Agglutination of Treponema Pallidum In Syphilitic Serums

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NUMEROUS unsuccessful attempts have been made to demonstrate specific agglutination of pathogenic *Treponema pallidum* in syphilitic serum. Most such attempts have met with failure due to the tendency of the organisms to agglutinate spontaneously. Interest in the subject was revived by the report of Tani (*1*) that Antiformin-killed spirochetes showed no such spontaneous clumping, but were agglutinated in syphilitic serum after an incubation period of 24 hours; and that organisms so treated provided satisfactory antigens for the serodiagnosis of syphilis. More recently, specific agglutination of *T. pallidum* was reported by Gain (*2*) who obtained antigens which showed no spontaneous agglutination from syphilomas of X-irradiated rabbits; and also by Hardy and Hollander (*3*) who prepared satisfactory heat-killed spirochete suspensions from lesions of syphilitic rabbits treated with cortisone.

The agglutination technique described in the present paper was devised by bringing together the findings of two separate lines of investigation. The experiments of Tani (*1*) were confirmed and extended. At the same time, there was in progress a study of complement in the *Treponema pallidum* immobilization test (TPI). In testing the fresh serum of various animal species for complement activity, it was noted that fresh steer, or other bovine, serums caused disappearance rather than immobilization of the spirochetes, and also caused agglutination of sensitized sheep cells. It was then found that the agglutination of *T. pallidum* in syphilitic serum was greatly enhanced by the addition of fresh steer serum. These effects of fresh steer serum in both the TPI test and the agglutination test were due to the presence of conglutinin (*4*) in addition to complement.

By means of the agglutination technique with fresh steer serum the presence of syphilitic antibody may be demonstrated in a test which utilizes killed spirochetes and is completed in only 2 hours. It will be shown that this reaction appears to have a specificity comparable with that of the TPI test, and sensitivity many times as great.

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Methods

The Nichols strain of *T. pallidum* was employed in all experiments. Serum samples from normal or syphilitic human donors were stored at −20° C. until tested. Fresh steer or fresh guinea pig serum was distributed in suitable amounts in small containers and stored in a CO_2 chest at −76° C., and samples were not thawed until immediately before use. The steer serum was frozen on the day the blood was collected; the guinea pig serum usually was frozen on the day following collection.

TPI tests were set up by the method of Nelson and Mayer (*5*) with modifications and controls

previously described (*6*, *7*). Unmodified Nelson's medium (*8*) was used in titering serum from patients with untreated primary, secondary, or latent syphilis. Five times the usual concentration of sodium thioglycollate (*9*) and 5 percent of inactivated normal rabbit serum were added to the medium in all other experiments. Each assay tube contained a total volume of 0.45 cc., which included 0.3 cc. spirochete suspension, 0.1 cc. of undiluted steer or guinea pig serum as complement, and 0.05 cc. of test serum or dilution. TPI assays were incubated for 16 hours at 37° C.

In tests with sheep erythrocytes, suspensions of cells and dilutions of serum were made in physiological saline (0.85-percent sodium chloride). Sensitized cells were prepared by mixing equal volumes of a 3-percent sheep cell suspension and a 1:500 dilution of rabbit amboceptor. (Ambocepter was obtained from the Venereal Disease Research Laboratory, Chamblee, Ga.) Unsensitized cells were similarly prepared with saline instead of amboceptor. Sheep cell suspensions were used in a volume of 0,4 cc. In testing for residual complement in TPI assays or in complement titrations, the tubes were incubated in a water bath at 37° C. for 30 minutes.

