutions of serum were then made and were tested with a third lot of antigen. The control serum and the serum absorbed with heated TPCF antigen were diluted 1:20 and 1:40, and were then further diluted in increments of 40 out through the 1:320 dilution. Pool E absorbed with unheated TPCF antigen was treated in a similar manner through the 1:160 dilution and was then diluted 1:320. The results of this experiment are shown in table 4. Both the control serum and the serum absorbed with heated TPCF antigen showed FTA titers of 1:280, while the serum absorbed with unheated antigen was reduced in titer to 1:80.

The results of the FTA tests on anti-TPCF serums prepared in rabbits against unheated or heated TPCF antigen are shown in table 5. This table also depicts the TPCF and TPI titers of the serums. The control syphilitic rabbit serum was reactive in all of the three tests; the anti-TPCF serum was reactive in the FTA test (titer 1:40) and the TPCF test, but

Table 2. Results of RPCF tests on syphilitic serum absorbed with TPCF antigen

Syphilitic serum pool E	TPCF titer	Results of RPCF tests on serum dilutions					
		1:20	1:40	1:50	1:60	1:70	1:80
Control—unabsorbed	1:160	R	R	R	R	R	N
Absorbed with TPCF	1:10	R	R	R	R	R	N

NOTE: R—reactive; N—nonreactive.

Table 3. Results of TPI tests on syphilitic serum absorbed with unheated or heated TPCF antigen

Serum pool E	TPCF titers		Results of TPI tests	
	Unheated antigen	Heated antigen	50 percent TPI titer [1]	95 percent confidence interval
Control—unabsorbed	1:160	1:40	1:74	1:65–1:84
Absorbed with—				
TPCF	1:10	1:10	1:57	1:48–1:67
Heated TPCF	1:160	1:10	1:58	1:53–1:64

[1] Calculated by Karber's method on actual dilutions of serum tested.

Table 4. Results of FTA tests on syphilitic serum absorbed with unheated or heated TPCF antigen

Serum dilution (pool E)	FTA titers		
	Control—unabsorbed (TPCF titer 1:160)	Absorbed with—	
		TPCF (TPCF titer 1:10)	Heated TPCF (TPCF titer 1:160)
1:20	Reactive	Reactive	Reactive.
1:40	do	do	Do.
1:80	do	Weakly reactive	Do.
1:120	do	Nonreactive	Do.
1:160	do	do	Do.
1:200	do		Do.
1:240	do		Weakly reactive.
1:280	Weakly reactive		Do.
1:320	Nonreactive	Nonreactive	Nonreactive.

was nonreactive in the TPI test. The antiheated TPCF serum was reactive only in the TPCF test. (It should be noted that the TPCF titer of the antiheated TPCF serum represented antibody to the heat stable portion of TPCF antigen.)

Discussion

The experiments presented here show that there is no cross relationship between TPCF antibody and RPCF antibody. Cannefax and Garson (*26*) earlier demonstrated that syphilitic serum absorbed with RPCF antibody was not reduced in TPCF titer, and that rabbits injected with RPCF antigen did not develop TPCF antibody. In the present study, serum absorbed with TPCF antigen was not reduced in RPCF titer, and rabbits injected with TPCF antigen had no increase in RPCF titer. These findings are of particular interest since Cannefax and Garson demonstrated that a cross relationship does exist between the Reiter antibody and the antibody to *T. pallidum* cryolysis protein (TPCP): absorption of syphilitic serum with RPCF antigen removed only RPCF antibody, while absorption with TPCP antigen removed both TPCP and RPCF antibody.

Table 5. FTA, TPCF, and TPI titers of serums from rabbits injected with unheated or heated TPCF antigen

Rabbit serum	Antibody titers		
	FTA	TPCF	TPI
No. 8748 (syphilitic control).	1:80	1:320	1:640
Anti-TPCF	1:40	1:160	Nonreactive.
Antiheated TPCF	Nonreactive.	1:40	Do.

