ON THE PRESENCE IN SYPHILITIC SERUM OF ANTIBODIES TO SPIROCHETES, THEIR RELATION TO SO CALLED WASSERMANN REAGIN, AND THEIR SIGNIFICANCE FOR THE SERODIAGNOSIS OF SYPHILIS

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When the Wassermann test was discovered, it was viewed as a specific antigen-antibody reaction. Spirochetal products present in an extract of fetal syphilitic liver were believed to function as antigen, reacting with an antibody to spirochetes supposed to be present in syphilitic serum to give the positive complement fixation reaction. The subsequent discovery that an alcoholic extract of normal mammalian tissue could be used as "antigen" instead of the aqueous extract of syphilitic liver apparently demolished that theory, and the Wassermann reaction has since remained a puzzling anomaly among serodiagnostic tests.

There has been considerable speculation (literature summarized in (1)) as to the nature of the constituent of syphilitic serum which reacts with tissue lipoids to give complement fixation (Wassermann) and aggregation (the flocculation tests: Eagle, Hinton, Kahn, Kline, Meinicke, Müller, etc.). These reactions have been held by some to be completely non-specific, evidence of a vague colloidal lability of the serum proteins. Against this theory may be adduced the finding that the reactive component of syphilitic serum (reagin) is a definite substance, a globulin which is present in minute but measurable amounts (2), and which can be absorbed by tissue lipoids. Moreover, in every respect susceptible of experimental study, reagin behaves like an antibody rather than an abnormally labile protein (3).

Other workers, notably Sachs, Klopstock, and Weil (4) have considered reagin to be an antibody to the host's own lipoids, liberated in the course of tissue destruction at foci of infection. These autogenous lipoids, otherwise non-antigenic, were believed by them to be activated by spirochetal protein to form a complete antigen. Although this theory has been widely accepted, it fails to explain the extraordinary specificity of the serodiagnostic tests for syphilis. There seems no *a priori* reason why spirochetal protein should uniquely activate the lipoidal tissue haptene and thus initiate antibody production, while in other conditions in which there is just as much tissue destruction and in which some other bacterial protein is available as the activator, the Wassermann remains consistently negative.

From time to time, various investigators have reported that syphilitic serum contains antibodies to spirochetes. Thus, syphilitic serum has been reported to agglutinate suspensions of cultured spirochetes in higher titres than do normal sera (5–9, 34). Similarly, several investigators have reported that syphilitic human sera give complement fixation with suspensions or extracts of cultured spirochetes (10–18). Gaehtgens in particular has developed a sensitive and specific diagnostic complement fixation test in which a phenolized culture of the Reiter strain of *Treponema pallidum* is used as antigen (19, 33). The question as to whether syphilitic serum contains spirocheticidal antibodies has been reopened by the recent work of Tani and his coworkers (20) and Turner (21), who have obtained suggestive evidence in that direction. Finally, spirochetes injected into experimental animals have been reported to cause the appearance not only of spirochetal antibodies, but of positive Wassermann tests (complement fixation with tissue lipoids) as well (13 *b*, 14, 15, 17).

Contrary to these reports, numerous other investigators have found no significant differences in the spirochetal agglutinating or complement fixing activity of normal and syphilitic serum (23–28, 32), and have been unable to produce regularly positive Wassermann reactions in either experimental animals or human beings by the injection of killed cultures of *T. pallidum* (27–31, 43). These discrepant results may well be due to the fact that there are numerous strains of cultured *T. pallidum*, all of which are non-pathogenic for rabbits.[1] There is accordingly good reason to doubt that any of these strains is actually *T. pallidum*, and it would not be surprising if they differed in their reactivity with syphilitic serum. The weight of the evidence just cited does indicate that syphilitic serum reacts with certain cultured strains of spirochetes to give both agglutination and complement fixation. In particular, the diagnostic utility of the complement fixation test with the cultured Reiter strain as described by Gaehtgens, has been amply confirmed (literature summarized in (33)). That same strain has been shown by Beck (34) and in this laboratory to be agglutinated in much higher titre by syphilitic serum than by normal serum.

