STUDIES ON TREPONEMA PALLIDUM AND SYPHILIS.

V. Further Studies on the Relation of Culture Pallida to Virulent Pallida and on Reinfection Phenomena.

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(Received for publication, June 26, 1916.)

The writers have already reported studies[1] on immune sera developed in rabbits and sheep by repeated injection of treponemata cultivated from the third generation of syphilitic rabbit orchitis. Although developing powerful agglutinating and treponemicidal powers for the culture *pallida*, these sera had practically no influence on virulent treponemata obtained from lesions, and had no protective value when allowed to act on virulent treponemata before inoculation into rabbits. These studies might be interpreted as indicating one of two things. Either they show that the culture *pallida* are not identical with the virulent *pallida* and that we did not have in our hands a true *Treponema pallidum*, or that during cultivation the *pallidum* had been so changed that it had lost not only its virulence, but certain protective attributes which in the virulent state preserved it from reaction with the serum. The former possibility, though logic and the desire for the omission of no possible factor force its consideration, is not likely for a number of reasons. In the first place, the culture treponema which was used was obtained after passing for three generations through rabbits, a period sufficiently removed from the human lesion to preclude the likelihood of having obtained an organism accompanying the *pallidum* in the human lesion. Furthermore, this organism, as will be shown, is in every respect identical

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

with the strains of *Treponema pallidum* isolated and placed at our disposal by Dr. Noguchi, and the sera which acted powerfully on this strain did so likewise for the Noguchi strains. Thus, this strain of culture *pallidum* is identical with the Noguchi strains, and these, although similarly deprived of virulence and having become subject to serum action, have in former experiments by Dr. Noguchi[2] been shown capable in their early generations of producing typical syphilitic lesions in the testes of rabbits. It has seemed more than likely to us, therefore, that the difference between the virulent and the culture treponemata was due to the fact that in its fully virulent condition, *Treponema pallidum* is in some way insulated from the defensive mechanism of the animal body. This may be due to protective structures analogous to the bacterial capsule, although we were unable by the Porges method of applying acid and heat, or by using sodium oleate as suggested by Lamar in the case of pneumococci, to render these treponemata serum-susceptible. It may, on the other hand, be due to the close biological adaptation of the virulent organisms to the animal body, whereby no reaction between the two takes place. In that case, the failure of susceptibility to antisera produced with non-virulent culture treponemata would imply a rather profound chemical change accompanying the adaptation to culture and the loss of parasitism. However this may be, the fact remains that with the sera so far produced by us, we have not been able to exert any influence on the virulent *pallida*, although the sera we have had have agglutinated the various cultures in dilutions up to 1:2,000 to 1:4,000. We have not yet been in the position to work with horses and to produce sera of highest possible potency.

In the present communication we wish to discuss further experiments bearing upon the problem of the differences between the virulent and culture treponemata, the interrelationship of various culture treponemata, and to report experiments aiming toward further comprehension of the problems of immunity in syphilis.

[2] Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

Group Agglutination of Treponemata.

Incidental to the general plan of our studies, we desired to determine the specificity of the agglutination of culture treponemata in order to find out whether the sera might be used for species identification. The immunization of rabbits with culture treponemata is not a simple matter inasmuch as many rabbits die during the process. It seemed at times as though the cultures were toxic, but we have not yet been able to determine definitely whether the accidents of death were due to a true toxicity or to a slow anaphylactic poisoning such as we have noticed in cases of animals treated with many bacteria, a problem upon which we hope to report in another communication. Rabbits were treated with suspensions of culture *pallidum* washed with salt solution and heated to 56°C. for half an hour. In the early experiments we used cultures made in sheep serum rabbit kidney broth under oil; in later experiments we employed young growths obtained without animal tissue, upon coagulated egg medium with ascitic broth, a method devised in this laboratory by Miss Ruth Gilbert and referred to in a previous paper. In all cases, we need hardly add, care was taken to carry out the final serum reactions against cultures grown on animal protein different from that in the cultures with which the animals were immunized, in order to avoid error by protein-antiprotein reactions. The following experiment represents a type of the results obtained by group agglutination experiments (Table I).

TABLE I.

Experiment with Serum 624.

Treponema agglutinated.	Titer of agglutination.
T. pallidum A (homologous)	1 : 2,000
" " (Noguchi 1)	1 : 400
" " (" 2)	1 : 1,000
" *calligyrum*	1 : 4,000
" *refringens*	1 : 200
" *microdentium*	1 : 1,000
" *mucosum*	Agglutinated spontaneously in salt solution controls.

