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IMMUNOLOGICAL STUDIES ON PURE CULTURES OF VARIOUS SPIROCHETES.

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INTRODUCTION.

As a sequel to the successful cultivation of *Treponema pallidum* and other allied forms of spirochetes,[1] an era of test-tube experimentation on the question of immunity in syphilis has been inaugurated. Previous to the time when these organisms were obtained in pure cultures, extensive experiments were carried out with suitable animals by various investigators, notably by Metchnikoff and Roux,[2] Neisser and his associates,[3] Landsteiner and Finger,[4] Uhlenhuth and Mulzer,[5] and others. Summing up their results, we are confronted with the fact that in syphilis no immunity, in the sense generally understood in bacterial infections, is demonstrable either in animals or in man.[6,7] The insusceptibility of animals or human beings who have once contracted the infection and have since been apparently cured of a subsequent infection with *Treponema pallidum*, a fact well recognized since the time of Ricord,[8] is no longer regarded as a state of immunity, but of an anergy,[3] which means that the same organism is not capable of responding to another infection as long as there is a preexisting infection. Moreover, clinical and experimental data show that when an individual is cured of an attack, he soon regains practically his original susceptibility to a second

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[1] Noguchi, H., *Münch. med. Woch.*, 1911, lviii, 1550; *J. Exp. Med.*, 1911, xiv, 99; 1912, xv, 81, 90, 466; xvi, 194.

[2] Metchnikoff, E., and Roux, E., *Ann. Inst. Pasteur*, 1903, xvii, 809; 1904, xviii, 1, 657; 1905, xix, 673; 1906, xx, 785.

[3] Neisser, A., Beiträge zur Pathologie und Therapie der Syphilis, Berlin, 1911; also *Arb. k. Gsndhtsamte.*, 1911, xxxvii, 569; *Deutsch. med. Woch.*, 1906, xxxii, 1, 97.

[4] Landsteiner and Finger, *Centr. Bakteriol., 1te Abt., Ref.*, 1906, xxxviii, Beil., 107. Landsteiner, K., *Centr. Bakteriol., 1te Abt., Ref.*, 1908, xli, 785.

[5] Uhlenhuth, P., and Mulzer, P., *Arb. k. Gsndhtsamte.*, 1913, xliv, 307.

[6] Zinsser, H., *J. Lab. and Clin. Med.*, 1916, i, 785.

[7] Levaditi, C., *Z. Immunitätsforsch., Ref.*, 1910, ii, 277–318.

[8] Ricord, P., Traité de la syphilis, Paris, 1845.

infection.[9] In this respect, syphilis bears more resemblance to a protozoan than to a bacterial disease. Attempts to study the immunity question *in vitro* have not been lacking, and numerous investigators claim to have observed diverse specific immunity reactions, such as precipitation,[10] immobilization,[11] complement fixation, [12, 13] etc., when the serum or cerebrospinal fluid had been brought in contact with a material containing the treponemata of syphilis. But analysis of these observations proved that many of these phenomena could not be confirmed, and none was strictly specific. The Wassermann reaction is very constant in syphilis, but not specific. It is evident, therefore, that there is no recognized specific antigen-antibody reaction in syphilis. Schereschewsky[14] tried agglutination tests with his impure cultures of a spirochete derived from syphilitic material, with inconclusive results. We cannot, however, accept the negative findings of earlier investigators as final until it has been demonstrated that the techniques employed cannot be further improved. The great obstacle in the way of satisfactorily testing immune reactions in syphilis lies in the fact that a sufficient quantity of virulent organisms free from tissue constituents is obtained only with difficulty. A pure culture would fill this requirement.

In 1912 Noguchi[15] had already commenced to employ his several strains of culture *pallidum* for the purpose of studying the various immunity problems which were awaiting solution by means of a culture material. The main efforts were directed to finding out whether a rabbit repeatedly inoculated with the pure *pallidum* cultures will become resistant to a subsequent inoculation with a virulent *pallidum*. The cultures used were already avirulent. For comparison, another series of rabbits was similarly treated with live and killed virulent *pallida* for the same length of time, which covered a period of 5 months. The mode of immunization consisted in intravenous and intratesticular inoculation, except in the case of the live virulent material, which could not be used intratesticularly on account of its tendency to start the infection in the organ. The aim of the intratesticular mode of immunization was to find out whether there is such a thing as a local immunity in syphilis. The immunized animals were tested with a virulent strain by inoculating it into their testes. It was found that six out of the twelve rabbits immunized with the culture *pallidum* intravenously took the inoculation, while five of the twelve rabbits receiving the virulent *pallidum* took. Those which were immunized with the *pallidum* emulsions (live culture and killed

[9] John, F., *Samml. klin. Vortr.*, 1909, 559 ff.

[10] Fornet, W., *Berl. klin. Woch.*, 1908, xlv, 85.

[11] Hoffmann, E., and von Prowazek, S., *Centr. Bakteriol., 1te Abt., Orig.*, 1906, xli, 741, 817.

[12] Detre, L., *Wien. klin. Woch.*, 1906, xix, 619.

[13] Wassermann, A., Neisser, A., and Bruck, C., *Deutsch. med. Woch.*, 1906, xxxii, 745.

[14] Schereschewsky, *Deutsch. med. Woch.*, 1909, xxxv, 1652.

[15] Noguchi, *J. Am. Med. Assn.*, 1912, lviii, 1163.

virulent strains) lost much of the glandular structure of their testes, some becoming mere strands of hard connective tissue. Positive takes were recorded in three of the six rabbits treated with the culture and two of the six treated with the killed *pallidum* from a syphilitic orchitis of the rabbit. As the number of animals was so small it was impossible to draw any conclusion. The results as compared with control series with normal rabbits were striking, since the takes in the normal animals were practically 90 per cent. There was an indication that the repeated inoculation of the rabbit with the *pallidum* material reduced susceptibility to a certain extent, although the difference may have been due to the altered structure of the inoculated testes. The immune sera obtained from these rabbits were also tested for agglutination, spirochetolysis, complement deviation, and opsonic property against their homologous and cross antigens. The results were at first encouraging, but repeated experiments soon showed them to be indecisive on account of the technical difficulties in the way of obtaining satisfactory antigens. This was so even with the culture *pallidum*, which at that period was either very difficult to obtain free of culture media or underwent spontaneous agglomeration.