The relationship of these spirochetal antibodies present in syphilitic serum to the so called "reagin" detected by the Wassermann and flocculation tests, has remained obscure. Kroó, Schulze, and Zander (35) have found that the spirochetal antibody, unlike Wassermann reagin, resists heating to 63°C.; and Gaehtgens (19) has reported that absorption of syphilitic serum with either spirochetes or beef heart lipoids removes only the one type of "antibody," without affecting the other. These workers have therefore concluded that syphilitic serum contains two distinct substances: antibodies to *T. pallidum* which cross-react with cultured spirochetes, and an unrelated, non-specific factor which inexplicably reacts with tissue lipoids to give the diagnostic flocculation and Wassermann tests. On the other hand, Eagle (1, 36) has suggested that so called Wassermann reagin and spirochetal antibody, instead of being independent substances with a different immunological background, are actually one and the same thing. On that theory, the primary serum change in syphilis, as in any other bacterial infection, is the development of antibodies to the infectious agent, in this case, antibodies to *T. pallidum*. The cross-reaction of those antibodies with mammalian tissue extracts (Wassermann and floccula-

[1] The occasional reports of pathogenic cultured organisms are poorly documented, and other workers using the same strains have obtained consistently negative results on attempts at animal inoculation (1, page 296).

tion tests) would depend on the fortuitous presence in mammalian tissue of lipoids closely related immunologically to an antigenic component of the spirochete.

The recent work of Beck (34) strongly supports this view. Absorption of syphilitic sera with some, but not all, strains of cultured spirochetes was found by him variably to diminish their Wassermann reactivity; moreover, alcoholic extracts of spirochetal suspensions were found to behave qualitatively like alcoholic tissue extracts in complement fixation tests with syphilitic serum.

The experiments to be reported here further support the thesis that the positive Wassermann and flocculation tests given by syphilitic serum with alcoholic tissue extracts are due simply to the fact that syphilitic serum contains antibodies to spirochetes, and that these antibodies happen to cross-react to a limited degree with mammalian tissue lipoids. The significance of these findings for the practical serodiagnosis of syphilis is discussed in the text.

Methods and Materials

Spirochetal Suspension.—The spirochetal suspension used in the experiments here described was the phenolized suspension of the Reiter strain,[2] recommended by Gaehtgens for use in the serodiagnosis of syphilis. This killed suspension contained approximately 10^9 organisms per cc., along with a considerable amount of debris which made the suspension as a whole appear granular. Morphologically, the organisms were both thicker and longer than *T. pallidum* as seen in rabbit or human lesions; the spirals were more shallow, and not as sharply angled or closely woven as the corkscrew spirals of the pathogenic spirochetes. Even the inexperienced would have little difficulty in distinguishing the two.

A second lot of Palligen subsequently obtained from the same source contained fewer organisms, and had a larger proportion of amorphous debris. Its reactivity also seemed less than that of the suspension used in the present study.

Complement Fixation.—In describing the complement fixation technique for use with this spirochetal suspension, Gaehtgens stressed the necessity for a preliminary titration to determine the amount of complement which suffices to counteract the anticomplementary activity of the suspension. However, it was found unnecessary to perform this preliminary titration. Palligen in the proper dilution was substituted for the cholesterolized alcoholic extract of beef heart used in the Wassermann test, and the technique was not otherwise altered. In the particular Wassermann technique used in this laboratory (1), all reagents are used in the same volume, and the complement is used in a fixed dilution, without titration. When substituted in that technique for ordinary Wassermann antigen, the spirochetal suspension was found to be partially anticomplementary in approximately a 1:2 dilution. Its complement-fixing activity, to be presently described, was found to decrease beyond a 1:10 dilution, and to disappear at approximately 1:20 to 1:30 dilutions. It was accordingly used in 1:6 to 1:8 dilutions in the actual test.[3]

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[2] Sold by the Sächsische Serumwerke at Dresden under the trade mark "Palligen."