The cultures here employed, apart from *pallidum* A, are cultures placed at our disposal by Dr. Noguchi, and used in these experiments because we could be absolutely sure of their source and of their being true representatives of their respective species, being, in fact, the cultures from which these species were first described by their discoverer. The differences which seem to exist between the three culture *pallidum* strains do not seem to us to mean very much, since minor variations in the character of suspension of each strain used will often change the result to a moderate extent. The treponemata are long and often tangled, and differences almost as great as those noticed above may be due to factors not connected with the specificity

TABLE II.

Absorption of Agglutinins.

Preliminary Titration of a Rabbit Immunized with Treponema pallidum. Strain A.

Suspension of	1:10	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000	Salt.
T. pallidum A.....	+	+	+	+	+	+	+	0	0
" *calligyrum*.....	+	+	+	+	+	+	+	0	0
" *refringens*.....	+	+	+	+	0	0	0	0	0
" *mucosum*.....	0	0	0	0	0	0	0	0	0
" *microdentium*.....	+	+	+	+	+	0	0	0	0
Tonsil organism.....	+	+	+	+	0	0	0	0	0

Agglutination in the Serum of the Same Rabbit (1:50) after Absorption with the Various Strains.

Tested against serum (1:50) absorbed with	Tested against suspension of			
	T. pallidum.	*T. calligyrum.*	*T. refringens.*	*T. microdentium.*
T. pallidum A.....	0	0	0	0
" *calligyrum*.....	0	0	0	0
" *refringens*.....	+	+	0	+
" *mucosum*.....	+	+		
" *microdentium*.....	+	+	+	0
Tonsil organism.....	+	+	+	+
Serum untreated.....	+	+	+	+
Salt solution.....	0	0	0	0

of the serum. For instance, in another experiment in which other suspensions of A and Noguchi 2 were used against the same serum, A, though showing a prezone, went only to 1:500, whereas Noguchi 2 went to 1:1,000. Moreover, experiments reported by us in a previous communication, have also brought out the similarity between the strains. Absorption experiments such as the ones reported here confirm the above (Table II). The close relation between the *pallidum* and the *calligyrum* is shown here.

From the preceding experiments the general impression is gained that the various strains of treponemata are closely related to each other. These observations correspond with cultural and other comparisons which are being carried out by Miss Gilbert with various strains of treponemata from many sources, studied and in part isolated by her. This work is not yet completed and will be reported in a subsequent paper. However, we believe that the work we have done indicates a close group relationship between the various microorganisms.

Action of the Serum of Syphilitic Animals and Man on Culture Pallida.

In studying the immunological problems of syphilis, one of the first hopes fostered is that of eventual success in utilizing the treponema cultures for diagnostic and therapeutic purposes. Our hope of influencing the disease in rabbits by passive immunization with culture antisera, had, of course, been indefinitely deferred by the failure of such sera to act upon or protect against the virulent organisms. However, it still seemed important to determine whether the sera of syphilitic animals and man would possess agglutinating power for the cultures. The ease and speed with which the cultures grow on the egg media might, then, supply a simple method of diagnosis, possibly analogous to the Widal reaction in typhoid. In fact, Kissmeyer[3] has recently made this claim and has expressed the belief that the method would prove of practical diagnostic value.

We may state incidentally that up to the present time, we have not found the sera of syphilitic human beings or animals to exert a considerable agglutinating or immobilizing effect upon virulent trepo-

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

nemata from rabbits. On one or two occasions, slight differences in this respect between the sera of normal and of syphilitic animals have been noticed, but up to the present time these have been too insignificant to be convincing, and this matter will have to be studied further.

To return to the agglutination of culture organisms by the sera of infected animals and man, we cite the following experiments (Table III).

TABLE III.

Agglutination of Treponema pallidum in Rabbit Sera.

Culture Strains.

A. Normal Rabbit Sera.

1:10	1:25	1:50	1:100	1:1,000
+	0	0	0	Not tested.
+++	+	0	0	" "
0	0	0	0	" "
+++	0	0	0	" "
+++	0	0	0	" "

B. Syphilitic Rabbit Sera.

Time since first appearance of syphilitic lesion.	1:10	1:25	1:50	1:100	1:1,000
11 days	+++	++	0	0	Not tested.
27 "	+++	0	0	0	" "
19 "	+++	+	0	0	" "
2 wks	++	++	+	0	" "
11 mos	+++	+	0	0	" "
11 "	++	++	+	0	" "

C. Serum of Rabbit Immunized with Culture Pallida.

1:10	1:25	1:50	1:100	1:1,000
+++	+++	+++	+++	+++

It appears from this experiment and others, similar in result, that the agglutination of the culture *pallida* by the sera of syphilitic rabbits is more regular and slightly higher than that with the sera of

normal rabbits. However, circulating antibody formation is, if it exists at all, very slight, and certainly on the basis of the experiments that we have done so far, we would not venture to distinguish a syphilitic rabbit from the normal rabbit, except in the form of a conjecture, on the basis of the agglutination of the culture organisms.