Craig and Nichols,[16] employing alcoholic extracts of pure cultures of the *pallidum*, *pertenuis*, and *microdentium*, furnished by Noguchi, reported that these antigens fixed complement with syphilitic sera very much as does an alcoholic extract of a congenitally luetic fetal liver.

Kolmer,[17] in the meanwhile, employing a strain of culture *pallidum* furnished by Noguchi, prepared immune sera in the rabbit and demonstrated the presence of agglutinins for the strain used. His most powerful serum agglutinated the *pallidum* in a dilution of 1 : 1,280. Kolmer, Williams, and Laubaugh[18] next studied a series of human and animal sera with regard to complement fixation, with the culture *pallidum* as antigen. Their positive findings, although more numerous, were nevertheless similar to those already obtained by Noguchi.[15] On the other hand, they noticed that rabbit immune sera not only fixed complement with their homologous antigens, but also with washed typhoid and cholera antigens. They point out, as was previously emphasized by Noguchi,[19] that these immune sera do not fix complement with the alcoholic extract of the culture *pallidum*.

Kissmeyer,[20] employing a strain of culture *pallidum* as antigen in the agglutination tests with human sera, obtained a consistent result when compared with the clinical and Wassermann reactions, but this apparent specificity was evidently

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[16] Craig, C. F., and Nichols, H. J., *J. Exp. Med.*, 1912, xvi, 336.

[17] Kolmer, J. A., *J. Exp. Med.*, 1913, xviii, 18.

[18] Kolmer, J. A., Williams, W. W., and Laubaugh, E. E., *J. Med. Research*, 1913, xxviii, 345.

[19] Noguchi, *J. Exp. Med.*, 1909, xi, 84.

[20] Kissmeyer, A., *Deutsch. med. Woch.*, 1915, xli, 306.

not confirmed by Zinsser, Hopkins, and McBurney.[21] The recent contributions made by these investigators[22] have advanced our knowledge of the immunity phenomena in connection with the cultivated avirulent and uncultivated virulent *pallidum* strains; comparing the behavior of both towards the immune sera prepared by means of the former. They came to the conclusion that the immune serum behaves in the same manner as a bacterial immune serum when tested upon the culture *pallidum*; that is, it produces agglutination, causes destruction of the organism with the cooperation of fresh complement, and fixes complement with the culture antigen. On the other hand, no immunity phenomena are demonstrated when the immune serum and the virulent uncultivated organisms of the same strain are brought together. They have not mentioned, however, whether or not an immune serum prepared by means of injecting the virulent material repeatedly will ever produce a protective or spirocheticidal principle.

The work of Kolmer and Zinsser, Hopkins, and McBurney, to which we have referred, has brought out a number of facts, particularly with regard to the relation between the avirulent culture and the virulent tissue *pallidum* strains, but the question is still far from being solved. It is not superfluous, therefore, that any data bearing upon this phase of the study should be published.

The present paper deals with the results of immunological studies which had been interrupted but were recently resumed. As will be seen from the following account, we have used a considerably larger number of culture spirochetes and of animals and continued the immunization longer than any worker has previously reported. We have purposely avoided touching the question of the relation between the cultivated and uncultivated strains, as we expect to consider that point in a future paper.

EXPERIMENTAL.

Material and Scope of Experiments.

In the present series of immunization experiments, rabbits of about 3 kilos were employed. Several rabbits were used for each of the four strains of *Treponema pallidum*, and several also for each of the following: *Treponema calligyrum*, *Spirochæta refringens*, *Treponema microdentium*, and *Treponema mucosum*. The mode of immunization

[21] Zinsser, H., Hopkins, J. G., and McBurney, M., *J. Exp. Med.*, 1916, xxiv, 561.

[22] Zinsser, H., and Hopkins, J. G., *J. Exp. Med.*, 1915, xxi, 576; 1916, xxiii, 323. Zinsser, Hopkins, and McBurney, *ibid.*, 1916, xxiii, 341.

consisted in the intravenous injection every week of 1.5 to 3 cc. of the fluid culture of each strain, the strains having been grown in a medium consisting of equal parts of normal rabbit serum and faintly alkaline bouillon, with a piece of fresh rabbit kidney. The ascitic fluid usually used was replaced by rabbit serum in order to avoid the production of a specific precipitin or of complement-binding antibodies for human proteins in the animals immunized against the spirochetal cultures. The organisms grew fairly well in this medium, owing undoubtedly to their gradual adaptation previously to various culture media which originally were unsuitable for their growth. The age of the cultures varied from 14 to 30 days, and the number of spirochetes was approximately thirty to forty per field when examined under the dark-field microscope (Leitz oc. 3, $\frac{1}{12}$ oil immersion).

The aim of these immunization experiments was to study the development of such agglutinins, complement-binding antibodies, opsonins, and spirocheticidal principles as may be demonstrated *in vitro* in the sera of rabbits repeatedly inoculated with spirochetes. The immunity reactions were first tested with the specific organisms and then with those not used for the production of the immune sera in question. The cross examinations, various non-specific as well as

TABLE I.

Immunization Tests with Treponema pallidum.

Rabbit No.	*T. pallidum.*	Period of immunization.	No. of injections.
		1915	
1	Strain McD.	Sept. 14–Dec. 17	13
2	" "	" 14– " 17	13
3	" "	" 10– " 17	11
4	" "	Oct. 20– " 17	8
5	Strain XI.	Sept. 14–Dec. 17	13
6	" XI.	" 14– " 17	13
7	" XI.	" 30– " 17	11
8	" XI.	" 30– " 17	11
9	Strain B29.	Sept. 14–Dec. 17	13
10	" B29.	" 30– " 17	11
11	Strain Z. A.	Sept. 14–Dec. 17	13
12	" "	" 14– " 17	13

the specific antigens being used against a given immune serum, were undertaken with a view to establishing the relation which may exist between different members of the group of spirochetes.

The protocols of immunization are given in Table I.

The results of experiments with the immune sera may be summarized under separate heads.

Agglutinins.

The mode of determining the content of agglutinins in each serum was conducted in the following manner.