In preparing the antigen used in agglutination tests with fresh steer serum, spirochetes were obtained from testicular lesions of rabbits inoculated 8 to 10 days earlier. The testicles showing 3+ induration were sliced in an egg cutter and extracted with 5 to 10 cc. of 0.85-percent sodium chloride per testis. Three such extractions were carried out for 1 hour at room temperature on a rotating shaker. The saline extracts were pooled and centrifuged for 10 minutes at a low rate of speed to sediment the larger tissue particles. The supernatant was then spun for 1 hour in a Sorval angle centrifuge (approximately 20,000 G) to sediment the spirochetes. The supernatant was discarded, and the organisms were resuspended in saline and killed by heating in a water bath at 56° C. for 40 minutes. The suspensions then were further diluted with saline to contain approximately 60 spirochetes per high power field, and were stored at 4° C. Antigens so prepared showed no spontaneous agglutination during storage periods which lasted for as long as 3 months. However, there often appeared, during storage, a finely granular precipitate in which some of the spirochetes became enmeshed. This material was present to a greater or less degree in the different suspensions, and usually could be removed or greatly reduced by centrifugation.

Agglutination Studies

In preliminary studies, the findings of Tani (*1*) with Antiformin-killed spirochetes were repeated, and other methods of killing the organisms were investigated. (Antiformin, which contained not less than 6 percent sodium hypochlorite, was purchased from American Antiformin Co., Brooklyn, N. Y.) unwashed spirochete suspensions were treated with Antiformin in final concentrations of 0.1 to 0.25 percent for a period of 30 minutes at room temperature. The organisms then were sedimented by centrifugation and resuspended in saline containing 0.5 percent phenol. One-tenth cubic centimeter of antigen was added to 0.1 cc. of syphilitic serum dilution, and the mixtures incubated in a water bath for 26 to 27 hours at 37° C. Specific agglutination was obtained by this method, and a positive control serum (human pool B) regularly showed 3+ to 4+ agglutination in the 1:32 dilution.

In further experiments, aliquot portions of the unwashed suspensions were subjected to different treatments. One portion was untreated and the spirochetes allowed to die on standing at 4° C. The organisms in the other portions were killed by treatment with Antiformin, by heating at 56° C. for 40 minutes, or by the addition of a final concentration of 1:4,000 Mapharsen, 0.2-percent phenol, or 0.1-percent formalin. Agglutination similar to that with Antiformin-killed antigens was obtained with the untreated, heated, or Mapharsen-killed organisms. All of the phenol-killed antigens showed some degree of spontaneous agglutination, and formalin-killed organisms failed to agglutinate.

Six different saline testicular extracts were used. Four of these provided satisfactory antigens. They were obtained from lesions of rabbits inoculated 7, 9, 11, or 11 to 13 days

earlier. Two extracts obtained from 9-day lesions or from pooled 7- to 14-day lesions were unsatisfactory, showing 1+ to 4+ spontaneous agglutination in all antigens except the formalin killed. The Antiformin-treated antigens showed the least amount of spontaneous agglutination, and the phenol-killed organisms showed the greatest amount.

Use of Fresh Steer Serum

In testing serum from numerous animal sources for complement activity in the TPI test (data to be published), considerable variation was noted in the serum from different species. While none of the serums showed immobilizing activity in the absence of hemolytic activity as measured by the usual rabbit amboceptor-sheep cell system, these two properties were not necessarily present in the same degree. Of particular interest in these studies was the finding that fresh steer serum caused complete disappearance of the spirochetes under the conditions of the TPI test. Also when a syphilitic serum was tested quantitatively with fresh steer serum, the spirochetes which reappeared at the end point of the titration were motile and there was no evidence that immobilization occurred between the stages of disappearance and reappearance. When tested under identical experimental conditions, the "disappearance titer" with steer serum and the immobilization titer with guinea pig serum were similar after 16 hours' incubation. However, it was found by reading the tests at 6 hours that disappearance proceeded more rapidly than immobilization. Disappearance occurred also when aliquot portions of the suspensions were heated at 56° C. for 40 minutes, and the tests incubated under either aerobic or anaerobic conditions.