The absence of antibodies in demonstrable amounts from the sera of syphilitic rabbits may be due to the fact that, in adult rabbits, generalization of syphilis is irregular and incomplete. However, we have so far been unsuccessful in producing agglutinins for the culture *pallida* by the intravenous injection of killed virulent treponemata. The virulent treponemata from lesions used in immunization were obtained by methods which we have previously described, and were killed by heating to 56°C. Two rabbits which received six injections each over a period of 7 weeks, showed no increase of agglutinins for the culture *pallida* as compared with a normal control. A third rabbit was more thoroughly treated. It received sixteen injections over a period of 10 weeks. Its serum compared with two normal controls showed apparently a slight increase in agglutinins (Table IV).

TABLE IV.

Agglutination of Culture Treponemata in the Serum of a Rabbit Immunized with a Virulent Organism.

	1:2	1:10	1:20	1:50	1:100	Control (salt solution).
Immune	+++	+++	+++	++	++	0
Normal 1	+++	++	++	+	+	
" 2	+++	+++	++	+	+	

The suspension here employed was agglutinated more readily than some other suspensions. This variation is one often noted by us and is due to peculiarities of the individual suspension, the cause for which is not entirely clear to us at present. At any rate, it is seen that the difference between the agglutination in the normal sera and in that of the rabbit immunized with dead virulent organisms is a slight one only. We are continuing experiments with sera produced in this way, but have mentioned this in passing since the observa-

tions indicate that the absence of agglutinins in syphilitic rabbits is due to the characteristics of the virulent treponemata, characteristics in which they differ from the culture organisms, rather than to the absence of generalization of the disease in these animals.

A series of similar tests has also been carried out with sera from human syphilis. This was done partly in the hope that serum antibodies might be produced in a species in which the disease is typically a systemic infection; also because we were naturally most interested in the possibility of utilizing such reactions for the diagnosis of human lues. The first experiments were done macroscopically on the sera of syphilitics giving positive Wassermann reactions. As controls the sera of normal individuals and of patients suffering from diseases not syphilitic and giving negative Wassermann reactions were used (Table V).

TABLE V.

Macroscopic Agglutinations.

	No agglutination occurred in	Agglutination occurred in serum 1:5 in	Agglutination occurred in serum 1:10 in	Agglutination occurred in serum 1:20 in
Tertiary syphilis, W. R. +, 23 cases.....	13 cases.	2 cases.	5 cases.	3 cases.
Non-syphilitic diseases, W. R. −, 5 cases	5 "	0 "	0 "	0 "
Normal individuals, W. R. −, 4 cases.....	2 "	1 case.	1 case.	0 "

This series seemed encouraging in as far as syphilitic sera agglutinated more regularly than did the normal, or those of the patients with non-syphilitic diseases, and in some of the syphilitic cases the titer was considerably higher than in the normal individuals that agglutinated. However, macroscopic tests we believe to be at the present time unreliable, and in the subsequent work we set up the tests in agglutination tubes, reading them first macroscopically, but confirming these readings by microscopic examination under the dark-field microscope. Such a large number of sera from individuals without syphilis agglutinated the culture treponemata in dilutions of 1:2, that in most of our subsequent experiments we did not include dilutions lower than 1:5. The following table gives the results in cases we have tested (Table VI).

TABLE VI.

Microscopic Agglutinations.

	No agglutination occurred in	Agglutination occurred in serum 1:2 in	Agglutination occurred in serum 1:10 in	Agglutination occurred in serum 1:20 in	Agglutination occurred in serum 1:50 in	Agglutination occurred in serum 1:100 in
Primary syphilis, W. R. +, 6 cases....	3 cases.	1 case.	2 cases.	0 cases.	0 cases.	0 cases.
Secondary syphilis, W. R. +, 18 cases...	8 "	4 cases.	3 "	1 case.	1 case.	1 case.
Tertiary syphilis, W. R. +, 64 cases...	27 "	7 "	23 "	3 cases.	2 cases.	2 cases.
Tertiary syphilis, W. R. −, 2 cases....	1 case.	0 "	0 "	1 case.	0 "	0 "
Congenital syphilis, W. R. +, 4 cases....	0 cases.	1 case.	3 "	0 cases.	0 "	0 "
Non-syphilitic diseases,* W. R. −, 37 cases...	22 "	4 cases.	7 "	1 case.	2 "	1 case.
Normal individuals, 40 cases.....	35 "	4 "	1 case.	0 cases.	0 "	0 cases.