[3] The relatively narrow zone within which Palligen gave reliable results (1:6 to 1:8 dilutions) is to be contrasted with the enormous range of dilutions over which the alco-

To 0.2 cc. of the inactivated serum or serum dilution were added 0.2 cc. of 1:10 pooled guinea pig complement, and 0.2 cc. of antigen (a 1:120 dilution of cholesterolized beef heart extract for the Wassermann test, or 1:6 to 1:8 dilutions of the spirochetal suspension). After 4 hours at 2–5°C., followed by ½ hour at 37°C., there were added 0.2 cc. of a 3 per cent suspension of sheep cells and 0.2 cc. of amboceptor diluted so as to contain 2½ hemolytic units. Actually, the latter two reagents were mixed, and 0.4 cc. of the sensitized cell suspension was added. Results were read after ½ hour at 37°C., and recorded as positive (no hemolysis), doubtful (partial hemolysis), or negative.

Spirochetal Agglutination.—To 0.2 cc. of inactivated serum (or serum dilution) were added 0.05 to 0.1 cc. of undiluted Palligen. The mixture was shaken for 3 to 5 minutes in a shaking machine at 240 to-and-fro movements per minute, with a 4 cm. stroke, and then centrifuged for 3 to 4 minutes at 1000 R.P.M. The sediment was gently resuspended, and the organisms examined under the dark field at 900 × magnification. The agglutination "titres" given in the tables are approximate only, as the end-point was difficult to establish. The clumps of spirochetes merely became smaller as the serum was progressively diluted; and even a small fraction of the serum concentration which caused the formation of definite coarse clumps sufficed to produce small aggregates of 2 or 3 spirochetes. The end-point was therefore far less sharp than in the case of the other three tests here studied (Wassermann; flocculation of beef heart lipoid; spirochetal complement fixation), all of which changed from definite positive to definite negative within a comparatively narrow range of serum concentrations.

Flocculation of Beef Heart Lipoid.—The serodiagnostic flocculation test discussed in the text was the Eagle modification, using as antigen an alcoholic extract of beef heart fortified with both cholesterol and corn germ sterol. All results were read both macro- and microscopically (37).

The Reactivity of Syphilitic Serum with Cultured Spirochetes

The observation of Gaehtgens that a phenolized suspension of cultured spirochetes (Reiter) gives positive complement fixation with syphilitic human serum, and consistently negative reactions with normal human sera, was readily confirmed. As will be indicated in detail elsewhere (33), a preliminary study of 1000 human sera confirmed the finding of Gaehtgens and many other German workers that this test compares favorably in both sensitivity and specificity with standard Wassermann and flocculation procedures.

In general, antibodies which give complement fixation in the presence of a specific antigen also cause the aggregation of that antigen (bacterial agglutination; protein precipitation). In this case also, as recently shown by Beck (34) and confirmed in this laboratory, syphilitic serum agglutinates the Reiter strain of cultured spirochetes (Table I, which gives as example the results in 9 sera out of approximately 100 tested). It is to be noted

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holic extracts of beef heart used in the Wassermann test continue to react with syphilitic serum. Attempts to increase that margin of safety are now in progress.

TABLE I

The Reactivity of 9 Syphilitic Human Sera with a Phenolized Suspension of Cultured Spirochetes (Palligen)

Serum titre* in			
Wassermann	Flocculation test (Eagle micro technique)	Spirochetal complement fixation reaction	Spirochetal agglutination
64–128	32	32–64	32–64
64–128	64	64–128	32
16–32	24	32–64	16–32
16–32	32	32–64	32
20–40	†	40–80	†
8	1	8–16	8–16
†	<8	†	8
Positive‡	1–2	Positive	8–16
"	2–4	"	8

* In the tables, the titre in each instance is expressed as the highest dilution of serum giving a definitely positive result. The double numbers (*e.g.*, 6–12) signify that the serum gave a positive result in a 1:6 dilution, and a doubtful result in a 1:12 dilution.

† Not done.

‡ Not titred.