In tests for residual complement there was 4+ agglutination but no hemolysis of sensitized sheep cells in tubes containing fresh steer serum. The tubes containing inactivated steer serum showed neither hemolysis nor agglutination. Table 1 shows the results of titrating steer serum against sheep cells as described under "Methods." Both sensitized and unsensitized cells were lysed in high concentrations of fresh steer serum, and were agglutinated in relatively high dilutions. The activity of the fresh serum was slightly higher against the sensitized than against the unsensitized cells. Inactivated steer serum showed no lytic activity, but a low degree of agglutinating activity against only the sensitized cells.

In further experiments with steer serum in the TPI test, it was noted that disappearance of the organisms sometimes took place in control tubes which contained spirochetes plus fresh steer serum, or spirochetes plus fresh steer serum and normal serum. Also a negative end point was not obtained in the titer of the positive control serum. In duplicate assays run

Table 1. Effect of steer serum on sheep cells

[0.4 cc. of serum or dilution +0.4 cc. of cell suspension]

Dilutions of steer serum	Fresh steer serum				Inactivated steer serum			
	Sensitized cells		Unsensitized cells		Sensitized cells		Unsensitized cells	
	Lysis	Agglutination	Lysis	Agglutination	Lysis	Agglutination	Lysis	Agglutination
0	3+	4+	3+	4+	Negative	2+	Negative	Negative.
1:2	1+	4+	±	4+	do	Negative	do	Do.
1:4	Negative	4+	Negative	3+				
1:8	do	4+	do	2+				
1:16	do	4+	do	1+				
1:32	do	2+	do	Negative				
1:64	do	Negative	do	do				
1:128	do	do	do	do				

NOTE: The designations 4+, 3+, 2+, or 1+ refer to the degree of hemolysis, or to the degree of agglutination of cells which resisted hemolysis.

Table 2. Results of TPI tests on serums from patients with suspected biological false-positive reagin tests, using fresh serum from both guinea pig and steer as sources of complement

Total serums tested	Results with guinea pig serum				Results with steer serum			
	Positive	Negative	Doubtful [1]	Nonspecific immobilization [2]	Positive	Negative	Doubtful [1]	Nonspecific immobilization
20	3	12	1	4	6	13	1	0

[1] The serum from this patient was positive in the agglutination test.
[2] Three of these patients were positive and one negative in tests with fresh steer serum.

with the same spirochete suspension and guinea pig complement, all controls were satisfactory. From the results with guinea pig complement it seemed clear that the spirochetes were not sensitized in vivo. It seemed more probable that steer serum contained a natural antibody to *T. pallidum* as well as to sheep cells, and that the effect of this antibody was apparent under the conditions of the TPI test only when the spirochete suspension contained relatively little anticomplementary or other interfering substances.

It was later found when washed killed antigens were used that disappearance of the organisms always occurred in fresh steer serum, and agglutination, but no disappearance, in heated steer serum. Immobilizing antibodies could not be demonstrated in heated steer serum when assayed in the TPI test with guinea pig complement even when twice the usual amount of serum was tested. The presence of "reagin" in steer serum was suggested by a positive Kahn test. However, VDRL and Mazzini tests were negative.

Serum samples from 20 patients with suspected biological false-positive reagin tests were examined by the TPI technique, using both fresh guinea pig and fresh steer serum as sources of complement. The two tests on the same patient were always run in the same assay. Table 2 shows the results of this study. There was agreement between the results obtained with the two methods except in four serums which showed nonspecific immobilization when assayed with guinea pig complement. Three of these serums were positive and one was negative when tested with steer serum. One serum which gave a doubtful result by both methods was later positive in the agglutination test with fresh steer serum.

Use of Syphilitic and Steer Serums

Spirochete suspensions washed in saline and heat killed, as described under "Methods," were used as agglutinating antigens. In studying the effect of adding fresh steer serum to the agglutination mixtures, it was noted that the organisms disappeared in controls of fresh steer serum, and showed 2+ agglutination in controls of heated steer serum. By suitably diluting the steer serum in saline, these effects were overcome, and sufficient activity remained to greatly enhance the agglutination of *T. pallidum* in syphilitic serum.