Into a series of small sterile test-tubes were measured quantities of the immune serum in amounts graduated from 0.1 to 0.000001 cc. for each tube. The distribution of the various amounts was carried out as is usual in such procedures; namely, by adequately diluting the serum with a 0.9 per cent saline solution and then taking out such quantities of each dilution as are required for titration. To each of the tubes containing various quantities of the serum was added 0.1 cc. of the spirochetal emulsion (as antigen), and the final volume was brought up to 1.5 cc. by adding the necessary quantities of 0.9 per cent saline solution to each tube. The content of the tubes was thoroughly mixed by shaking and the tubes were placed in a water incubator at 37°C. At least two tubes containing the spirochetal emulsion alone were prepared at the same time and served as controls. Concentrated and washed suspensions of various spirochetes derived from pure cultures in a fluid medium were used as antigens. 0.1 cc. of the suspension of each strain in 0.9 per cent saline solution was used for each tube. The number of spirochetes varied in different suspensions, but there were over 100 per field (Leitz oc. 3, $\frac{1}{12}$ oil immersion, dark-field illumination), and the addition of 0.1 cc. of the antigen emulsion to each tube (total volume of fluid, 1.5 cc.) produced grayish white, semitranslucent turbidity. The turbidity in the control tubes gradually subsided while standing in the incubator, but was never completely cleared up, even after 24 hours' standing. Readings of the results were made twice, once after 2 hours' incubation, and again at the end of 24 hours at a temperature of 15°C. The sera were used without any modification, such as inactivation at 56°C.

As may be seen from Table II, we first tested the agglutinating powers of various immune sera for their homologous strains; that is, for the strains which had been used for producing them in the rabbits by repeated intravenous injections. Thus, eleven sera were titrated with four different strains of the *pallidum*, two to four immune sera for each strain. As in the case of the *pallidum* the remaining

immune sera, eight in number, were tested with their homologous species, the *calligyrum*, *refringens*, *microdentium*, and *mucosum*, which had been employed for their production.

It may be mentioned that we had to make a number of preliminary experiments before we felt assured of obtaining fairly uniform and reliable results. The reading of a strong agglutination was quite easy, as the organisms rapidly settled down, and the sediment adhered firmly to the side and bottom of the tube, but the less intense reaction was not as clear as we wished; hence, our reading of the minimum zone of the reaction was more or less arbitrary. The titers of agglutinins in different sera read after 2 hours' incubation were somewhat lower than those recorded after the same set of tubes had been left at room temperature for 24 hours longer. It was always necessary, in order to detect a slight degree of agglutination, to shake up the sediment and compare the granulation or clumping of the suspension with a control tube without any serum. We have resorted solely to the macroscopic reading of the reaction. A microscopic examination of the sediment adhering rather firmly to the side and bottom of the test-tubes where a definite agglutination occurred revealed enormous masses of entangled spirochetes, some apparently undergoing morphological modifications, as shown by a granular appearance or by relaxation of curves.

In Table II several facts seem to stand out conspicuously. In the first place, the titers of agglutinins developed in the rabbits treated with the saprophytic species, namely, the *calligyrum*, *refringens*, *microdentium*, and *mucosum*, are decidedly lower than those found in the sera produced by immunizing the rabbits with the *pallidum*. Secondly, the amounts of agglutinins in different immune sera are not in direct proportion to the number of injections or the duration of the immunization. Thus, the highest titer was found in Serum 4, in which 0.00001 cc. caused a definite, and 0.000025 cc. a slight agglutination, while Serum 9, notwithstanding the fact that the animal had five more injections than the former, required 0.0025 cc. for a definite reaction. Again, it is evident, with regard to the production of the agglutinins, that different rabbits react differently to the same spirochetal antigens. The apparent difference between the titers of the *pallidum* group and those of the other group requires an ex-

TABLE II.

Tubes Incubated for 2 Hours in the Water Bath. Antigen: 0.1 Cc. of Emulsion of Fluid Cultures 2 Months Old. Total Volume 1.5 Cc.

Antigens.	Immune sera.	Titers of agglutinins.
		cc.
T. pallidum, Strain McD.	No. 2	0.000025
	" 3	0.0001
	" 4	0.00000375
" XI.	" 5	0.00005
	" 6	0.0005
	" 7	0.00004
	" 8	0.000025
" B29.	" 9	0.001
	" 10	0.00005
" Z. A.	" 11	0.001
	" 12	0.000025
T. calligyrum	" 13	0.0005
	" 14	0.001
S. refringens	" 15	0.00125
	" 17	0.001
T. microdentium	" 18	0.0025
	" 19	0.001
T. mucosum	" 20	0.001
	" 21	0.01

planation. Whether the phenomenon is due to the greater amounts of agglutinins produced in the rabbits immunized with the *pallidum* or to a physical or possibly also chemical factor inherent in the *pallidum* antigen has not been established. It is also possible that our results with the saprophytic varieties happened to be inferior merely because the animals used were unfavorable individuals. The results might have been different if we had employed more animals.

Of more interest are the results of cross examinations of various immune sera with regard to their specificity towards the homologous and heterologous antigens. Eight immune sera were chosen to be tested, each with six strains of *Treponema pallidum* and one of *Treponema calligyrum, Spirochæta refringens, Treponema microdentium,* and *Treponema mucosum,* or ten different antigens in all. Stated in more detail, the procedure was as follows:

Serum 4 was produced by injecting the animal with Strain McD. of the *pallidum* and was tested not only with the emulsion of the same strain (homologous), but also with those of the Strains XI, R, C_2, B30, and Z. A. of the *pallidum*, as well as with those of the *calligyrum*, *refringens*, *microdentium* and *mucosum* (heterologous and of different species). Serum 8, produced with Strain XI, and Serum 12, produced with Strain Z. A. of the *pallidum*, were likewise tested against their homologous as well as their heterologous antigens, while Serum 10, the homologous antigen of which was Strain B29, was tested with all ten heterologous antigens, since there was no homologous one in the series. Sera 14 and 17 of the *calligyrum* and *refringens* of the non-pathogenic spirochetes of the genitalia, and Sera 18 and 20 of the *microdentium* and *mucosum* of the buccal cavity were each tested with one homologous and nine heterologous antigens. In order to secure as closely comparable results as possible, all the tests were carried out in parallel series on the same day and with the same materials. The first reading was recorded after completion of the 2 hour incubation in the water thermostat and the second at the end of the 24 hour period at room temperature. The latter readings are given in Table III.