The results of TPI tests on the absorbed serums in the present study suggested that the heat stable moiety in TPCF antigen reacted to some degree with TPI antibody. Although anti-TPCF serums prepared in rabbits were nonreactive in TPI tests, this could have been due to insufficient immunological stimulus from the relatively small amount of antigen injected. It was shown in the second paper of this series that the antibody to heated TPCF antigen (like TPI antibody) arose later in syphilitic infection and declined less rapidly after treatment than the antibody to the heat labile portion of TPCF antigen. However, other previous work (*11*) indicated that TPI antigen was relatively heat labile, since minimum antigenic doses of *T. pallidum* declined in ability to produce TPI antibodies in mice in direct proportion to the temperature at which the suspensions were heated.

The significance of these various findings cannot at present be explained. It is possible that TPI antigen may be partially heat stable, or that immobilization of the treponeme may be accomplished by the interaction of several antibodies rather than by the action of a single

substance. TPI antibody alone has never been absorbed from syphilitic serum, and the present experiments offer merely suggestive evidence that TPI antigen was extracted from *T. pallidum* as a contaminant in TPCF antigen. Further investigations of the mechanism of immobilization would seem highly desirable.

The results of FTA tests on the absorbed serums indicated that a definite relationship exists between the antibody to the heat labile portion of TPCF antigen and the FTA antibody. The serum absorbed with unheated TPCF antigen showed a reduction of more than 70 percent in FTA activity, compared with the control serum. The serum absorbed with heated TPCF antigen was not reduced in FTA titer. Also, anti-TPCF serum prepared in rabbits was reactive in FTA tests, while antiheated TPCF serum was nonreactive. Since the antibody to heat labile TPCF antigen is most active in the TPCF test, it appears from our results that the TPCF test and the FTA test measure to a large degree the same antibody in syphilitic serum.

Unpublished experiments by Winter, Cannefax, and McLeod have shown that the FTA test also measures RPCF antibody. FTA tests were performed on two anti-RPCF rabbit serums using the same lots of antigen and conjugate which were employed in testing the anti-TPCF serums (table 5). One serum (RPCF titer 1:80) gave a reactive FTA test when undiluted, and the other serum (RPCF titer 1:640) gave a reactive FTA test in the 1:20 dilution. It appears from these results that the FTA antigen, as well as the TPCF antigen, represents a composite of reacting substances.

Summary

It was demonstrated that no cross relationship exists between the TPCF antibody complex and RPCF antibody.

The antibody to the heat stable substance in TPCF antigen may be related in some degree to TPI antibody.

The antibody to the heat labile portion of TPCF antigen, the most active antibody in the TPCF test, appeared to be identical with a major part of the antibody complex which is measured by the FTA test.

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Medical Counseling for Selective Service Rejectees

A plan to help young men rejected for military service because of health defects has been developed by the Federal Interdepartmental Committee on Children and Youth. Secretary of Health, Education, and Welfare Abraham Ribicoff, chairman of the committee, stated that the key element in the plan is the stationing of State or local health department personnel at Armed Forces examining centers. They will counsel Selective Service registrants failing to meet medical standards and, whenever possible, refer them to their private physicians. When this is not possible, the rejectees will be referred to appropriate voluntary or public community health facilities.

The committee emphasized that the response of the rejectees will be entirely voluntary but that health departments may conduct followup programs to learn the extent of the response.

Demonstration projects and service programs will be initiated in special areas throughout the country during 1962. Any interested State or local health department, as well as voluntary agencies, may participate in the program. The Public Health Service will offer consultative assistance and maintain liaison between health departments and the participating Federal agencies.

The plan for medical guidance of Selective Service rejectees was developed as a result of a recommendation of the 1960 White House Conference on Children and Youth.

Program Notes

An estimated 6,000 students were exposed to information about the health profession at the second annual Health Futurama at St. Petersburg Junior College, sponsored by the Community Welfare Council, the Pinellas County Board of Public Instruction, and the *St. Petersburg Times*.