TABLE II

The Reactivity of 15 Normal Human Sera with a Phenolized Suspension of Cultured Spirochetes (Palligen)

Serum titre in			
Wassermann	Flocculation test (Eagle micro technique)	Spirochetal complement fixation reaction	Spirochetal agglutination
0	0	0	6–12
0	0	0	3–6
0	0	0	3–6
0	0	0	6
0	0	0	6–12
0	0	0	6
0	0	0	3–6
0	0	0	6–12
0	0	0	3–6
0	0	0	12
0	0	0	3–6
0	0	0	6–12
0	0	0	8–16
0	0	0	8–16
0	0	0	8–16

that the serum agglutinin titre roughly paralleled the complement-fixing activity and that both titres were of the same order of magnitude as those obtained with the ordinary Wassermann and flocculation tests.

TABLE III

The Reactivity of Normal and Syphilitic Rabbit Sera with a Phenolized Suspension of Cultured Spirochetes (Palligen)

Type of serum	Wassermann titre	Flocculation titre (Eagle micro technique)	Spirochetal complement fixation titre	Spirochetal agglutination titre, approximate
Normal rabbit sera	2–4	0	8–16	8–16
	0	0	2–4	4–8
	0	0	8–16	8
	0	0	1–2	8
	0	0	4	8
	0	0	4–8	8–16
	1–2	0	16	4–8
	2–4	0	8–16	4–8
	0	0	0	16–32
	0	0	8–16	8
	0	0	4–8	4–8
	1–2	0	8	2–4
	0	0	8–16	4–8
	0	0	0	8–16
	0	<1*	4–8	32
	2–4	0	8–16	16
	0	<1	8–16	8–16
	0	0	0	4–8
	4–8	0	4–8	16–32
	2–4	0	8–16	16
	0	0	8–16	16–32
	0	0	4	8–16
	4–8	0	4–8	16–32
Serum of syphilitic rabbits taken 3 wks. after intratesticular inoculation	2	2–4	4–8	8
	2–4	0	8–16	8
	4	16	16–32	16
	2–4	0	8	8
		2–4	16–32	8–16
	0	0	0	4–8
	<2	4–8	16	8–16
	<2	4–8	16–32	16
	16	32–64	32–64	32–64
	4–8	32	32–64	64
	4	32	16–32	32–64
	2–4	4–8	8–16	8–16
Serum of syphilitic rabbits taken 5 wks. after intratesticular inoculation	128	48	64–128	32–64
	28	16	16–32	16–32
	96		32–64	32–64
	32	32	16–32	16–32
	6		16–32	64
	48	48	64–128	64–128

* A doubtful result when tested as whole serum.

As stated by Beck (34), the diagnostic utility of this spirochetal agglutination is unfortunately vitiated by the fact that normal human serum regularly contains small amounts of spirochetal agglutinin (Table II). Although with the particular technique here used, this normal agglutinin titre rarely exceeded a 1:12 dilution, in contrast with the higher titres obtained in some syphilitic sera, it follows that only strongly positive sera could be identified with certainty as syphilitic. Those sera which fix complement and give agglutination only in, *e.g.*, 1:4 to 1:8 dilutions, would clearly be indistinguishable from normal serum by the agglutination procedure.

The spirochetal antibody of normal human serum differs qualitatively from that elaborated in the course of syphilitic infection in that it fails to give a positive complement fixation reaction. In contrast, normal rabbit serum gives both agglutination and weak complement fixation with the spirochetal suspension (Table III). Although this normal reactivity of rabbit serum increases markedly in the course of syphilitic infection, the difference is apparently one of degree, and not of kind.

The Relationship of the Spirochetal Antibody of Syphilitic Serum to So Called Wassermann and Flocculation Reagin

Relative Thermolability of Reagin and Spirochetal Antibody.—The question arises as to the relationship of the spirochetal antibody readily demonstrable in the serum of syphilitic human beings by agglutination and complement fixation, to the so called reagin which reacts with mammalian tissue lipoid to give positive Wassermann and flocculation tests. It has been stated (19, 35) that the two antibodies have nothing in common save their simultaneous presence in syphilitic serum, that they differ in their thermolability (35), and that absorption of the serum with either the lipoid or a spirochetal suspension removes only the antibody for the one, without affecting the other (19).

