

XXIV.—IMMUNITY STUDIES IN EXPERIMENTAL SYPHILIS *

II. SPIROCHETICIDAL PROPERTIES OF SERUMS IN LATENT AND EXPERIMENTAL SYPHILIS WITH SOME OBSERVATIONS ON IMMUNITY

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INTRODUCTION

The vast amount of literature dealing with the problem of immunity in syphilis may be grouped into a series of extensive clinical observations, on the one hand, and on the other, the report of a considerable amount of experimental work which received its first impulse from the pioneer studies on syphilis transmission in chimpanzees by Metchnikoff and Roux¹ two years prior to Schaudinn's discovery of *Spirochaeta pallida*. The conclusions, which were drawn from both the clinical and experimental data that had accumulated during a period of years, were, in the first instance, the result of observations not subject to control, and, with the causative agent yet unknown, could not be interpreted scientifically; in the second instance, the experimental findings, without exception, were based on what is now known to be an erroneous conception of the nature and behavior of the syphilitic virus within the animal body.

Reinoculation in Syphilis.—From the clinical studies of Levaditi,² Neisser,³ Finger and Landsteiner⁴ and others, we reach the conclusions that in the syphilitic patient definite resistance to reinoculation is acquired shortly after the appearance of the primary lesion; that the resistance is manifest throughout the secondary stage and in the tertiary stage in which, however, reinoculation becomes more frequently possible, after a certain time; that susceptibility to reinfection may

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* Studies, reports and observations from the dermatological department of the Barnard Free Skin and Cancer Hospital and the Washington University School of Medicine, St. Louis, Mo., U. S. A., service of Drs. M. F. Engman and W. H. Mook.

1. Metchnikoff and Roux: Ann. de l'inst. Pasteur **17**:809, 1903; **18**:1, 659, 1904; **19**:673, 1905; **20**:875, 1906.

2. Levaditi: Ztschr. f. Immunitätsforsch., 1910, Ref., Pt. 2, p. 277.

3. Neisser: Beiträge zur Pathologie und Therapie der Syphilis, Berlin, Julius Springer, 1911.

4. Finger and Landsteiner: Arch. f. Dermat. u. Syph. **78**:335, 1906; **81**: No. 1, 1906.

return even though the syphilis has been entirely cured, although in this regard a difference in degree of susceptibility prevails as compared with the susceptibility of a normal person.

Animal experimentation has led to definite conclusions which are correlated in the main with the earlier clinical observations and indicate a state within the animal body comparable with ideas of immunity expressed in the foregoing, in which progressively increasing resistance is developed as the syphilitic virus becomes generally distributed, until during the early tertiary stage this resistance reaches a maximum. In reviewing the work done by different investigators, we find two distinct points of view expressed. Neisser, for instance, is representative of those who believe that no immunity to syphilis exists, and that failure to reinoculate means an existing syphilis rather than any definite immune condition. In support of this theory he has adduced a number of positive inoculations with emulsions of bone marrow and spleen from monkeys which had resisted a second infection. On the other hand, animals which had been cured by drugs could be reinoculated. Finger and Landsteiner⁵ have taken a different view, and much of the later experimental work in syphilis is in accord with their idea that immunity increases gradually with the onset of the disease and becomes absolute during the "second latent period" preceding the tertiary stage when gummatous lesions may be developed. It is to be noted, in this place, that the interpretation of "immunity" phenomena has rested primarily on the principle of resistance which is developed in the host after the syphilitic virus has become generally distributed. This idea, although reasonably consistent with ordinary immunity phenomena, was based, however, on inadequate knowledge.