* The diseases showing high agglutination were heterogeneous, including such conditions as arthritis, tuberculosis, gastro-enteritis, glaucoma, etc., and there seemed to be no relation between disease and agglutination.

An analysis of this table yields the following summary. Agglutination in a dilution of 1:10 or above was obtained in:

Primary syphilis.....	in	2	out	of	6	cases,	or	33	per	cent.
Secondary syphilis.....	"	6	"	"	18	"	"	33	"	"
Tertiary syphilis.....	"	31	"	"	66	"	"	47	"	"
Non-syphilitic diseases.....	"	11	"	"	37	"	"	30	"	"
Normal persons.....	"	1	"	"	40	"	"	2	"	"

Before we included non-syphilitic diseases in our plan of experimentation we were hopeful of positive results, since a small percentage of normal people agglutinated the culture *pallida* in dilutions as high as 1:10, whereas a considerable percentage of syphilitics, especially of the tertiary stage, gave such agglutinations. However, sera from various other diseased conditions, for reasons not clear to us at present, also agglutinated the culture *pallida* in a percentage not far removed

from that resulting from the syphilitic serum agglutinations. It is not impossible that some of these, because of the group reactions indicated above, may have been due to the presence of foci of spirochete infection either in the throat, teeth, or other locations in these patients. However, this is a mere assumption, and the fact remains that the occurrence of such agglutinations in non-syphilitic sera detracts considerably from any diagnostic value such reactions might have. We believe that our experiments as far as they have gone in this direction, tend to indicate a slightly increased agglutination power of syphilitic serum for the culture *pallidum*. We do not think, however, that, as at present performed, agglutination of culture *pallida* can be claimed to have any diagnostic value. These results in a general way are in harmony with the specific complement fixations obtained by Noguchi,[4] Craig and Nichols,[5] and Kolmer, Williams, and Laubaugh,[6] as well as with work done on the same problem by us by somewhat different methods.[7] The experiments, as a group, seem to indicate that if circulating antibodies for the culture *pallida* are found at all in the course of syphilis, they are in amount so small that they cannot be definitely determined by available methods. As far as our own experiments have gone up to the present time, the same is true for the virulent *Treponema pallidum*.

Attempts to Protect with Culture Pallida.

Although we had unsuccessfully tried and reported experiments in which attempts were made to protect passively with the sera of rabbits immunized with culture treponemata, we thought it worth while to carry out a few experiments in which inoculation was practiced directly on rabbits actively immunized with such cultures. The following represents an experiment of this kind (Table VII).

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

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

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

[7] In a later publication we intend to report extensively on this phase of our work.

Immunization with Culture before Inoculation.

A.

Nov. 14, 1914. Five rabbits were injected with a good suspension of sheep serum kidney cultures heated to 56°C. plus 0.5 per cent phenol. No bacteria present. Good material.

Injections were made intravenously Nov. 14, 21, and 30.

Inoculated Dec. 11 with six controls, with virulent material from a lesion in Rabbit 12.

TABLE VII.

Treated rabbits.	Injections of culture treponemata.				Results.
	Nov. 14.	Nov. 21.	Nov .23.		
	cc.	*cc.*	*cc.*		
1	3	1	1	All inoculated with virulent material, Dec. 11, 1914.	Doubtful.
2	3	1	1		+
3	3	1	1		+
4	3	1	1		Chancres.
5	3	1	1		+
Controls.					
6					+
7					+
8					+
9					+
10					Died in less than a month after inoculation.
11					Died in less than a month after inoculation.

B.

Two rabbits were immunized with dead ascitic culture material, highly concentrated. One of these died in the course of immunization, the other one, No. 13, received twelve injections between Dec. 18 and Feb. 19. On Feb. 23 it was inoculated with virulent material into both testes. On Mar. 6 it developed a lesion in the left testis which was removed for other experiments,[8] and on Mar. 18 it developed a large diffuse lesion in the right.

Since these experiments take a considerable amount of time, and since, in the last case at least, extensive and satisfactory immunization with the culture *pallidum* had been practiced, we feel justified in reporting these few experiments as indicating that intravenous treatment with culture *pallida* is not likely to confer any considerable degree of resistance upon rabbits.

[8] The removal of testes for the obtaining of virulent material for rabbits is always carried out under ether anesthesia.

In the hope that localized immunization might result more favorably, we treated two rabbits intratesticularly with living ascitic egg cultures from Jan. 21, 1916, to Apr. 21, 1916, a total of eight injections being given. On May 3 they were inoculated with virulent treponemata together with two controls. On May 23 one of these rabbits developed lesions on both sides. The other one developed a lesion on June 25. One of the controls only has shown a lesion to date.