In setting up agglutination tests, 0.1 cc. antigen, 0.1 cc. of 1:7 fresh steer serum, and 0.1 cc. of inactivated test serum or dilution were mixed in Wassermann tubes and incubated for 2 hours in a shaking machine at 37° C. In reading the tests, 0.01 cc. of each mixture was measured onto a slide and examined under a 22 x 22 mm. cover slip with the high dry objective.

In reading the tests, consideration was given both to the number of unagglutinated spirochetes per field, and to the proportion of organisms which were agglutinated. This is illustrated in table 3 which also shows a typical titer obtained on pool B, a positive human control serum. There were 20 spirochetes per field with no agglutination in the controls of antigen plus saline, antigen plus active or inactive complement, or antigen plus normal serum with active complement. In the control of heated complement and undiluted pool B, one-half the total number of spirochetes were unagglutinated, and one-half were agglutinated into

small clumps. This was designated as 2+ agglutination.

In the titer of pool B with active complement, there appeared to be complete disappearance of the spirochetes in the undiluted serum. In the 1:10 and 1:20 dilutions, there was 4+, or practically complete, agglutination with only one or less unagglutinated spirochete per field. The agglutinated organisms were contained in a few large, very tightly packed clumps which were usually located by searching the slide with the low-power objective. In the 1:40 dilution, there was an average of two single spirochetes per field, and 3+ to 4+ agglutination. The clumps in this dilution were less tightly packed, smaller, and more numerous than in the two preceding tubes. The 1:80 dilution showed 2+ agglutination with approximately one-half the organisms agglutinated in small- and medium-sized lacy clumps. In higher dilutions, there were 18 to 20 single spirochetes per field, and little or no agglutination.

At the same time pool B was run with fresh guinea pig serum which was active in the TPI test, and a titer only slightly higher than that without complement was obtained. Strongly positive agglutination occurred in the undiluted syphilitic serum after 2 hours' shaking, and in the 1:10 dilution after 23 hours' shaking. In the absence of shaking, agglutination occurred less rapidly with both steer and guinea pig complements.

Experiments were then set up to study the mechanism by which fresh steer serum enhanced spirochetal agglutination. Pool B was titered as described above with fresh steer serum, with fresh guinea pig serum, and with fresh guinea pig serum plus 0.05 cc. of a 1:3 dilution of steer serum which had been heated for 30 minutes at 56° C. The tests were read after shaking for 2 hours at 37° C. In the titer with fresh steer serum, the 1:40 dilution of pool B showed 3+ agglutination; in the test with fresh guinea pig serum, only the undiluted pool B showed 4+ agglutination; and when tested with fresh guinea pig serum plus heated steer serum, 4+ agglutination was obtained in the 1:40 dilution. The control of undiluted pool B plus heated serum showed only 2+ agglutination. It seems clear from these results that the property of steer serum which enhanced agglutination was conglutinin (*4*).

Using the technique with fresh steer serum, pool B has been titered a total of 24 times, with six different antigens. The reproducibility of the titers was well within the limits of technical error, and different antigens did not vary greatly in sensitivity. The dilution giving 3+ to 4+ agglutination ranged from 1:40 to 1:80 in 13 tests with 2 antigens, and from 1:80 to 1:160 in 11 tests with 4 antigens.

The specificity of the agglutination test is shown in table 4 which contains a comparison of the results of standard serologic tests (STS), TPI, and agglutination tests on serum from 154 presumably nonsyphilitic human donors. The

Table 3. Agglutination of *T. pallidum* in the presence of syphilitic serum and fresh steer serum

[Shaken 2 hours at 37° C.]