Localized Reactions and Tissue Immunity.—Early investigations by Metchnikoff, Roux, and Neisser had brought out the fact that experimental syphilis in monkeys gave rise to immune properties of the skin similar to that found in man; that is, reinfections were not possible at a time when a primary lesion was manifest. In confirming these observations an attempt was made by Kraus and Volk⁶ to explain the paradoxical reaction in persons with generalized syphilis in whom cutaneous infections could rarely, if ever, be elicited by reinoculation. It appeared that resistance developed slowly and was at its height only after a long time. Superinfections were possible up to about three weeks after the first inoculation and prior to the appearance of a primary lesion. Reinfection failed, as a rule, after the lesion had persisted for several days. Under these conditions superinfections were characterized by

5. Finger and Landsteiner: *Verhandl. d. deutsch. Dermat. Gesellsch.*, IX Kongress, Springer, 1907, p. 25.

6. Kraus and Volk: *Wien. klin. Wchnschr.* 19:621, 1906.

their relatively benign appearance and much smaller size as compared with the ordinary initial lesion. That the size and type of lesions may be influenced by preexisting ones has been mentioned by Nichols,⁷ and Brown and Pearce⁸ in later studies. The possible influence of primary foci on the subsequent development of later lesions had already been suspected by Kraus and Volk,⁶ and in a few of their experiments rudimentary manifestations were observed when lesions were excised from seven to fourteen days after their appearance. From this it was concluded that a skin immunity appeared to prevail in the same degree as before and that a partial immunity seemed to develop as a result of inoculation of the surface of the skin. An explanation of the previously described phenomenon of resistance to reinfection by way of the skin and mucous membrane at a time when certain organs and tissues continue to undergo injury must be sought for in the behavior of certain tissues, such as the rabbit testicle, for example. It has been found that apparent recovery from a previous lesion develops a resistance to subsequent inoculation, although ordinarily no generalized resistance in rabbits appears to develop during the disease, since the opposite testicle can be successfully inoculated before, during and after the existence of a lesion on the other side. Observations of many investigators point to the probability of a localized reaction existing in the animal body, in consequence of a mechanism by which active invasion with *Spirochaeta pallida* leads to a state of nonsusceptibility. Neisser has called this condition "Anergie." It is conceivable that organisms which may gain entrance at this time may remain uninjured and yet be capable of setting up lesions in other parts of the body. There is evidence from recent studies on the dissemination of *Spirochaeta pallida* and the so-called "carrier state" in latent syphilis that such may be the case. At all events, it seems to be of some importance to differentiate between the classic conception of immunity to syphilis and the state of affairs which is probably more consistent with present-day knowledge of infection with this disease.

Our own investigations in latency in man⁹ and in experimental syphilis, together with the observations of Brown and Pearce,¹⁰ have

7. Nichols, Henry J.: Observations on the Pathology of Syphilis, J. A. M. A. **63**:466 (Aug. 8) 1914.

8. Brown and Pearce: J. Exper. M. **32**:445, 1920.

9. Eberson, F., and Engman, Martin F.: An Experimental Study of the Latent Syphilitic as a Carrier, J. A. M. A. **76**:160 (Jan. 15) 1921. Eberson, F.: Dissemination of Spirochaeta Pallida in Experimental Syphilis, Arch. Dermat. & Syph. **3**:111 (Jan.) 1921; XXIII.—Immunity Studies in Experimental Syphilis. Infectivity and Survival of Spirochaeta Pallida in Rabbits, with Observations on Some Strains from Latent Syphilis, Ibid. **3**:775 (June) 1921.

10. Brown, W. H., and Pearce, L.: Note on Dissemination of Spirochaeta Pallida from Primary Focus of Infection, Arch. Dermat. & Syph. **2**:470 (Oct.) 1920.

established the significance of the lymphatic glands as foci from which *Spirochaeta pallida* may be discharged. The rôle which this type of localization may play in immunity may be conjectured. It is not mere speculation to attribute an immunity response to the presence of these organisms since we know that resistance to reinoculation is associated with the presence of spirochetes in some part of the body. Foci in which "latent" spirochetes are localized may act as centers from which antibodies are slowly elaborated and then discharged into the blood stream. From a consideration of an extreme type of localization, such as that found in glands, we next pass on to a study of the immunity phenomena which may be seen to occur in single lesions.