Had experiments such as those reported immediately above shown more favorable results, we should feel unjustified in publishing on the basis of so small a series. However, a negative result in an experiment of this kind is far more convincing than a positive one, and, although this work is being continued, we feel at present that it is not likely that either local or general treatment with culture *pallida* will protect.

Incidentally, the last experiment, together with two other rabbits not yet reinoculated, shows that repeated local injection of living culture treponemata will not produce a lesion, and that, as far as the rabbit testis is concerned, our cultures have lost all virulence.

Local and general immunization with suspensions of dead virulent treponemata are going on, but these are slow owing to the difficulty of obtaining material for injection and because of the frequent death of rabbits during the process. We are not yet, therefore, prepared to report on this work. However, the work of Uhlenhuth and Mulzer indicated that vaccines prepared with dead virulent material conferred no protection.

Reinfection Experiments in Rabbits.

The work of Uhlenhuth and Mulzer[9] has given us a thorough knowledge of the conditions prevailing in rabbits that have been inoculated with syphilitic virus. It is well known that in man and in monkeys a resistance is acquired during the course of syphilis which seems to protect against superinfection during a period which begins with, or shortly after, the development of the chancre, lasts throughout the secondary period, and, though waning, through the tertiary. It is also generally held that complete cure reestablishes susceptibility and that true reinfection, though rare, probably because of the relative infrequency of complete cure, is, nevertheless a fact. The extensive tabulations of John[10] may be referred to for confirmation of this statement. In rabbits the conditions are not the same, inasmuch as superinfection and reinoculation have almost invariably been suc-

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

[10] John, F., *Samml. klin. Vortr.*, 1909, N. F., *Inn. Med.*, No. 157–64, 559.

cessful even during the existence of syphilitic lesions. Bertarelli[11] succeeded in reinoculating the cornea of a rabbit which had been inoculated with syphilis on a previous occasion. Uhlenhuth and Mulzer, Fontana,[12] Neisser,[13] and Pürckhauer,[14] have also found that infections of the eye were possible at a period during which the opposite eye was still syphilitic, the last named authors even showing that the cornea of the same eye could be twice inoculated. Tomasczewski[15] found that the corneal infection did not protect against scrotal infection, and, *vice versa*, although scrotal infection conferred a skin immunity. Ossola[16] and Truffi[17] also found that successful skin inoculations in rabbits made reinfection of the skin difficult although the protection so conferred was not absolute. In estimating the value of the last experiments, it should not be forgotten that successful skin inoculations in rabbits is not a thing to be obtained with regularity.

Uhlenhuth and Mulzer in extensive experiments on rabbits came to the following conclusions. (1) Syphilis of the testis and of the eye in rabbits does not protect against reinfection, and this holds good in all cases whether or not the syphilitic testicular or eye lesions have healed spontaneously, have been cured by specific drugs, or are still existing. (2) The pathological lesions resulting from such reinoculations do not differ from, and are not less severe than those following the first inoculation.

In only two animals did they notice resistance to reinoculation and these were very young rabbits that had been generally syphilized by the intracardial injection of the virus. In immunization experiments, they found that repeated injections of rabbits with the serum of rabbits intravenously treated with virulent material did not protect and that no vaccine produced with luetic material had any effect on subsequent inoculation.

In general, we may subscribe to these results of Uhlenhuth and Mulzer on the basis of our own experience in a large number of experiments. However, in one important particular, our results seem to differ distinctly from the conclusions of Uhlenhuth and Mulzer, and that is in the acquisition of a powerful localized resistance by the particular tissues which have been the seat of a syphilitic lesion at some time. After obtaining the results which we tabulate below (Table VIII), we went over the protocols of Uhlenhuth and Mulzer and found that in a number of cases reported by them, their results bear out our own, although apparently in their summaries, they overlooked the significance of their data.

[11] Bertarelli, E., *Centr. Bakteriol., 1te Abt., Orig.*, 1908, xlvi, 51.

[12] Fontana, A., *Riv. ig. san. pubb.*, 1907, xviii, 646.

[13] Neisser, A., Beiträge zur Pathologie und Therapie der Syphilis, Berlin, 1911, 569; also *Arb. k. Gsndhtsamte.*, 1911, xxxvii, 569.

[14] Pürckhauer, R., *Arb. k. Gsndhtsamte.*, 1911, xxxvii, 576.

[15] Tomasczewski, E., *Berl. klin. Woch.*, 1910, xlvii, 1447.

[16] Ossola, cited by Truffi below.