In the analysis of Table III, several points are brought out. In the first place, there is a pronounced degree of specificity of each characteristic for its homologous group of antigens. Thus, the immune sera belonging to the *pallidum* group agglutinated most strongly when brought together with the emulsion of the *pallidum* strains, but not at all when mixed with the *microdentium* or *mucosum*. The reverse is also true; that is, the immune serum produced by means of the *microdentium* agglutinated none of the other groups, except for a slight reaction with the *mucosum*. The *mucosum* immune serum showed its strongest action on the *mucosum* antigen, although there was a more or less feeble reaction with some of the other spirochetes, especially the *microdentium*. The relation between the *calligyrum* and *refringens* seems very close, considered from the point of view of the agglutination reaction. They did agglutinate mutually to such an extent that they might be included in the same group. As already mentioned above, they showed no affinity whatever for the group of the mouth spirochetes. On the other hand, there existed a certain degree of so called group reaction between the *pallidum* and the *calligyrum* groups. It may be pointed out that in spite of the close relation between the *calligyrum* and the *refringens*, the immune sera pertaining to the *pallidum* did not noticeably agglutinate the *refringens*, while the same sera invariably agglutinated the *calligyrum*

TABLE III.

Specificity of Immune Sera towards the Homologous and Heterologous Antigens. Results of Incubation for 24 Hours at Room Temperature.

Antigens.	Immune sera.							
	No. 4 homologous with Strain McD.	No. 8 homologous with Strain XI.	No. 10 homologous with Strain B29.	No. 12 homologous with Strain Z. A.	No. 14 homologous with *T. calligyrum.*	No. 17 homologous with *S. refringens.*	No. 18 homologous with *T. microdentium.*	No. 20 homologous with *T. mucosum.*
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
T. pallidum.								
Strain McD.....	0.0005	0.0005	0.0005	0.0025	0.05	Trace at 0.05.	None at 0.05.	None at 0.05.
" XI.....	0.0005	0.0005	0.0005	0.005	0.05	Trace at 0.05.	None at 0.05.	None at 0.05.
" R.....	0.0005	0.005	0.005	0.005	0.05	0.05	None at 0.05.	Trace at 0.05.
" C_2.....	0.0005	0.001	0.0005	0.001	0.01	Trace at 0.05.	None at 0.05.	Trace at 0.05.
" B30.....	0.0005	0.0025	0.0005	0.005	0.05	Trace at 0.05.	None at 0.05.	Trace at 0.05.
" Z. A.....	0.0005	0.0005	0.0005	0.0005	0.05	0.05	None at 0.05.	Trace at 0.05.
T. calligyrum.....	0.05	0.05	0.025	0.025	0.0005	0.0075	None at 0.05.	Trace at 0.05.
S. refringens.....	None at 0.05.	None at 0.05.	None at 0.05.	None at 0.05.	0.005	0.0025	None at 0.05.	Trace at 0.05.
T. microdentium...	None at 0 05.	None at 0.05.	None at 0.05.	None at 0.05.	None at 0.05.	None.	0.001	0.01
T. mucosum.....	None at 0.05.	None at 0.05.	None at 0.05.	None at 0.05.	None at 0.05.	"	0.05	0.001

in doses between 0.05 and 0.025 cc., that is, in a dilution of 1:20 to 1:40. This group reaction is, of course, far below the titers of these sera for the *pallidum* antigens, in which they varied from 0.005 (1:200) to 0.0005 cc. (1:2,000), and still further down to 0.00001 cc. (1:100,000) (Table II). Conversely, the *calligyrum* serum, which agglutinated its own antigen in a dose of 0.0005 cc. (1:2,000) produced a group reaction with the *pallidum* emulsions in doses ranging from

0.05 (1:20) to 0.01 cc. (1:100). In this respect, the behavior of the *refringens* serum was somewhat similar to that of the *calligyrum*, as it also produced a distinct agglutination with some of the *pallidum* antigens when employed in a concentration stronger than 1:20, sometimes even in a dilution of 1:40.

As to the results obtained among the *pallidum* antigens with the corresponding and cross immune sera, it will be noticed that the reaction between the homologous antigens and immune sera are, as a rule, stronger than those which occurred when the former were mixed with the sera produced by the other strains. On the other hand, a serum which strongly agglutinates a certain strain or strains may not necessarily agglutinate others, while the latter may yet be the most readily and strongly agglutinated by another serum. In other words, our present study indicates that the agglutinin titers of these sera are variable according to the differences in the individual strains of the same group. This phenomenon has long been recognized in the agglutination of various bacteria by their immune sera, and apparently it holds good in the case of the spirochetes. The complex composition of agglutinins, such as partial agglutinins of different affinities or still only scantily understood factors in agglutination reactions, is accountable for the intricacy of the so called specific as well as group reactions.

Influence of Time upon the Agglutinins in Vitro and in Vivo.

The immune sera used in the foregoing experiments were preserved in a refrigerator at the temperature of 6°C. for 3 months and then examined for their strength. In the meantime, the rabbits immunized were not given any further injection of the spirochetal emulsions for the same length of time, and then their sera were drawn for the titration of agglutinins. Table IV shows the titers of these sera as compared with their original strength.

The rates with which the agglutinin contents of the immune sera lost strength during the 3 months seem to be irregular and show no constant proportion to the original titers of the sera. There is, however, a general tendency of the agglutinins to disappear from the serum more rapidly *in vivo* than *in vitro*. The titers of the ag-

TABLE IV.

Effect of Time upon Agglutinins in Vitro and in Vivo.

Immune sera.	Titer		
	Original.	After 3 mos. kept at 6°C.	After 3 mos. in the animal.
	cc.	*cc.*	*cc.*
No. 2, *T. pallidum*, Strain McD	0.000025	0.00025	0.001
" 8, *T.* " " XI	0.000025	0.0005	0.001
" 10, *T.* " " B29	0.00005	0.0001	0.001
" 12, *T.* " " Z. A	0.000025	0.0001	0.001
" 14, *T. calligyrun*	0.001	0.01	0.1
" 15, *S. refringens*	0.00125	0.01	0.1
" 19, *T. microdentium*	0.001	0.01	<0.1
" 20, *T. mucosum*	0.001	0.01	<0.1

glutinin in the sera derived from these rabbits at the end of 3 months after discontinuation of immunization were reduced to about one-one hundredth in Nos. 14, 15, 19, 20; one-fortieth in Nos. 2, 8, 12; and one-twentieth in No. 10, while the original sera when preserved in the refrigerator during the same period of time lost their strength down to about one-tenth of the initial titers, except in No. 8, where it was almost twice as much weakened as in the rest. It may therefore be assumed that during the first 3 months after the cessation of immunization, the disappearance of the agglutinins for various spirochetes was almost ten times as fast in the animal body as in the test-tubes kept at a temperature of 6°C.