Local Lesions and Inhibitory Effects on Development of Lesions Elsewhere.—Some of the early experiments made with monkeys demonstrated the inhibitory effect exercised by lesions in one organ on the development of lesions in other parts of the body (Metchnikoff and Roux). Using the rabbit testicle for his study, Nichols⁷ found that unilateral inoculation when followed by castration of the infected testicle promptly gave rise to lesions in the opposite testicle. The fundamental importance of these observations has led to intensive study of the cyclic changes in syphilis and to those factors which tend to shed much light on immunity reactions in the experimental animal. From the standpoint of immunity response to infection it would be of great value to interpret types of lesions as evidence of a certain kind of reaction, although the change in the character of lesions makes such interpretation difficult. There is evidence, however, that resistance of the animal to *Spirochaeta pallida* is intimately connected with the nature and extent of the reaction taking place at the site of inoculation, and any influence which is capable of modifying the reaction might be expected, according to Brown and Pearce,¹¹ to react on the phenomena of the infection as a whole. In their opinion, generalization is the measure of resistance. The protection which is developed from any given focus may not affect all tissues or all parts of the body to the same degree, but it may be extended progressively from primary lesions to the successive groups of tissues and manifest protective action in one group at a time when other groups of tissues may be afforded little or no protection. The fact that tissue immunity may manifest itself by extension from group to group has direct bearing on the view which we have expressed regarding the elaboration of antibodies by way of localized areas into the blood stream.

11. Brown, W. H., and Pearce, L.: Experimental Production of Clinical Types of Syphilis in the Rabbit, *Arch. Dermat. & Syph.* **3**:254 (March) 1921.

Cyclic Reactions in Syphilis and Analogous Phenomena of Trypanosome and Spirillary Diseases.—Cyclic phenomena in syphilis find a counterpart in other diseases which are caused by spirochetes. In relapsing fever the periodic functioning of a protective mechanism is known to take place in the blood of infected individuals, or as Levaditi¹² believes, to manifest itself as a phagocytic reaction which is independent of serum properties. African tick fever offers analogous phenomena, and although recurrences are more common, susceptible animals are immune for a period following a second attack. A similar immunity follows recovery from spironematosis in geese and other fowls. The mechanism on which such immunity depends has been studied by numerous investigators, notably by Gabritschewsky,¹³ Marchoux and Salimbeni,¹⁴ Levaditi,¹² Levaditi and Manouelian,¹⁵ Mesnil and Nicolle,¹⁶ Neufeld and von Prowazek,¹⁷ and others. To Gabritschewsky we owe our knowledge of the bactericidal content of blood in relapsing fever in which the protective property was found to be at a maximum after the crisis, diminished during apyrexia and increased again during the next attack. Notwithstanding these fluctuations, immunity as a whole increased in the course of the disease. The protection afforded by active immunity in these different diseases is not wholly dependent on bactericidal properties in the serum, for as Novy and Knapp¹⁸ have pointed out, other immune bodies may play an important part in the mechanism of defense. More recently Inada and his collaborators¹⁹ demonstrated in the blood serum of patients with Weil's disease the presence of a specific lysin for the spironema. Immune bodies may be present four years or more after the attack of disease.

In diseases caused by trypanosomes the facts are similar to those given in regard to spirochete infections. The attributes common to all of these seem to be a resistance to reinfection which coexists with the presence of the living organisms in the host. Syphilis in this respect is analogous in its behavior, not only from the standpoints which have been discussed, but also in regard to certain localizing characteristics of *Spirochaeta pallida* and the reactions attending their dissemination.

12. Levaditi: Compt. rend. Soc. de Biol. **60**:134, 1906; Ann. de l'inst. Pasteur **20**:41, 1906.

13. Gabritschewsky: Ann. de l'inst. Pasteur **10**:629, 1896.

14. Marchoux and Salimbeni: Ann. de l'inst. Pasteur **17**:569, 1903.

15. Levaditi and Manouelian: Ann. de l'inst. Pasteur **20**:593, 1906.

16. Mesnil and Nicolle: Ann. de l'inst. Pasteur **20**:513, 1906.

17. Neufeld and von Prowazek: Centralbl. f. Bakteriol. Ref., Abt. 1 **41**:754, 1908.

18. Novy and Knapp: J. Infect. Dis. **3**:291, 1906.

19. Inada, Ido et al: J. Exper. M. **33**:377, 1916.

Analogies may be found again, for example, in diseases of fowls in which *Spirochaeta gallinarum* has been shown to localize in certain viscera and to enter the blood stream by way of diverse glandular tissues.¹⁵