Tubes [1]	Single *T. pallidum* per field	Agglutination
1. Saline+saline	20/1	No clumps.
2. Heated steer serum (1:7)+saline	20/1	Do.
3. Active steer serum (1:7)+saline	20/1	Do.
4. Active steer serum (1:7)+normal serum	20/1	Do.
5. Heated steer serum (1:7)+pool B [2] undiluted	10/1	2+small and medium clumps.
1. Active steer serum (1:7)+pool B undiluted	0/25	Disappearance.
2. Active steer serum (1:7)+pool B 1:10	1/10	4+tightly packed clumps.
3. Active steer serum (1:7)+pool B 1:20	1/1	Do.
4. Active steer serum (1:7)+pool B 1:40	2/1	3+-4+ clumps less opaque.
5. Active steer serum (1:7)+pool B 1:80	10/1	2+ lacy clumps.
6. Active steer serum (1:7)+pool B 1:160	18/1	Occasional small clump.
7. Active steer serum (1:7)+pool B 1:320	20/1	Do.

[1] Each tube contained a total volume of 0.3 cc. composed of 0.1 cc. antigen +0.1 cc. of each indicated reagent.
[2] Pool of positive human syphilitic serum.

Table 4. Results of qualitative serologic tests on presumed nonsyphilitic donors

Donors	Total	STS			TPI		Agglutination		
		Positive	Negative	Not tested	Positive	Negative	Positive	Negative	Doubtful
Medical students	46	--------	--------	46	0	46	0	43	3
VDEL staff	29	--------	--------	29	0	29	0	29	0
Hospital patients	66	0	66	0	0	66	0	66	0
Hospital employees	12	0	12	0	0	12	0	12	0
Blood donor	1	0	1	0	0	1	1	0	0
Total	154	0	79	75	0	154	1	150	3

donors included medical students, Venereal Disease Experimental Laboratory staff, hospital patients with diseases other than syphilis, hospital employees, and one blood donor. In VDRL tests, there were no positives, 79 serums were negative, and 75 not tested. In TPI tests, there were no positives, and all 154 serums were negative. In the agglutination tests, there was 1 positive test, 3 doubtful reactions, and 150 negatives. The positive serum was obtained from the one blood donor, and the three doubtfuls, from medical students.

Table 5 shows a comparison of the results of STS, TPI, and agglutination tests on serum from patients with untreated primary, secondary, or latent syphilis. There were serums from 12 cases of darkfield positive primary syphilis. In STS tests, 8 were positive, 3 were negative, and 1 was not tested. In the TPI tests, 5 were positive, 6 negative, and 1 showed nonspecific immobilization. In the agglutination tests, 10 were positive and 2 negative. The serums from the 66 secondary and 33 latent syphilis patients were positive in all of the three tests.

A comparison of the results of quantitative STS, TPI, and agglutination tests on serum from 15 patients with untreated primary, secondary, or latent syphilis is shown in table 6. Agglutination titers on the 10 positive primary serums ranged from 1:20 to 1:160. Agglutination titers on the 15 secondary serums ranged from 1:20 to 1:2560; and on the 15 latent serums ranged from positive with undiluted serum to positive in the 1:1280 dilution. There was no correlation between the agglutination titers of these serums and the TPI or STS titers.

The result of measuring the relative sensitivity of the TPI test and the agglutination test by another method is shown in table 7. Two-fold dilutions of syphilitic serums were made in saline, and these saline dilutions were then further diluted twofold in normal human serum, with similar dilutions in saline as controls. These second dilutions were tested quantitatively in the TPI and agglutination tests. It is apparent that dilution with human serum did not interfere with the antibody titration, and that the agglutination titer of the syphilitic serum was significantly higher than the TPI titer.

Table 5. Results of qualitative serologic tests on human syphilitic serum

Serum	Total tested	STS			TPI			Agglutination	
		Positive	Negative	Not tested	Positive	Negative	Nonspecific immobilization [1]	Positive	Negative
Primary	12	8	3	1	5	6	1	10	2
Secondary	66	66	0	0	66	0	0	66	0
Latent	33	33	0	0	33	0	0	33	0

[1] In tube containing inactive complement.