Complement Fixation.

The immune sera were also tested for their property of binding complement when mixed with their homologous as well as heterologous antigens. The technique of carrying out the tests was that usually employed in such experiments.

The immune sera were inactivated at 56°C. for half an hour before use. The antigens were prepared from the pure cultures of various spirochetes grown in a fluid medium consisting of equal parts of rabbit serum and slightly alkaline bouillon, with a piece of normal rabbit kidney. The spirochetes were collected from cultures which had been vigorously growing for 4 weeks. For the purpose of concentration and purification, the spirochetes were collected by a prolonged

centrifugation and washed again in 0.9 per cent saline solution. The sediment, resuspended in an adequate volume of 0.9 per cent saline solution, showing about twenty to thirty spirochetes under dark-field illumination, was used as the antigen. The relative quantities of different factors were as follows: The immune serum in quantities of from 0.1 to 0.0001 cc., complement 0.04 cc. (2 units), antigen 0.2 cc., sheep corpuscles 5 per cent, anti-sheep amboceptor 0.0002 cc. (corresponding to 2 units), total volume made to 1.5 cc. with 0.9 per cent saline solution. The first incubation was for 1 hour (at 36°C. in the water bath), and the second also for 1 hour. The quantities of immune serum and the spirochetal suspensions chosen for the above experiments were previously tested and found to be not anticomplementary in themselves. The results were recorded after allowing the tubes to stand for 2 hours longer at room temperature.

TABLE V.

Complement Fixation with Homologous Antigens.

Antigens 0.2 cc.	Immune sera.	Titers of fixation.
		cc.
T. pallidum.		
Strain McD	No. 2	0.005
	" 3	0.002
	" 4	0.005
" XI	" 5	0.005
	" 6	0.1
	" 7	0.005
	" 8	0.008
" B29	" 9	0.007
	" 10	0.003
" Z. A.	" 11	0.002
	" 12	0.0003
T. calligyrum	" 14	0.01
S. refringens	" 15	0.1
	" 17	0.03
T. microdentium	" 18	0.007
	" 19	0.05
T. mucosum	" 20	0.002
	" 21	0.005

Table V shows that the titers of the immune sera derived from different rabbits vary considerably according to the individual variations that exist among the animals. For example, Immune Sera 6 and 15 showed an extremely low power for complement fixation, in spite of the fact that the rabbits in the same groups, particularly

in the Strain XI series, produced fairly high titers. The titers of the *pallidum* group were in the main quite high, while the *calligyrum* and *microdentium* remained much less developed. It is interesting to note that of the two animals treated with the *mucosum*, both responded with high titers. When compared with the agglutinin titers of the same sera, the fixation titers are much lower but seem to run fairly parallel, in the sense that a serum with stronger agglutinating power had also a higher titer for complement fixation. There were a few exceptions to this rule.

For determining the question of specificity of the complement fixation reaction with the spirochetes a series of experiments was performed in which a certain number of the immune sera were tested, each in turn, with the entire set of antigens. Table VI gives the results. The technique used was the same as before.

Table VI shows that various immune sera fixed complement more strongly with their corresponding antigens than with a heterologous one. Among the *pallidum* strains, the reaction is fairly interchangeable, although some immune sera (Nos. 3 and 7) have caused only a weak fixation with a certain antigen (Strain B29). The *calligyrum* serum produced a marked fixation, not only with its own antigen, but also with certain *pallidum* strains (Strains XI and Z. A.) to such an extent that in a dilution of 1: 30 no difference could be distinguished between the *calligyrum* and these two *pallidum* strains. In this connection, mention may be made of an analogous instance where one of the *pallidum* immune sera (No. 3) fixed complement with the *calligyrum* antigen distinctly in a dilution of 1: 30. The *refringens* serum fixed complement best with its own antigen, but also feebly with all the other antigens except that of the *mucosum*. A similar group reaction was evident with the *calligyrum* and *mucosum* sera. In spite of the presence of a certain amount of group reaction, the specific character of the complement fixation phenomenon seems to hold. At least, the differences between the *pallidum*, *refringens*, *microdentium*, and *mucosum* are sufficient for considering the reaction specific. The *calligyrum* showed much affinity for the *pallidum* on the one hand and for the *refringens* on the other.

TABLE VI.

Complement Fixation with Heterologous Antigens.

Immune sera.		Antigens used for cross titration.							
		T. pallidum strain.				*T. calligyrum.*	*S. refringens.*	*T. microdentium.*	*T. mucosum.*
		McD.	XI.	B29.	Z. A.				
	cc.								
No. 3 homologous with Strain McD.	0.1	++	++	<+	++	+	−	−	<<+
	0.03	++	++	−	++	<+	−	−	−
	0.01	+	+	−	++	−	−	−	−
No. 7 homologous with Strain XI.	0.1	+	++	++	++	+	−	−	−
	0.03	+	++	<+	+	−	−	−	−
	0.01	<+	+	−	<+	−	−	−	−
No. 10 homologous with Strain B29.	0.1	++	++	++	++	<+	−	−	−
	0.03	++	++	++	++	−	−	−	−
	0.01	++	+	++	++	−	−	−	−
No. 11 homologous with Strain Z. A.	0.1	+	++	++	++	<+	<+	−	−
	0.03	<+	+	+	++	−	−	−	−
	0.01	−	<+	−	++	−	−	−	−
No. 14 homologous with *T. calligyrum.*	0.1	<<+	++	<+	+	++	<+	<<+	<<+
	0.03	−	<+	−	<+	<+	−	−	−
	0.01	−	−	−	−	<<+	−	−	−
No. 17 homologous with *S. refringens.*	0.1	<+	+	<+	<+	+	+	<<+	−
	0.03	−	−	−	−	<<+	<<+	−	−
	0.01	−	−	−	−	−	−	−	−
No. 18 homologous with *T. microdentium.*	0.1	−	−	−	<+	<<+	−	++	<+
	0.03	−	−	−	−	−	−	++	−
	0.01	−	−	−	−	−	−	<+	−
No. 21 homologous with *T. mucosum.*	0.1	<+	<+	<+	<+	<<+	−	<+	++
	0.03	−	−	−	−	−	−	−	++
	0.01	−	−	−	−	−	−	−	++

Spirocheticidal Properties.