In weighing the analogy of syphilis to other diseases, one must not lose sight of the fact that the ordinary conception of immunity defines a condition which is the result of a refractory state incompatible with the existence of the organism causing the disease. From the biologic point of view, the cycle in syphilis would tend to disprove the theory that immunity is not possible at any stage of the infection.

EXPERIMENTAL IMMUNITY IN SYPHILIS

Antibody Phenomena.—Serologic studies in experimental syphilis have been concerned mainly with the development in serums of agglutinating and complement-fixing bodies. Attempts to demonstrate specific agglutinins in human and experimental syphilis were made by Hoffmann,²⁰ Landsteiner and Mucha,²¹ Hoffmann and von Prowazek,²² Uhlenhuth and Mulzer,²³ Metchnikoff and Roux,¹ and others, with negative or indecisive results. Following Noguchi's discovery, in 1911, of a method for isolating *Spirochaeta pallida* in pure culture, he was able to demonstrate in immunized rabbits the presence of agglutinins and complement-fixing substances for cultivated strains. The first successful experiments were reported by Kolmer²⁴ and were followed soon afterward by those of Nakano²⁵ and Kissmeyer.²⁶ With pure cultures of the spirochetes it was possible to develop highly agglutinating serums from rabbits. Serums from individuals in the primary, secondary and tertiary stage and from congenital syphilis were found to contain agglutinins in about 50 per cent. of the cases. These antibodies were shown to be present in highest concentration during the later stages of the disease and in the greatest percentage of cases among persons who showed late primary, secondary, tertiary and latent syphilis (Kolmer, Broadwell and Matsunami²⁷). That the reactions of culture *Spirochaeta pallida* differed from those which were obtained from human lesions was next shown by Zinsser, Hopkins and McBurney,²⁸

20. Hoffmann: Dermat. Ztschr. **13**:561, 1906.

21. Landsteiner and Mucha: Centralbl. f. Bakteriöl., Ref. **39**:540, 1907.

22. Hoffmann and von Prowazek: Arb. a. d. k. Gsndhtsamte **37**:205, 1911.

23. Uhlenhuth and Mulzer: Arb. a. d. k. Gsndhtsamte **33**:183, 1909.

24. Kolmer: J. Exper. M. **18**:18, 1913.

25. Nakano: Arch. f. Dermat. u. Syph. **116**:265, 1913.

26. Kissmeyer: Deutsch. med. Wchnschr. **41**:306, 1915.

27. Kolmer, Broadwell and Matsunami: J. Exper. M. **24**:333, 1916.

28. Zinsser, Hopkins and McBurney: J. Exper. M. **23**:341, 1916.

in a study of agglutinins in serums from animals immunized with culture spirochetes.

Protective Properties of Serum in Human and Experimental Syphilis.—Few references are found in the literature pertaining to protection experiments made in vitro and in vivo with serums from persons or infected animals. Metchnikoff and Roux found serums from monkeys which had been systematically treated with attenuated syphilitic virus ineffective in preventing animal infection with material obtained from active lesions. They believed, however, that they had demonstrated in two instances a definite protective antibody in serum from a monkey which had received subcutaneous injections with syphilitic blood for a period of eight months. A chimpanzee that had been inoculated with a mixture of such serum and material from a chancre remained negative for thirty-eight days, when death from pneumonia supervened. Another monkey, inoculated similarly, evidenced slight lesions as compared with a control. These results appear to be indecisive. Two therapeutic experiments with serums from infected monkeys, reported by Finger and Landsteiner, cannot be interpreted owing to the technic employed. Local subcutaneous injections of serum in a total amount of 59 c.c. failed to prevent roseola which developed eight weeks after primary genital lesions. In both patients the primary lesions were excised previous to administration of the serum. The generalization which follows as a result of extirpation of the primary foci would tend to mask the result of these therapeutic measures.