Professional representatives from more than two dozen fields were present during the 2-day exhibit to answer questions and give advice.

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Employees of State and local governments covered both by old age, survivors, and disability insurance and by staff retirement systems number 2,600,000. These represent about 41 percent of all State and local government employees, as of January 1961, according to the March 1962 *Social Security Bulletin.*

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"Water Resources in Rensselaer County," published by the New York State Department of Health, is a complete survey of the topographic, geographic, and population factors bearing upon effective usage of water for domestic, industrial, fish and wildlife, and recreational purposes in the county.

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Continuous steady noise for several hours or more each day, 5 days a week, causes significant hearing loss to workers exposed to it, with maximum damage accruing in 10 to 12 years, according to research carried out by Dr. Aram Glorig, Dr. W. Dixon Ward, and James Nixon, and reported in the October 1961 issue of the *A.M.A. Archives of Otolaryngology*. However, adherence to hearing conservation criteria proposed by the researchers would prevent such deafness. The study was supported by a grant from the National Institute of Neurological Diseases and Blindness.

A 75-patient Alcoholic Treatment Center opened in February at Central Islip State Hospital, Long Island, New York, with a program of research and rehabilitation for alcoholics who are not psychotic.

Each patient will receive a week of concentrated treatment—psychotherapy, medical help, religious counseling, and Alcoholics Anonymous techniques—and then a staff conference will be held to determine which specific treatment has proved most suitable.

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Under a new inspection program, the equipment of itinerant food vendors in Philadelphia must be examined at district health centers by the city health department before the vendors can be issued a license or a renewal.

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In support of Philadelphia's Poison Information Center, Poison Prevention Week posters, window streamers, and leaflets have been displayed by some 1,250 of the city's pharmacists. The city's 330 Cub Scout Packs have distributed pressure-sensitive labels bearing the health department's poison information telephone number.

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The Center of Alcohol Studies has been moved from Yale University to Rutgers, the State University of New Jersey. The move was financed by the National Institute of Mental Health. The annual summer school of alcohol studies will be held at Rutgers July 1–26, 1962.

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Beginning July 1, 1962, a new test for phenylketonuria in newborn infants will be tried on some 400,000 babies in a mass screening effort throughout the nation. The test, devised by Dr. Robert Guthrie, University of Buffalo, can be used in the first few days after birth.

An 8-week seminar for clergymen, "Pastoral Care of the Alcoholic and His Family," has been conducted in Washington, D.C., by the School of Alcoholic Studies of the Pastoral Institute, co-sponsored by the Washington Area Council on Alcoholism. The seminar included instruction in modern methods of treating alcoholics and discussions of ministers' practical experience in dealing with the problem.

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The Mental Research Institute of the Palo Alto Medical Research Foundation, Palo Alto, Calif., and the Family Institute, New York, N.Y., announce the publication of *Family Process*, a semiannual, multidisciplinary journal. The first issue appeared in March 1962.

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The problems encountered in attempting to fluoridate North Carolina's water are discussed in the January 1962 issue of the *Health Bulletin*, the official publication of the North Carolina State Board of Health.

The issue reprints a paper by Arthur S. Flemming, former Secretary of the Department of Health, Education, and Welfare, criticizing the opposition to fluoridation.

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The December 1961 issue of *Phoenix* is devoted to a history of schistosomiasis (bilharziasis) and to research directed at finding a vaccine or cure for this disease.

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Two awards, $1,000 and $500, will be given for the outstanding research projects in accident prevention conducted since 1959. The awards, administered by the National Safety Council under a grant from the Metropolitan Life Insurance Company, will be made to encourage research workers in the various disciplines to include accident causation in their studies.

Further information may be obtained from Dr. Murray Blumenthal, Director, Research Division, National Safety Council, 425 North Michigan Ave., Chicago 11, Ill.