The data to be here reported are not in accordance with those findings. In the first place, contrary to Kroó, Schulze, and Zander (35), and confirming Hoeltzer and Ssuschkowa (38), there was no significant difference in the rate of destruction at 63°C. of the spirochetal antibody and of the reagin which determines Wassermann and flocculation tests. One of three experiments on this point, all with qualitatively similar results, is summarized in Table IV.

Absorption Experiments.—The supposed demonstration by absorption experiments that these two antibodies are independent has been called into question by the recent work of Beck (34), who noted a partial disappearance

of the Wassermann reactivity of syphilitic serum after absorption with some strains of cultured spirochetes, and who further noted a qualitative similarity between alcoholic extracts of cultured spirochetes and the tissue extracts used in Wassermann tests. Our own absorption experiments indicate that the positive complement fixation and flocculation tests given by syphilitic serum with tissue lipoids, as well as the similar reactions with spirochetal suspensions, are all due to the presence in syphilitic serum of antibodies to spirochetes. There is apparently no Wassermann or flocculation reagin as distinct from those spirochetal antibodies.

TABLE IV

Showing That There Is No Significant Difference in the Thermolability at 63°C. of Spirochetal Antibody and So Called Wassermann and Flocculation Reagin

Time for which syphilitic serum was kept at 63°C.	Titre of serum as determined by			
	Reagin		Spirochetal antibody	
	Wassermann test	Flocculation test	Spirochetal complement fixation reaction	Spirochetal agglutination*
min.				
0	156	96	112	56
3	112	96	96	56
15	40	48	48	32
30	32	24	32	10
60	10	1.5	12	1
120	4–8	†	4–8	†

* Approximate only.

† Reading precluded by partial coagulation of serum protein.

One of four experiments on this point, all with qualitatively similar results, is summarized in Tables V and VI. If syphilitic serum is absorbed with a large excess of beef heart lipoid, it becomes, as expected, both Wassermann- and flocculation-negative, *i.e.*, all the so called reagin has been bound by the lipoid. As stated by Gaehtgens and by Beck, the reagin-free filtrate gives both agglutination and complement fixation reactions with the spirochetal suspension in the same titre as the original serum (Table V). If, however, the same serum is absorbed with successive increments of the spirochetal suspension, all its reactivity, whether with beef heart lipoid or with a spirochetal suspension, disappears. The absorbed serum gives negative flocculation and Wassermann tests for syphilis, and loses its ability to agglutinate or give complement fixation with the spirochetes (Table VI).

TABLE V

Illustrating the Effect of Absorption with Beef Heart Lipoid on the Reactivity of Syphilitic Serum

(Same serum as that used in the experiment of Table VI)

Flocculation antigen (37) was diluted with 1.5 volumes of 4 per cent NaCl solution. 10 cc. of inactivated, Wassermann-positive syphilitic serum were absorbed with the sediment from (*a*) 2.5 cc. of the antigen dilution, and (*b*) 15.0 cc. of the antigen dilution. Each mixture was brought up to 20 cc. with 0.85 per cent NaCl, shaken, and centrifuged at high speed to remove most of the lipoidal particles. The supernatant fluid was then passed through a dry Berkefeld filter to remove the remaining lipoid. A control consisted of the original serum similarly diluted with salt solution and similarly filtered. The various tests indicated in the table were carried out on the Berkefeld filtrate.

Serum tested	Wassermann titre	Flocculation titre	Spirochetal complement fixation titre	Spirochetal agglutination titre	Conclusion
Original serum	16–32	32	32–64	32	
Serum absorbed with 2.5 cc. flocculation antigen	0*	2–3	32–64	32	Even 6 times the amount of flocculation antigen which sufficed to remove 90 per cent of so called reagin failed to affect the spirochetal complement fixation or agglutination titre
Serum absorbed with 15 cc. flocculation antigen	0*	0*	32–64	32	

* Negative in the 1:2 dilution tested.

TABLE VI

The Effect of Absorption with Cultured Spirochetes (Palligen) on the Reactivity of Syphilitic Serum

(Same serum as that used in the experiment of Table V)

Inactivated Wassermann-positive syphilitic serum was absorbed with the sediment obtained on the centrifugation of Palligen (4 cc. Palligen per cc. serum). The mixture was then centrifuged at high speed to remove the agglutinated organisms. This removal was so complete as to render filtration unnecessary. The absorption was repeated 4 times, an aliquot portion of each supernatant fluid being reserved for the performance of the various serologic tests indicated in the table.