Spirocheticidal activity of serums has been studied in rare instances. Finger and Landsteiner⁵ reported negative results with serums from florid stages of syphilis. In vitro experiments, described by Zinsser and Hopkins,²⁹ demonstrated spirocheticidal properties for culture strains in the serum of rabbits and sheep that had been immunized with cultures of *Spirochaeta pallida*. With virulent spirochetes obtained directly from lesions, this protective property was not manifested by serum produced with culture spirochetes.²⁸ These results were confirmed by Noguchi,³⁰ who found, in addition, that serum from a syphilitic rabbit destroyed culture spirochetes.

EXPERIMENTAL

The experiments which are reported in this paper have been based on the idea that syphilis may offer immunity phenomena analogous or similar to those that are found in other infections which tend to assume a latent or carrier state. In order that the nature of a protective mechanism might be studied more effectively and be subject to control,

29. Zinsser and Hopkins: J. Exper. M. **23**:323, 1916.

30. Noguchi: J. Exper. M. **25**:765, 1917.

the method of experimental infection was designed so as to permit a study of serum properties at different stages of the disease. These animal experiments were planned to supplement the data which might be obtained from an investigation of serums taken from persons having latent syphilis. The protective property of specimens of serums was measured by the presence or absence of spirocheticidal activity when combined with living, virulent *Spirochaetae pallidae* derived from active syphilitic lesions in rabbits. It is recognized that there is great difficulty in determining an effective combination of dosage of spirochetes, volume of serums, time of incubation and the amount of the mixture to be injected finally as a test of antibody content. Since no attempt was made to minimize the chances of infecting the experimental animal, this should be borne in mind when weighing the negative results in which the given stage of infection might have a direct bearing on the protective property of such serum as was used in the test. Detailed quantitative procedures that would measure different degrees of spirocheticidal activity against diminishing doses of virulent organisms could not be followed owing to lack of space and material. The numerous control experiments in which normal serums were used against fixed doses of spirochetes in a manner comparable with the test serums in all particulars were the criteria in every series.

Material and Scope of Experiments.—A study was made of the spirocheticidal property of different serums from patients known to have latent syphilis with a history of old as well as more recent infection, and from experimentally infected rabbits. In the group of animal experiments a considerable amount of material was available for the study of protective serum properties during the various stages of syphilitic infection. The patients who were selected for study were free from visible syphilitic manifestations or symptoms and were for the most part untreated cases.

Technic.—Blood from patients was drawn with sterile precautions by means of a glass syringe from the median vein of the arm. Specimens were allowed to stand at room temperature until the clot was formed and then kept in the ice chest for one half hour before centrifugation. The clear serum was next heated at 54 C. for twenty minutes and stored in hermetically sealed tubes until needed. Serum was from one to four weeks old and never exceeded six weeks at the time experiments were made. Experimentally infected rabbits were bled from the heart, under ether anesthesia, and the serum treated similarly to that which was obtained from patients. Rabbit serums were from two to five weeks old at the time tests were made.

A combined in vitro and in vivo method was used in determining the spirocheticidal activity of serums which were combined in amounts

of 2 c.c. with 0.1 c.c. of a suspension of virulent *Spirochaeta pallida*. The mixtures were incubated in a water bath at 37 C. for one and one half hours and then injected intratesticularly into normal rabbits, in duplicate series for each strain of spirochetes which were used in the test. Normal human and normal rabbit serums served as controls and were subjected to every step in the technic in parallel with the test serums.

Animals Used.—Medium-sized gray and brown rabbits with well developed testicles were used. At the time of inoculation the animals were from 5 to 6 months old, as a rule.