Table 6. Results of quantitative serologic tests on serum from patients with untreated syphilis

Patient No. and stage of syphilis	STS		TPI titers [1]	Agglutination titers [1]			
	VDRL titers	Kahn units					
Primary							
18821	1:64	256	>1:16	1:80	4+	1:160	2+
19529	1:16	32	1:5	1:80	4+	1:160	2+
19574	1:16	64	1:25	1:40	4+	1:80	2+
20495	1:8	16	Positive undiluted	1:40	4+		
20307	1:16	64	do	1:20	4+	1:40	2+
21279	Positive	1	do		Unsatisfactory.[3]		
18415	1:64	512	Unsatisfactory [2]	1:20	3+	1:40	2+
19521	1:4	16	Negative	1:40	4+		
20039	1:4	16	do	1:20	3+	1:40	2+
18588	1:4	8	do		Unsatisfactory.[3]		
19538	1:2	8	do		Do.		
19884			do	1:40	3+		
20321	Negative	Negative	do	1:20	3+	1:40	1+
20073	do	do	do		Negative.		
20011	do	do	do		Do.		
Secondary							
19957		32	1:4	1:1280	3+	1:2560	2+
19307	1:32	64	1:4	1:640	3+	1:1280	2+
19639	1:64	128	1:8	1:320	3+	1:640	2+
19623	1:128	256	1:4	1:640	4+	1:1280	2+
19044	1:32	64	1:16	1:640	4+	1:1280	2+
21296	1:16	64	1:8	1:640	3+	1:1280	1+–2+
18520	1:16	16	1:90	1:80	3+	1:160	Negative.
18982	1:32	64	1:96	1:20	4+	1:40	2+
19354	1:32	128	1:9	1:80	3+	1:160	Negative.
19450	1:32	128	1:34	1:80	4+	1:160	2+
19591	1:8	64	1:64	1:40	3+	1:80	2+
18906	1:64	128	1:25	1:80	3+	1:160	2+
19762	1:16	256	1:125	1:40	3+	1:80	2+–3+
19793	1:64	256	1:125	1:640	3+	1:1280	–1+
19802	1:16	32	1:125	1:40	3+–4+	1:80	2+
Latent							
18794	1:32	64	1:8	1:320	3+	1:640	Negative.
18695	1:16	32	1:25	1:1280	3+	1:2560	1+
18567	1:8	16	1:16	1:320	4+	1:640	2+
19287	1:4	16	1:32	1:320	3+	1:640	2+
19065	1:32	64	1:16	1:320	3+	1:640	2+
19500	Positive	4	1:32	1:160	3+	1:320	2+
18905	1:2	2	1:4	1:320	4+	1:640	2+
18288	1:16	32	1:125	1:640	3+	1:1280	2+
18287	1:8	16	1:94	1:80	3+	1:160	1+
18481	1:32	64	1:75	1:80	4+	1:160	1+
18411	1:16	32	1:25	1:160	3+	1:320	–1+
18425	1:4	4	1:25	1:10	3+	1:20	2+
18582	1:32	128	1:25	1:40	3+–4+	1:80	2+
L. J.			1:2	Undiluted	4+	1:10	1+
18523	1:8	16	1:5	Do.	4+	1:10	1+

[1] Titers expressed as the actual dilution of serum tested.

[2] Unsatisfactory in TPI test. Serum showed nonspecific immobilization in control tube containing inactive complement.

[3] Unsatisfactory in agglutination test. Serum too grainy and amount insufficient to centrifuge and repeat test.