Number of absorptions with Palligen	Wassermann titre	Flocculation titre	Spirochetal complement fixation titre	Spirochetal agglutination titre	Conclusion
Original serum	16–32	32	32–64	32	Absorption with the spirochetal suspension removed all reactivity, with beef heart lipoid and spirochetes alike
1	8–16	12	8–16	8–16	
2	<2*	4–6	<2	2–4	
3	<2	0	<2	<2	
4	<2	0	<2	<2	

* Absorbed serum negative in a 1:2 dilution.

In summary, beef heart lipoid removes from syphilitic serum only so called reagin, the substance which determines the positive Wassermann and flocculation tests, without demonstrably affecting the reactivity of the serum with a spirochetal suspension. The spirochetal suspension, on the other hand, removes all the antibodies, both for the spirochetes and for tissue lipoids.

It is difficult to explain these observations on any basis other than (*a*) that the cultured strain of spirochete used in these absorption experiments is so closely related immunologically to pathogenic *T. pallidum* that the antibodies to the latter elaborated in the course of syphilitic infection cross-react with the non-pathogenic cultured organism, which may or may not be *T. pallidum*; and (*b*) that both the pathogenic and the cultured Reiter strains of spirochete contain, among other antigenic factors, one which is similar to an ubiquitous lipoid present in almost all mammalian tissue. As serum antibodies are elaborated to the various antigenic components of *T. pallidum*, antibodies therefore appear which cross-react with that tissue lipoid. The absorption of syphilitic serum with the tissue lipoids apparently removes only those cross-reacting antibodies, and does not affect the antibodies to the other antigenic components of the spirochete. The absorbed serum therefore continues to agglutinate and give complement fixation with the spirochetal suspension. On the other hand, absorption with the spirochetal suspensions removes all the antibodies, to lipoids and other factors as well, and renders the serum completely inactive with that same spirochetal suspension.

The Production of Antibodies to Cultured Spirochetes by Immunization with Tissue Lipoids

Since syphilitic sera become Wassermann- and flocculation-negative after absorption with a washed suspension of cultured spirochetes, it follows that the antigenic factor of the spirochete which is serologically related to tissue lipoids is present on the surface of the organisms. One would accordingly expect that antibodies to tissue lipoids would combine with spirochetes to give agglutination and complement fixation. This has been found to be the case. As previously shown (4), the lipoidal floccules formed in the various diagnostic flocculation tests for syphilis, when washed free of excess serum and injected intravenously into rabbits, cause the appearance of antibodies to lipoids, *i.e.*, positive Wassermann and flocculation tests, in high titre. Presumably, the lipoidal tissue haptene is activated by the foreign protein (the reagin-globulin of syphilitic human serum) with which it is in intimate combination, to form a complete antigen (40–42.)

As shown in Table VII, the antisera so produced agglutinate and give complement fixation with suspensions of cultured spirochetes in titres far exceeding the reactivity of normal rabbit sera. When these antisera are absorbed with tissue lipoids, by the method described in Table V, their

TABLE VII

The Presence of Spirochetal Agglutinins and Complement-Fixing Antibodies in Rabbits Immunized to Beef Heart Lipoid

100 cc. of flocculation antigen (37) were diluted with 150 cc. of 4 per cent salt solution. The resulting milky suspension was centrifuged and the sediment added to 2 liters of Wassermann-positive syphilitic serum, previously inactivated at 56°C. for 30 minutes. The suspension was placed in a shaking machine for 5 minutes, diluted with 4 liters of 0.85 per cent NaCl, and centrifuged for ½ hour. The sediment, consisting of the lipoid and the reagin with which it had combined, was washed with 200 cc. of salt solution and resuspended in 100 cc. 6 rabbits were injected intravenously three times weekly for 5 weeks with gradually increasing amounts of suspension (0.4 cc., increasing to 0.8 cc.).