Strains of Spirochaeta Pallida Used.—Three strains of *Spirochaeta pallida* were isolated originally from a penile chancre (No. 170), the gland of a patient with latent syphilis (No. 137) and from the semen of a patient with latent syphilis (No. 117), respectively. Spirochetolytic properties of serums from the latent cases were studied against at least two strains and in some instances with three. Of these strains, No. 170 was used constantly along with either the latent strain No. 137 or No. 117. In this place mention may be made of the fact that no difference in the protective property of the serum was found to exist for any special organism. When definite spirocheticidal activity was present, all of the strains failed to infect, irrespective of their origin. This was not the general rule, however, with serums that were taken from experimentally inoculated rabbits, as will be shown later. Emulsions of *Spirochaeta pallida* were prepared from testicular lesions in rabbits with active syphilis. The strains were transferred from animal to animal at stated intervals and were uniformly and constantly virulent. A small amount of material was taken directly from the lesion by means of a finely drawn out capillary glass pipet. Warm salt solution was added in a small volume to the testicular puncture fluid which was expelled into a sterile Petri dish with the aid of a rubber nipple attached to the pipet. A sterile glass rod, flattened at one end and bent at a convenient angle, was used to grind up the sticky mass in the smallest possible volume of solution. This was next drawn up into a pipet through a thin layer of sterile absorbent cotton placed in the homogenous emulsion. The perfectly clear fluid containing the organisms free from any tissue or foreign material was finally added to the serum and the protective property next studied. Suspensions of this material averaged from 15 to 20 spirochetes per microscopic field.

The rabbits were examined daily after two weeks had elapsed. The testicles of animals that remained negative for two months or longer were punctured at different intervals and dark-field examinations made of the testicular fluid over a period of six months. In a number of instances such animals were anesthetized and the emulsions of testicle,

liver and spleen, and in some cases the inguinal glands were injected into a series of normal rabbits to ascertain the fate of *Spirochaeta pallidae* which were introduced with the serums. At no time were these subinoculations into the testicle successful, showing that those animals were negative which failed to develop lesions synchronously with or approximately at the time the control animals were found positive.

RESULTS OF EXPERIMENTS WITH HUMAN SERUMS

Eighteen specimens of blood serum from persons having latent syphilis were found to have spirocheticidal properties for different strains of *Spirochaeta pallida*. In those cases in which true latency did not exist, no protection was manifested by the serum. A history of recent infection invariably confirmed the experimental results in these instances.

TABLE 1.—RESULTS IN EXPERIMENT 1.

Serum	Spirochete			Result
	Amount c.c.	Suspension c.c.	Strain of <i>S. pallida</i>	
H. Mc. D.....	2.0	0.1	170, 137	Negative; animals remained well
W. M.....	2.0	0.1	170, 137, 117	Negative; animals remained well
H. A.....	2.0	0.1	170, 117	Negative; animals remained well
H. B.....	2.0	0.1	170, 137	Negative; animals remained well
J. A.....	2.0	0.1	170, 137	Negative; animals remained well
C. B.....	2.0	0.1	170, 117	Negative; animals remained well
Normal human..	2.0	0.1	170, 137, 117	Positive lesions after 28, 34, 36 days
Normal rabbit...	2.0	0.1	169, 170 137, 117	Positive lesions after from 4 to 5 weeks, on the average

In Experiment 1 are tabulated the results which were obtained with serums from persons whose infection dated back from three to twenty-five years. The blood Wassermann reactions in these and succeeding protocols refer to specimens which were taken just prior to the experiment, unless specified otherwise.

Clinical Data.—M. Mc. D., a man, aged 55, gave a history of a primary lesion twenty years ago. The history as to antisyphilitic treatment was vague. His wife had had one stillbirth. The blood Wassermann reaction was + + + +.

W. M., a man, aged 45, gave a history of a penile chancre twenty-five years ago. The blood Wassermann reaction was + + + + in the cholesterin antigen. There was clinical evidence of hepatic cirrhosis.

H. A., a man, aged 32, had had a chancre eight or nine years ago, with secondary eruption at the time. The blood Wassermann reaction was + + + +.

H. B., a man, aged 29, gave a history of a chancre on the penis five years ago. His wife had had no miscarriages. The blood Wassermann reaction was + + + +.