In order to determine whether or not these immune sera have a destructive power upon the spirochetes *in vitro*, several series of tests were carried out. Because of the part played by complement in a bacteriolytic process, our experiments were so arranged that in one set the immune serum was allowed to act alone, while in another, both complement and immune serum were put together with the spirochetes.

The immune sera were inactivated at 56°C. for 30 minutes before use, and the spirochetes were from recent fluid cultures and were very active. The amount of immune serum was graduated from 0.1 down to 0.00005 cc., while spirochetes and complement were used in a quantity of 0.1 cc. each for each test-tube. The volume of the resulting fluid in each tube was made uniformly 1.1 cc. by adding 0.9 per cent saline solution. After all the factors, namely, spirochetes, immune serum, and complement, had been mixed, the tubes were placed in a water bath incubator at 37°C. for 1 hour, and then the whole contents were used for cultivation in the usual ascitic agar tissue medium. The results were recorded at the end of 2 or 3 weeks of incubation at 36°C. Normal rabbit serum was also tested as control.

The results of experiments in which most of the antisera were tested show that the anti-*pallidum* Serum 12 suppressed the growth of its homologous strain Z. A. in a dose of 0.001 cc. in the presence of complement, while scanty growth was observed in all the tubes where there was no complement added to the immune serum. Normal rabbit serum had no appreciable effect on the growth of this strain. It was noted also that the number of spirochetal colonies diminished almost proportionately with the gradual increase of the specific immune sera, until, in quantities of serum beyond a certain limit, no growth was observed. With the anti-*pallidum* Serum 4, and possibly also No. 8, the titer was 0.0003 cc. It was not rare for the anti-*pallidum* sera to attain the titer of 0.001 cc. On the other hand, the immune sera for the *refringens*, *calligyrum*, and *mucosum* were far inferior in their restraining influence upon their homologous species, none of them being strong enough to inhibit the growth in a dose below 0.01 cc. In the *mucosum*, 0.01 cc. of the antiserum failed to kill the organism.

In parallel series of experiments without the addition of complement, it was found that the powerful immune sera (Nos. 4 and 8) caused a considerable restraint upon the growth of the homologous strains, the only difference between these sera and those containing complement being that their effects are less marked. When used in quantities below 0.03 cc., neither of these sera caused a complete suppression of the growth of organisms acted upon by them. The question naturally presents itself as to whether the suppression of growth in cases where complement and the immune sera were employed was due to the actual destruction of the organisms or merely

to the agglutination. The latter assumption may be dismissed, as the phenomenon does not occur when the immune sera alone (containing the agglutinins) are used. Of course, the sparseness of colonies in these tubes must be partly due to the agglutination, which tends to clump many thousand spirochetes to start a single colony. Yet a total suppression cannot be explained on this ground, since a single colony, once it has begun to grow, will finally form a diffuse growth throughout the medium. As will be described later, the failure of the spirochetes to grow after being subjected to the action of complement and immune serum was largely due to the actual destruction of the spirochetes. Syphilitic rabbit serum was also tested and showed that there was an unmistakable destruction of the *pallidum* strain in quantities above 0.03 cc. Normal rabbit serum showed a slight restraint upon growth of this strain when used in doses of 0.1 cc. Neither of these sera had any ill effect upon the spirochetes when complement was omitted from the mixture.

Microscopic Observations on the Effect of the Immune Sera upon Spirochetes in Vitro.

Immune Sera 4 (Strain McD.), 8 (Strain XI), 10 (Strain B29), and 12 (Strain Z. A.) were chosen for a series of observations in which we followed the changes which take place in *Treponema pallidum* when the organisms have been subjected to the action of these sera. Three strains of the *pallidum*, McD., R, and Z. A., were used for the purpose. As in the previous experiments, the action of the immune sera was studied with and without the addition of complement (guinea pig fresh serum).

The sera were inactivated at 56°C. for 30 minutes. The mixture of the serum, spirochetal suspension (McD.), and complement (in cases where it was added) was incubated in a water bath at 37°C. for 3 hours, and microscopic examinations were made during and after incubation. Similar results were obtained with the strains of R and Z. A.

Table VII demonstrates the fact that *Treponema pallidum* undergoes a fundamental change in its morphology when acted upon by its immune sera and complement at an optimum temperature. The phenomenon is similar to the dissolution or disintegration of various

TABLE VII.

Effect of Immune Sera upon Spirochetes in Vitro.

Strain McD. 0.1 cc. + Immune Serum 4 (Strain McD.) 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + Immune Serum 8 (Strain XI) 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + Immune Serum 10 (Strain B29) 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + Immune Serum 12 (Strain Z. A.) 0.1 cc. + complement 0.1 cc.	The organisms were strongly agglutinated within 1 hr., then they became granular in appearance, and only a few retained their form.
Strain McD. 0.1 cc. + Immune Serum 4 (Strain McD.) 0.1 cc. + no complement. Strain McD. 0.1 cc. + Immune Serum 8 (Strain XI) 0.1 cc. + no complement. Strain McD. 0.1 cc. + Immune Serum 10 (Strain B29) 0.1 cc. + no complement. Strain McD. 0.1 cc. + Immune Serum 12 (Strain Z. A.) 0.1 cc. + no complement.	The agglutination was even stronger than in the above series. At the end of 24 hrs. the organisms kept their form.
Strain McD. 0 + no serum + complement. " " 0 + " " + no "	No noticeable changes.

microorganisms under the influence of an immune serum. In the absence of complement, the organisms were strongly agglutinated, but not dissolved, even after 24 hours.

In the following experiment, attempts were made to study the part, if any, played by leukocytes in the destruction of the spirochetes *in vitro*. Leukocytes both from normal and from immunized rabbits were collected by Wright's method. The citrate blood was briefly centrifuged, and the leukocytic layer of the sediment carefully skimmed and put into another centrifuge tube. In ordinary opsonin work, the cells thus collected are used without washing, but in the present experiment, they were washed with a 0.9 per cent saline solution by a renewed centrifugalization. The immune leukocytes were obtained from a rabbit which had been immunized with the Z. A. strain (No. 12).