[17] Truffi, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1909, lii, 555; 1910, liv, 337.

TABLE VIII.

Reinoculation of Rabbits.

Rabbit No.	1st inoculation.	Lesion.		Interval.	2nd inoculation.	Results.
		First appearance.	When healed.			
14	Nov. 6, 1914. Both testes. Strain F.	Jan. 30, 1915. Nodules in both testes.	May 10.	6 mos.	Nov. 30. Both testes. Strain A.	Negative until Jan. 11, 1916. Died.
15	July 8, 1915. Both testes. Strain S.	Aug. 25, Nodules in right testis. Left negative.	Sept. 10.	6 wks.	Nov. 30. Both testes. Strain A.	Testes negative until Feb. 23, 1916. Lost. Jan. 6. Developed keratitis in left. Treponemata found in nasal secretion. Feb. 23. Keratitis gone.
16	Apr. 2, 1915. Both testes. Strain A.	May 2. Right scrotal lesion. Nodule in left testis.	Not examined until Sept. 10. Negative.	At least 6 wks., probably much longer.	Nov. 24. Both testes. Strain A.	Negative until Apr. 10, 1916. Died. No lesions except partial loss of hair on back, Jan. 24.
17	Nov. 21, 1914. Both testes. Strain T.	Dec. 22. Nodule in left testis. Jan. 6, 1915. Diffuse orchitis in right.	Sept. 10, 1915.	At least 6 wks., probably much longer.	Nov. 24. Right testis. Strain A.	Negative until Jan. 18, 1916, when doubtful nodule was noted, probably scar of the old lesion. Jan. 24, negative. Thereafter negative until Apr. 10. Died.
18	July 8, 1915. Both testes. Strain S.	Aug. 28. Nodule in left.	Sept. 10. Gone.	6 wks.	Nov. 24. Both testes. Strain A.	Negative until May 15, 1916. Died.

19	Nov. 10, 1915. Both testes. Strain F.	Nov. 24. Diffuse orchitis, left. Dec. 1. Right and left. Positive to puncture.		Less than 2 wks.	Dec. 18. Both testes. Strain A.	Jan. 12, 1916. Left testicle positive on puncture; unusually large number of spirochetes. Feb. 9. Nodule on both sides. Apr. 25. " " right.
20	Dec. 11, 1914. Intravenously immunized with culture treponemata and inoculated in both testes. Strain A.	Mar. 11, 1915. Nodule in left testis.		About a year.	Mar. 14, 1916. Left testis. Strain A.	Negative until Apr. 19, then very small nodule (left) which showed no treponemata on puncture. Died June 1. Negative.
21	May 26, 1915. Both testes. Strain A.	June 30. Diffuse orchitis in right. Sept. 2. Small nodule in left.	Jan. 24, 1916.	7 wks.	Mar. 14. Both testes. Strain A.	Doubtful nodule on right, Mar. 28. Negative on puncture. Negative within few days, then remained negative (June 20).
22	Sept. 8, 1915. Both testes. Strain L.	Oct. 4. Small nodule in left. Oct. 7. Small nodule in right and left. Nov. 26. Right excised.	Jan. 6, 1916.	2 mos.	Mar. 14. Left testis. Strain A.	Negative until May 29. Died.

TABLE VIII—*Concluded.*

Rabbit No.	1st inoculation.	Lesion.		Interval.	2nd inoculation.	Results.
		First appearance.	When healed.			
23	Oct. 21, 1915. Both testes. Strain F.	Nov. 24. Diffuse orchitis in right and left. Left removed.	Chancre until Feb. 23, 1916. Healed.	3 wks.	Mar. 14. Right testis. Strain A.	Apr. 19. Small nodule on right. Negative on puncture. Negative until June 5. Died.
24	Oct. 21, 1915. Both testes. Stain F.	Nov. 24. Nodule in left. Feb. 23, 1916. Doubtful nodule in right.	Dec. 2. Left healed.	14 wks.	Mar. 14. Both testes. Strain A.	Apr. 11. Died. No lesion.
25	Oct. 4, 1915. Both testes. Strain T.	Nov. 24. Nodule in right. Jan. 28, 1916. Nodule in left. Right excised.	Feb. 9.	5 wks.	Mar. 14. Left testis. Strain A.	Mar. 28. Doubtful small nodule. May 5. Negative on puncture. Remained negative (June 20).
26	June 9, 1915. Both testes. Strain A.	Aug. 25. Nodule in left excised. Jan. 27, 1916. Nodule in right.	Feb. 9.	5 wks.	Mar. 14. Right testis. Strain T.	Apr. 11. Distension of right testis. Negative to puncture. Apr. 23. Doubtful nodule, negative to puncture. May 23. Died. Negative.
27	Nov. 18, 1915. Both testes. Strain A (+ immune serum).	Jan. 4, 1916. Nodules in right and left. Right positive on puncture.	Feb. 23. Right healed.	3 wks.	Mar. 18. Both testes. Strain T.	Apr. 3. Chancre, right and left. Negative on puncture. Apr. 26. Nodules right and left. Negative to puncture. May 1. Died. No lesion.