J. A., a man, aged 26, had had a chancre four or five years ago, with no eruption at the time. The blood Wassermann reaction was + + + + in the cholesterin antigen and + in the noncholesterin antigen.

C. B., a man, aged 31, gave a history of a chancre three years ago. There was no eruption at the time. His wife had had no miscarriages. The blood Wassermann reaction was + + + + in the cholesterin antigen and + + in the noncholesterin antigen.

The next experiment illustrates the spirocheticidal activity in serum from a patient who had received antisyphilitic treatment until the Wassermann reaction was persistently negative. Another interesting observation brought out in this series is that spirocheticidal bodies may be present in the serum of an infant in common with those present in the mother's serum.

Clinical Data.—M. W., a woman, aged 45, had been taking antisyphilitic treatment for an indefinite period up to about fourteen months prior to the time her serum was obtained for the experiment. Her blood Wassermann reactions were successively negative at intervals of six months and she was classified as "serologically well."

TABLE 2.—RESULTS IN EXPERIMENT 2.

Serum	Spirochete			Result
	Amount c.c.	Suspen- sion c.c.	Strain of <i>S. pallida</i>	
M. W.....	2.0	0.1	170, 137	Negative; animals remained well
M. L. D.....	2.0	0.1	170, 137	Negative; animals remained well
D. L. D.....	2.0	0.1	170, 137	Negative; animals remained well
Normal rabbitN.	2.0	0.1	170, 137, 117	Positive lesions after 4 to 7 weeks
Normal human..	2.0	0.1	170, 137, 117, 169	Positive lesions after 4 to 7 weeks

M. L. D., a woman, aged 34, gave no history of infection and had received no treatment. Her blood Wassermann reaction was + + + + in the noncholesterin antigen.

D. L. D., a girl, aged 18 months, whose mother is M. L. D., gave a + + + + Wassermann reaction in the noncholesterin antigen only. She was apparently healthy and free from syphilis.

Typical cases of latent syphilis are those in which the patient has no history of an infection, no history of treatment for syphilis at any time, and in which there are no signs or manifestations of the disease. Such persons usually find their way to the clinic for syphilis by way of other clinics to which they may have come for examination. These patients, as a rule, give a positive blood Wassermann reaction in one antigen only, generally the cholesterin antigen. The following experiment which was performed with such a group of patients gave uniform results for spirocheticidal properties in the serums tested.

Clinical Data.—R. C., a woman, aged 31, gave no history of infection, and the patient had never taken any treatment. The blood Wassermann reaction was + + + + in the noncholesterin antigen only.

R. O'N., a woman, aged 33, gave no history of infection. She had had two miscarriages. The blood Wassermann reaction was + + + + in the cholesterin antigen only.

G. B., a woman, aged 33, gave no history of infection or treatment. The blood Wassermann reaction was + + + + in the cholesterin antigen only.

A S., a woman, aged 27, gave no history of infection or treatment. The blood Wassermann reaction was + + + + in the cholesterin antigen only.

TABLE 3.—RESULTS IN EXPERIMENT 3.

Serum	Amount c.c.	Spirochete Suspension c.c.	Strain of <i>S. pallida</i>	Result
R. C.....	2.0	0.1	170, 137, 117	Negative; animals remained well
R. O'N.....	1.5	0.1	170, 137	Negative; animals remained well
G. B.....	1.5	0.1	170, 117	Negative; animals remained well
A. S.....	2.0	0.1	170, 117	Negative; animals remained well
Normal human..	2.0	0.1	170, 137, 117	Positive lesions after 29, 32 and 35 days, respectively
Normal rabbit...	2.0	0.1	170, 137, 117	Positive lesions after 30, 28 and 30 days, respectively

In Experiment 4 are summarized the results of protection tests for another group of patients who gave no history of infection and whose blood Wassermann reactions were positive in both antigens, or, as in the majority of cases, slightly positive in either one or the other in inverse relationship.

TABLE 4.—RESULTS IN EXPERIMENT 4.