The technique was as follows: To 0.1 cc. of a suspension of one of the *pallidum* strains were added 0.1 cc. of the leukocytic suspension, 0.1 cc. of each of the immune sera, and 0.1 cc. of complement. The mixtures, after being well stirred,

were incubated in a water bath at 37°C. and examined at various intervals; for example, 30 minutes, 1 hour, 2 hours, and 4 hours (kept at room temperature). The examinations were made without staining under the dark-field microscope, and also as film preparations stained by Giemsa as well as by a modified Fontana silver impregnation. Films prepared from a mixture in which unwashed citrate leukocytic suspension was used washed off the slides very readily and could not be depended upon for accurate results. Normal as well as immune serum and normal leukocytes were also used (Table VIII).

TABLE VIII.

Effect of Leukocytes upon Spirochetes in Vitro.

Strain McD. 0.1 cc. + immune leukocytes (No. 12) 0.1 cc. + Immune Serum 4, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + immune leukocytes (No. 12) 0.1 cc. + Immune Serum 8, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + immune leukocytes (No. 12) 0.1 cc. + Immune Serum 10, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + immune leukocytes (No. 12) 0.1 cc. + Immune Serum 12, 0.1 cc. + complement 0.1 cc.	Strong agglutination and gradual lysis, many spirochetes adhering to the leukocytes, into which some were definitely ingested. Total disappearance of spirochetes in 24 hrs.
Strain McD. 0.1 cc. + normal leukocytes 0.1 cc. + Immune Serum 4, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + normal leukocytes 0.1 cc. + Immune Serum 8, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + normal leukocytes 0.1 cc. + Immune Serum 10, 0.1 cc. + complement 0.1 cc. Strain McD. 0.1 cc. + normal leukocytes 0.1 cc. + Immune Serum 12, 0.1 cc. + complement 0.1 cc.	Strong agglutination and general disintegration of spirochetes but decidedly less than in the preceding series. Phagocytosis present.
Strain McD. 0.1 cc. + no leukocytes + Immune Serum 4, 0.1 cc. + complement 0.1 cc.	Much lysis in 24 hrs.
Strain McD. 0.1 cc. + immune leukocytes 0.1 cc. + no immune serum + complement 0.1 cc.	No striking changes.
Strain McD. 0.1 cc. + immune leukocytes 0.1 cc. + no immune serum + no complement.	No lysis or phagocytosis.
Strain McD. 0.1 cc. + immune leukocytes 0.1 cc. + normal serum 0.1 cc. + complement 0.1 cc.	No lysis or phagocytosis.
Strain McD. 0.1 cc. + immune leukocytes 0.1 cc. + normal serum 0.1 cc. + no complement.	No lysis or phagocytosis.

Parallel series of experiments were also carried out with Strains R and Z. A. with similar results. It seems apparent, therefore, that some of the spirochetes are readily taken up by immune as well as normal rabbit leukocytes, when an immune serum and complement are simultaneously added to the mixture. The number of spirochetes taken up by phagocytes is small compared with what we are accustomed to see with bacteria. It may be due to the filamentous feature of the organisms, which prevents the cells engorging them with readiness, or what appears still more probable is that they form enormous masses of entangled nets too large to be taken up by the phagocytes, and then gradual lysis occurs. It is also possible that the engorged spirochetes quickly disappear through intracellular digestion. At all events, the agglutinated masses become gradually granular and indistinct and finally undergo dissolution. The completeness with which this process proceeds is most marked when immune leukocytes are used. Perhaps there exists within these cells certain elements which render the lytic processes more energetic than when only immune serum and complement are used. In the absence of complement, the immune serum produced only a slight degree of phagocytosis and no lysis. The organisms, after being acted upon by the leukocytes in the immune serum and complement mixture, lose their property to take Giemsa stain as strongly as those not so treated. Their curves are seen to be flattened in many specimens. They still retain their affinity for the silver precipitation method (Fontana). It has been difficult to stain the spirochetes distinctly within the phagocytes, although they could be seen in fresh preparations by means of dark-field illumination. The large number of spirochetes adhering to the leukocytes prevents a clear appearance of the phenomenon.

A series of experiments was performed with normal human leukocytes in conjunction with the immune rabbit sera and complement. The results were similar to those recorded for normal rabbit leukocytes.

Effects of Immune Sera and Leukocytes upon the Uncultivated Strain of Treponema pallidum.

The action of the sera derived from rabbits immunized with cultivated strains of *Treponema pallidum* may also be studied. It may be tested by subjecting a suspension of a virulent testicular material rich in the *pallidum* to the effect of the immune sera and then inoculating certain animals susceptible to experimental syphilis. Another procedure would be to study the immunity phenomena which follow the mixing of the *pallidum* and the serum *in vitro*. Agglutination, immobilization, granular disintegration, dissolution, or complement binding may be studied. The first method is subject to the difficulties inherent in experiments in which the susceptibility of different individuals constitutes an inconstant factor. In fact, our preliminary experiments indicated that study with extensive material is required. This phase of the study has been under investigation by one of us since 1911 and will have to be continued for a longer time. The test-tube phenomena were better observed.

To 0.1 cc. of a rich suspension of virulent *pallidum* from syphilitic orchitis of a rabbit, 0.1 cc. of each of the several immune sera, comprising Nos. 4 (Strain McD.), 8 (Strain XI), 12 (Strain Z. A.), and 10 (Strain B29) was added, and 0.1 cc. of a 40 per cent solution of fresh guinea pig serum as complement. The mixture was made up to 1 cc. by adding a 0.9 per cent sterile saline solution and then placed in a water bath kept at the temperature of 37°C. The mixture, an opalescent fluid, was examined from time to time for the agglutination, motility, or disintegration of the *pallidum* under the dark-field microscope. Controls with the *pallidum* alone, the *pallidum* with the immune serum (inactivated), and the *pallidum* with the complement were provided.