28	Dec. 2, 1915. Both testes. Strain S.	Dec. 28. Small nodule in left testis. Jan. 4, 1916. Diffuse orchitis (?) in right.	Feb. 9.	5 wks.	Mar. 18. Both testes. Strain T.	Apr. 6. Chancres on right and left testes. Apr. 11. Nodules on right and left. Non-motile treponemata on puncture.
29	Dec. 6, 1915. Both testes. Strain T.	Dec. 28. Nodules in right and left. Jan. 8, 1916. Right removed.	Jan. 27.	7 wks.	Mar. 18. Left testis. Strain T.	Apr. 26. Doubtful nodule, left. Negative on puncture May 2. Negative (?) May 5. Negative on puncture.
30	Aug. 4, 1915. Both testes. Strain F.	Sept. 8. Diffuse orchitis in right and left. Right excised.	Nov. 4.	19 wks.	Mar. 18. Left testis. Strain T.	May 23. Nodule (left); many motionless treponemata.

Rabbits 14 and 15 were controlled by Rabbit 31, inoculated with the same material, which developed bilateral lesions.

Rabbits 16, 17, and 18 were controlled by Rabbit 32, inoculated with the same material, which showed bilateral lesions.

Rabbits 20, 21 22, 23, 24, and 25, were controlled by Rabbit 33, which showed a positive lesion on one side.

Rabbits 26, 27, 28, 29, and 30, were controlled by Rabbit 34, which showed bilateral lesions.

During the period of reinoculation of these rabbits (Nov., 1915, to Mar., 1916), 30 rabbits were inoculated for other purposes by the same technique. These showed 97 per cent positive results, 80 per cent of the rabbits developing bilateral lesions. In contrast to this, the above group of 17 reinoculated rabbits showed in 1, or 6 per cent, typical lesions; in 2, or 12 per cent, small lesions positive to puncture, in 8, or 47 per cent, small doubtful nodules, negative to puncture and probably never syphilitic in nature; and 6, or 35 per cent, were absolutely negative.

The protocols of Uhlenhuth and Mulzer to which we have referred[18] deal with a series of eleven rabbits inoculated in the testis and reinoculated after apparent recovery from first infection. Of this series, three, inoculated for the first time on one side only, were negative after the second inoculation. Two were inoculated first in the right, and then in the left from the lesion developed in the right testis, and therefore have no bearing on our experiments. Two were inoculated in both testes, but developed lesions only on the left. On reinoculation into both testes they developed lesions only on the right. One gave no response to the first inoculation but developed lesions on both sides after the second.

Only three out of the eleven developed lesions on reinoculation on the same side as that on which the first lesion had appeared. One of these showed a diffuse orchitis after the first inoculation but only a localized sac lesion on reinoculation. Another, although reinoculated on the left side only, subsequently developed lesions on both sides, indicating that the original infection had not yet run its course. The third, which developed diffuse bilateral lesions after the first inoculation, and a diffuse lesion on the left after the second inoculation, is the only one of the series which seems to have developed a second satisfactory lesion on reinoculation at the site of the original disease.

Thus, in a general way, although not so interpreted by them, the results of Uhlenhuth and Mulzer are not out of harmony with the results obtained by us in the seventeen rabbits reported above. Of these seventeen reinoculations, twelve failed to show lesions in testes which had shown lesions before. Another one (No. 20) developed a small nodule which was negative to puncture, but we shall not include this with the twelve as entirely negative, as we wish to be particularly cautious. The occasional small nodules which developed in the testis and were negative to puncture, in two or three of these rabbits, even if they had proved to be syphilitic by successful puncture, would indicate a considerably less extensive lesion than is ordinarily found in such inoculations, and at present we rarely fail to obtain spirochetes on puncture in syphilitic rabbits. Rabbit 27 developed chancrous lesions of the skin, therefore histologically not on the site of the previous lesion of the testis, and in this case, no treponemata could be found. Rabbit 28 undoubtedly developed small lesions positive to puncture, although the treponemata found were non-motile, an occurrence which we cannot at present satisfactorily explain. But in this rabbit, the first inoculation products were doubtful. No. 30 developed a small nodule in a testis which had

[18] Uhlenhuth and Mulzer, *Arb. k. Gsndhtsamte.*, 1913, xliv, 453.

previously been the seat of a diffuse orchitis. But the lesion was small and limited in extent and the treponemata found on puncture were motionless. One rabbit in this series (No. 19), although exhibiting definite lesions on the first inoculation, developed frank lesions after the second inoculation. It will be noticed that this is the only rabbit in which reinoculation was undertaken less than 2 weeks after apparent healing of the first lesion.