Rabbit No.	Time of bleeding	Reactivity of serum with			
		Tissue lipoids		Spirochetal suspension	
		Wassermann titre	Flocculation titre	Complement fixation titre	Agglutination titre
24-05	Before immunization	0	0	<4	6
	After "	256–512	128	64–128	32–64
24-23	Before "	0	0	<4	3
	After "	512	256	64–128	64–128
24-36	Before "	0	0	6	2
	After "	512	256	128–256	128
24-47	Before "	12	0	6	4
	After "	256–512	128–256	64–128	64
25-11	Before "	3	0	6	3
	After "	512–1024	256	64–128	64–128

reactivity with spirochetes almost completely disappears.[4] It seems clear that antibodies to tissue lipoids cross-react with the Reiter strain of cultured spirochetes to give complement fixation and agglutination.

[4] Small amounts of spirochetal agglutinin persist after absorption with tissue lipoids, the titre corresponding to the reactivity of normal rabbit serum (Table III). These residual spirochetal antibodies cannot be removed even by a large excess of tissue lipoids and probably are the normal antibodies, which are similarly unaffected by absorption with tissue lipoids.

It is noteworthy that the Wassermann and flocculation titres of the anti-lipoidal sera greatly exceed the spirochetal agglutination and complement fixation titres (*cf.* Table VII). This is contrary to the results obtained in syphilitic serum, with which spirochetes often give a higher titre than do tissue lipoids. This qualitative difference between anti-lipoidal sera and syphilitic sera is in keeping with the dual thesis of the present paper (*a*) that *T. pallidum*, as well as cultured spirochetes, contain an antigenic factor resembling, but not necessarily identical with, the reactive constituent of tissue extracts, and (*b*) that this factor is only a part of the antigenic complex of the organism. Syphilitic sera often give higher titres with the spirochetal suspension than they do with tissue lipoids, first, because the spirochetal suspensions contain reactive factors other than the lipoid, and second, because the spirochetal lipoid may be, and probably is, more closely related to the lipoidal component of *T. pallidum* than is the tissue extractive. Conversely, antibodies to the tissue lipoid would be expected to react more strongly with the same lipoidal suspension than they would with a spirochetal suspension the lipoidal component of which may differ qualitatively from the tissue extractive, and at best constitutes only a part of the antigenic complex on the surface of the organism.

The immunological response of rabbits to cultured spirochetes, in particular, the paradoxically low titred Wassermann reaction given by spirochetal antisera (*cf.* page 216), will be considered in a following paper.

DISCUSSION

The data here presented strongly support the thesis (1, 34, 36) that the serum change in syphilis is primarily an antibody response to *T. pallidum*, and that the reactivity with tissue lipoids which underlies the diagnostic Wassermann and flocculation tests is a chance cross-reaction due to the presence of a serologically related substance in *T. pallidum* and in mammalian tissue extracts. The disappearance of all Wassermann and flocculation reactivity on the absorption of syphilitic serum with cultured spirochetes (Reiter strain), and the production of spirochetal agglutinins and complement-fixing antibodies by the immunization of rabbits with tissue lipoids, clearly indicate that serologic relationship. The recent demonstration by Beck (34) that cultured spirochetes contain a substance extracted by alcohol and presumably lipoidal, which qualitatively resembles tissue lipoids in its reactivity with syphilitic serum, is further evidence in that direction. It should be emphasized that the component of the spirochetes which cross-reacts with tissue lipoids is only a part of the antigenic complex of the organism. Syphilitic sera absorbed with tissue lipoids

continue to give agglutination and complement fixation in undiminished titre with spirochetal suspensions.