The results of our observations were in the main similar to those obtained by Zinsser, Hopkins, and McBurney, except for a few points. It was noticed in our experiments that the particular strain of *Treponema pallidum* employed became sooner or later immobilized by the addition of the immune sera, and none was motile after 3 hours at 37°C. In the control tubes containing plain saline or complement solution, the organisms were still active for several hours longer. In some control tubes, there were a few motile *pallida* at the end of 24 hours. The immobilizing effect was augmented by the presence of complement. There was no definite agglutination or dissolution of the *pallidum* except in cases of Sera 4 and 8, where there were small clumps of entangled immobilized organisms suggesting a slight agglutination. But we were unable to find definite disintegration of the *pallidum*.

Studies were also made to discover whether or not leukocytes from an immune or normal rabbit ingested the tissue *pallidum* under the influence of the immune serum, but the results were inconstant. Although there was an unmistakable phagocytosis in the presence of the immune serum, it was slight in comparison with the cultivated strains, and there was no general disintegration of the organisms. In considering the above results, it appears as though the immune sera prepared by injecting the rabbit with avirulent strains of culture *pallidum* exerted much less effect upon a virulent strain derived from a syphiloma in a rabbit. Whether or not this is due to unsuitable immunization or to a modification in strains is not shown by the present experiments. The failure of agglutination of the tissue *pallidum* may have been due to the presence of various tissue proteins simultaneously introduced into the mixture, for these apparently indifferent substances can often interfere with agglutination or complement fixation.[23] The entire question as to the relation between the uncultivated virulent strains and those which had become avirulent through cultivation is still under investigation.

CONCLUSIONS.

Experiments were carried out for the study of culture spirochetes in their relation to various immunity reactions *in vitro*. Several strains of *Treponema pallidum* and one each of *Treponema calligyrum*, *Spirochæta refringens*, *Treponema microdentium*, and *Treponema mucosum* were used. Tests were made of immune substances responsible for agglutination, complement fixation, spirocheticidosis, and opsonization. In cases of agglutination and complement fixation, cross titrations were made.

1. In the sera derived from rabbits immunized with various spirochetes agglutinins were demonstrated in varying quantities for the homologous antigens. The amounts of agglutinins developed were considerably higher in the *pallidum* immune sera than in the other groups. There was no parallelism between the amounts of antigens injected and the amounts of agglutinins developed.

2. Cross titrations among different *pallidum* strains revealed that

[23] Noguchi, H., and Bronfenbrenner, J., *J. Exp. Med.*, 1911, xiii, 92.

the agglutination is not necessarily strongest when homologous antigens and immune sera are brought together.

3. On the other hand, the reactions between the immune sera and antigens belonging to different species were sufficiently specific to justify the grouping.

4. Certain degrees of group reaction were observed between the *pallidum* immune sera and the *calligyrum*, and occasionally very faintly also between the *pallidum* and the *refringens* antigens and *vice versa*. There was a much more pronounced group reaction between the *calligyrum* and *refringens*. The immune serum and antigen of the *microdentium* showed a slight affinity for the *mucosum* but none for the *pallidum*, *calligyrum*, or *refringens*, while the *mucosum* immune serum caused a slight agglutination with many members of the other groups. Hence, it appears that the *pallidum* is more or less related to the *calligyrum*, while the affinity between the *calligyrum* and *refringens*, and possibly also between the *calligyrum* and *mucosum* in a much smaller degree, seems close. The *microdentium* showed the least relation to any other spirochetes.

5. Titration of agglutinins in the sera obtained 3 months after the cessation of immunization revealed that the agglutinin contents were already greatly reduced, having fallen roughly to 0.01 of the original strength. The rates of disappearance were irregular in different animals and bore no direct relation to the initial titers. Titration made of the immune sera which had been preserved aseptically in a refrigerator (6°C.) during the same period (3 months) indicated that the original strength of these sera was reduced to about one-tenth. The agglutinins for spirochetes disappear from the rabbit's body much more rapidly than they are reduced in the separated sera by deterioration on standing at 6°C.

6. Titration of the immune sera for complement fixation power showed with a few exceptions, in which there was only slight complement binding, that the titers were high enough to indicate the presence of this principle. The anti-*pallidum* sera possessed higher average titers than the other immune sera tested with correspondingly homologous antigens. The least active were the anti-*refringens* sera.

7. Cross titration of anti-*pallidum* immune sera for complement fixation showed that a given serum with a high titer for its own strain of antigen was also strong with most of the other strains of the

pallidum. Instances occurred also in which the titers with heterologous *pallidum* antigens fell far below those of the homologous. Group reactions between the different spirochetes, such as the *pallidum* and the *calligyrum*, the *calligyrum* and the *refringens*, and the *microdentium* and the *mucosum*, were also indicated. The *mucosum* and the *pallidum* showed a slight degree of group reaction. No anti-*pallidum* serum fixed complement with the *microdentium*.

8. The immune sera were tested for their spirocheticidal properties *in vitro* against the correspondingly specific and heterologous varieties with and without the addition of complement. Many of the anti-*pallidum* sera killed their own strains. Normal rabbit serum exhibited only a slight degree of inhibition. Without complement, the immune sera caused a considerable reduction in the number or density of colonies, but not a complete suppression of growth. Complement alone had no injurious effect upon the *pallidum* strains. The antisera for the *calligyrum*, *refringens*, and *mucosum* showed feeble spirocheticidal action, while the antisera for *microdentium* was stronger. A syphilitic rabbit serum tested against a strain of culture *pallidum* gave a feeble inhibitory effect.

9. Under the influence of immune sera and complement, the spirochetes undergo within a few hours complete disintegration or granular degeneration. Without complement, they are more powerfully agglutinated, but no disintegration occurs, even after 20 hours, and complement alone has no effect.

10. In the presence of homologous immune serum and complement, the culture *pallidum* may be ingested by the leukocytes, but phagocytosis is slight, possibly on account of the filamentous nature of the organisms. The spirochetes in such a mixture disintegrate within a few hours, disintegration being especially rapid when the immune leukocytes are used. In the absence of immune serum, phagocytosis is not noticeable, while without complement but in the presence of immune serum and leukocytes, some phagocytosis, without subsequent lysis, occurs.

A virulent strain of *pallidum*, obtained from syphilitic orchitis in a rabbit, exposed to agglutination, lysis, and phagocytosis by an immune serum prepared by means of culture *pallidum* strains, showed only slight agglutination and phagocytosis but rapid immobilization without disintegration in the presence of complement.