If we analyze these results together with the few data gathered from the paper of Uhlenhuth and Mulzer, they seem to us to furnish evidence that there is a distinct local resistance to reinfection developed at the site of a previous lesion. In rabbits the conditions are peculiarly favorable for experiments bearing on this point. Although generalization of the *pallida* probably occurs fairly regularly in very young intracardially inoculated rabbits, and to a limited extent in intratesticularly inoculated adults, the treponemata do not easily form lesions on other sites or active pathological reactions in many of the organs of rabbits. It is known of these animals as we have stated above that the existence of a lesion in one testis does not appear to confer resistance in other parts of the body, and we cannot speak in rabbits as we can in human beings and monkeys, of resistance to superinfection coincident with the existence of the disease. Since the experiments cited above, then, seem to point out that this resistance is conferred upon the site of pathological reaction, we are inclined to deduce that resistance to syphilis is a localized process which affects the tissues in which the reaction to the treponemata has taken place, and is not a property conferred, as in resistance to some bacterial infections, by a diffusion of antigen and a consequent production of sessile and circulating antibodies in parts remote from the point of actual infection. This complements our results and those of others who have failed to find any considerable amount of circulating antibodies, and would explain the universal failure to produce immunization either by treatment with dead syphilitic virus or, passively, with the sera of syphilitic or vaccinated human beings and animals.

The difficulty which arises in interpreting these experiments lies in determining whether the testes, after reinoculation, still harbored spirochetes from the first inoculation (the reinoculation being really

a superinfection), or whether they had completely recovered. It is practically impossible to determine this, since negative puncture is insufficient to permit conclusions as to the absence of treponemata from the entire testis. However, in some of our rabbits, 2 to 3 or 6 months, or nearly a year had elapsed, and to ordinary examinations the testis seemed normal at the time of reinoculation. We believe that the reaction capacity of the tissue cells must be the important element and not the possible persistence of a limited number of treponemata which are no longer capable of inducing reaction. As far as the analysis of the immunological condition is concerned, it is, after all, of chief importance whether the treponemata persisting or newly introduced can or cannot cause pathological injury.

CONCLUSIONS.

1. Immune sera produced in rabbits by treatment with our Culture Strain A of *Treponema pallidum* agglutinated not only the homologous strain, but also the Noguchi strains, and indicate a close group relationship of other non-pathogenic treponemata. Absorption experiments confirmed this, indicating a close relationship between the *pallidum* and the *calligyrum*.

2. Culture treponemata are not agglutinated to a much greater extent by the sera of syphilitic rabbits than they are by those of normal rabbits.

3. Culture treponemata are not agglutinated to any considerable extent by the sera of rabbits immunized with virulent treponemata.

4. The sera of syphilitic patients, especially those in the tertiary stages, agglutinate culture *pallidum* to a slightly greater extent than do those of normal individuals, but the culture *pallidum* is agglutinated to an almost equal degree by the sera of many individuals with diseases other than syphilis. We do not think that we could definitely distinguish the syphilitic from the non-syphilitic serum by the agglutination of the culture *pallida*, and therefore we do not believe that the reaction has any diagnostic value at present.

5. Immunization with culture *pallidum*, either general or local, does not seem to confer upon rabbits any considerable degree of resistance to inoculation with virulent treponemata.

6. Rabbits that have once exhibited lesions in the testis are not easily reinfected at the same site if reinoculation is practiced more than a month or so after apparent healing of the lesion. We believe that the experiments above recorded strongly suggest that resistance to syphilis in rabbits is a localized cell phenomenon not dependent upon a generalized reaction on the part of tissues remote from the site directly involved in reaction with the invading treponemata. Antibodies analogous to those formed in most bacterial infections appear in the general circulation in slight amount, if at all. The finding of many motionless treponemata in a few of the small lesions following reinoculation suggests the possibility of a purely localized formation of antibodies. This was expressed by Landsteiner some years ago when he spoke of the localized formation of agglutinins when they were absent in the general circulation.

We hesitate to apply these results too generally to the conditions prevailing in human syphilis, but they contain the possibility of an explanation for the apparent skin immunity of the secondary period, and the later successive involvement of some organs and tissues when others remain normal and when external superinfection is successfully resisted.