The Reiter strain of cultured spirochetes used in these studies is not unique in its reactivity with syphilitic serum, as other cultured strains have been found by both Gaehtgens and Beck to behave similarly with syphilitic serum. Although the non-pathogenicity of these cultures raises serious doubts as to the propriety of their identification as *T. pallidum*, one must suppose that despite their non-pathogenicity and despite their morphological differences from true *T. pallidum*, some of these cultured strains are serologically so closely related to that organism as to be agglutinated by, and give complement fixation with, syphilitic sera. The possibility must be considered that pathogenic *T. pallidum* may contain antigenic factors over and above those present in these cultured strains. In that case, syphilitic serum absorbed with cultured strains would continue to react with true *T. pallidum*, and serologic reactivity would disappear completely only after absorption with the latter. These questions could be resolved by appropriate experiments with suspensions of the pathogenic organisms as obtained from rabbit or human lesions. However, technical difficulties have so far prevented the preparation of such suspensions sufficiently free from tissue extractives and sufficiently concentrated to permit their use in experiments along these lines.

In the meantime, until pathogenic *T. pallidum* can be regularly cultivated in quantity on artificial media, the use of cultured spirochetes may be of value for the routine serodiagnosis of syphilis. The fact that these organisms contain antigenic factors which react with syphilitic serum, some of which are not present in alcoholic beef heart extracts, suggests that many cases of syphilis which are negative to the Wassermann and flocculation tests now in use may be seropositive when tested with spirochete suspensions. In a small series recently studied from this point of view (*cf.* 33) this occurred with surprising frequency. Conversely, in conditions other than syphilis the serum may conceivably give false positive Wassermann or flocculation reactions with tissue extracts, and yet be negative when tested with a spirochetal suspension. This has already been stated as true for leprosy (39). Indeed, the discrepancies between the two types of test (with lipoidal tissue extracts and with spirochete cultures) may prove so marked that it may be necessary to revise our ideas with respect to the incidence of serologic "cure," and even more important, with respect to the occurrence of false positive reactions in conditions other than syphilis.

Such studies are now handicapped by the fact that only one proprietary

and costly preparation of cultured spirochetes is available for general use; and even that is so anticomplementary and of such varying activity as to enjoin caution in its routine use. Attempts to overcome these difficulties are now in progress.

SUMMARY AND CONCLUSIONS

1. In confirmation of Gaehtgens, syphilitic human sera give positive complement fixation with cultures of so called *T. pallidum* (Reiter strain). Syphilitic rabbit sera are equally reactive. Syphilitic human and rabbit sera agglutinate these cultures, often in high titre (Beck).

2. Normal rabbit sera react weakly with the culture to give both agglutination and complement fixation in low titre. Normal human sera, despite the fact that they contain agglutinins in low titre, fail to fix complement with the Reiter strain of cultured spirochetes. Confirming Gaehtgens, the latter reaction is therefore of practical utility for the serum diagnosis of syphilis.

3. When syphilitic serum is heated at 63°C., there is no demonstrable difference in the thermolability of the antibody to spirochetes, and of the reagin which determines the Wassermann and flocculation tests.

4. (*a*) The absorption of syphilitic serum by spirochetal suspensions removes all reactivity, not only for the spirochetes, but for tissue lipoids (alcoholic beef heart extract) as well; the sera become Wassermann- and flocculation-negative. (*b*) Absorption of syphilitic serum with tissue lipoids renders the Wassermann and flocculation tests negative, but does not demonstrably change the reactivity of the serum with spirochetes. (*c*) Rabbits immunized to beef heart lipoid develop spirochetal agglutinins and complement-fixing antibodies (Reiter strain) in high titre.

5. It is concluded that these cultured spirochetes contain antigenic material serologically related to a substance present in mammalian tissue, as well as other antigenic factors not present in such extracts, but equally reactive with syphilitic serum.

6. These findings support the thesis that the primary serologic change in syphilis is the development of antibodies to *T. pallidum*. The Wassermann and flocculation tests would be explained on the basis that the tissue extracts used as "antigen" in these tests contain one or more substances serologically related to antigenic components of *T. pallidum*. Similarly, the cultured Reiter strain of spirochete is apparently sufficiently close serologically to *T. pallidum* to be agglutinated by and to give complement fixation with the antibodies to *T. pallidum* present in syphilitic serum.

7. Since suspensions of cultured spirochetes contain antigenic factors which react specifically with syphilitic serum, some of which are not present in ordinary Wassermann and flocculation "antigens," they may prove even more valuable than those tissue extracts in the serodiagnosis of syphilis.

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