Table 8 shows the results of TPI and agglutination tests on serums from patients with suspected biological false-positive reagin tests. A total of 69 serums was examined. In the TPI test, 28 were positive, 15 negative, and 26 gave nonspecific immobilization which was not prevented by the addition of penicillinase (*7*). Of the 28 serums positive by the TPI test, 27

Table 7. Effect of diluting syphilitic serum in saline or in normal human serum on the sensitivity of the TPI and agglutination tests

Original dilution of serum in saline	TPI titers[1]—Original saline dilutions further diluted in—		Agglutination titers[1]—Original saline dilutions further diluted in—	
	Saline	Serum	Saline	Serum
1:2	1:4	1:4	1:80	1:80.
1:4	1:2	1:4	1:20	1:20.
1:8	Undiluted	Undiluted	1:20	1:40.
1:16	Negative	Negative	1:40	1:20.
1:32	do	do	1:10	1:10.
1:64	do	do	Undiluted	Undiluted.
Control of undiluted serum titrated in saline	1:8		1:160	

[1] Expressed as the actual dilution of serum tested.

were positive by the agglutination test, and 1 was negative. Of the 15 serums negative by the TPI test, 4 were positive by the agglutination test, and 11 were negative. Of the 26 serums giving nonspecific immobilization in the TPI test, 13 were positive by the agglutination test, and 13 were negative.

Discussion

A method has been described for enhancing the agglutination of *Treponema pallidum* in syphilitic serum by the addition of fresh steer serum. By means of this technique the presence of syphilitic antibody may be demonstrated in a specific test which is completed in only 2 hours. While it is not the object of the present paper to propose the use of this technique as a diagnostic test at present, it has certain obvious advantages over the serologic procedures currently used in the diagnosis of syphilis. Killed spirochetes are used as the antigen, the antigens may be stored for periods of months in the refrigerator or at −20° C., and the materials could be made available to any serologic laboratory. Studies to date indicate that the agglutination test may have a specificity comparable with that of the TPI test. It also appears to have greater reproducibility than the TPI test, and several times the sensitivity.

The mechanisms involved in the reactions of *T. pallidum* with syphilitic antibody and steer serum are under continued study. It was shown in experiments reported here that the enhancing effect of fresh steer serum in the agglutination test was due to the presence of conglutinin (*4*). It was found in later experiments not described here that the disappearance of the organisms under the conditions of the TPI test was also caused by conglutinin. It is not yet known whether the disappearance of the spirochetes under the conditions of either the TPI test or the agglutination test is a result of lysis or of

Table 8. Results of TPI and agglutination tests on serum from patients with suspected biological false-positive reagin tests

Total serums tested	TPI positive—28		TPI negative—15		TPI nonspecific[1]—26	
	Agglutination positive	Agglutination negative	Agglutination positive	Agglutination negative	Agglutination positive	Agglutination negative
69	27	1	4	11	13	13

[1] Nonspecific immobilization in tube containing inactive complement.

unusually strong agglutination. Although results with syphilitic serum absorbed with lipoidal antigen indicate that the agglutination test is probably not a measure of reagin, the identity of the antibody has not been determined. The possible identity of this agglutinating antibody with the TPI antibody (*5*) or other agglutinating antibodies (*1–3*) is now being investigated.

Summary

A study has been made of the effects of adding fresh steer serum to mixtures of *T. pallidum* and syphilitic serum. Because of its content of conglutinin, the steer serum caused disappearance rather than immobilization of the spirochetes under the conditions of the TPI test, and greatly accelerated and enhanced the clumping of the organisms in the agglutination test. The agglutination test performed by this method appears to compare favorably both in specificity and sensitivity with the TPI test.

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Venereal Disease Postgraduate Course

The 21st venereal disease postgraduate course will be held at Chapel Hill, N. C., from September 28 through October 2 under the cosponsorship of the United States Public Health Service and the North Carolina University Schools of Medicine and Public Health. The course is open to all physicians. Applications for enrollment should be sent to Dr. Harold Magnuson, Director, Venereal Disease Experimental Laboratory, Box 687, Chapel Hill, N. C. The courses previously were held twice annually at the United States Public Health Service Medical Center at Hot Springs, Ark., until the center was closed last June.