Serum	Amount c.c.	Spirochete Suspension c.c.	Strain of <i>S. pallida</i>	Result
S. S.....	2.0	0.1	170, 137	Negative; animals remained well
J. S.....	2.0	0.1	170, 137	Negative; animals remained well
E. O'N.....	2.0	0.1	170, 137, 117	Negative; animals remained well
L. C.....	2.0	0.1	170, 117	Negative; animals remained well
S. W.....	2.0	0.1	170, 137, 117	Negative; animals remained well
Normal human..	2.0	0.1	170, 137, 117, 169	Positive lesions after 4 to 6 weeks
Normal rabbit...	2.0	0.1	170, 137, 117, 169	Positive lesions after 4 to 6 weeks

Clinical Data.—S. S., a man, aged 28, gave no history of infection. The blood Wassermann reaction was + + + + in the cholesterin antigen and + + in the noncholesterin antigen.

J. S., a woman, gave no history of infection, and her husband's blood Wassermann reaction was negative. The blood Wassermann reaction of the patient was + + + +.

E. O'N., a woman, gave no history of infection or eruption, and there had been no miscarriages. The patient came to the clinic for trigonitis. The blood Wassermann reaction was + + + + in the cholesterin antigen and + in the noncholesterin antigen.

L. C., a woman, gave no history of infection or eruption. She had had one miscarriage. The blood Wassermann reaction was + + + + in the cholesterin antigen and + in the noncholesterin antigen.

S. W., a man, aged 30, gave no history of infection. His wife and only child were well and free from syphilis. The blood Wassermann reaction was + + + + in the cholesterin antigen and + in the noncholesterin antigen.

THE BLOOD SERUM IN EARLY SYPHILIS AND THE LATENT STAGE OF RECENT INFECTIONS

The presence of a protective property in serums from cases of syphilis would seem to depend on the stage of infection. A difference is to be noted between latency in the sense defined as occurring after a very old infection and the period of latency which occurs early in the disease and as an interval between the possible flaring up of a train of typical symptoms.

The serums from a group of seven patients with histories of recent infection were found uniformly negative for spirocheticidal activity. These results were in marked contrast to those obtained in the other series, and are summarized in Experiment 5.

TABLE 5.—RESULTS IN EXPERIMENT 5.

Serum	Spirochete			Result
	Amount c.c.	Suspension c.c.	Strain of <i>S. pallida</i>	
L. M.....	2.0	0.1	170, 137	Positive lesions after 32 to 38 days
D. W.....	2.0	0.1	170, 137	Positive lesions after 40 to 44 days
J. B.....	2.0	0.1	170, 117	Positive lesions after 48 to 50 days
E. L.....	2.0	0.1	170, 137, 117	Positive lesions after 50 to 52 days
C. W.....	2.0	0.1	170, 117	Positive lesions after 38 to 40 days
F. B.....	2.0	0.1	170, 137	Positive lesions after 55 to 57 days
N. N.....	2.0	0.1	170, 137	Positive lesions after 42 to 44 days
Normal human..	2.0	0.1	170, 137, 117, 169	Positive lesions after 40 to 42 days
Normal rabbit..	2.0	0.1	170, 137, 117, 169	Positive lesions after 36 to 40 days

Clinical Data.—L. M., a woman, aged 32, had had two miscarriages. Her history as to infection was vague. She showed definite signs of active pulmonary tuberculosis. The blood Wassermann reaction was + + + + in both antigens.

D. W., a man, aged 39, gave no history of infection. The blood Wassermann reaction was + + + + in the cholesterin antigen. The patient gave clinical evidence of prostatitis.

J. B., a man, had had a primary lesion on the penis two months ago. The blood Wassermann reaction was + + + + in both antigens.

E. L., a man, aged 30, gave a history of infection eight months ago with a + + + + blood Wassermann reaction at the time. Two months later the reaction was + + + + in the cholesterin antigen. One month afterward, following two weeks of mixed treatment, the reaction was +; a month later it was again + + + +. At the time a specimen of serum was taken for the experiment the Wassermann reaction was negative.

C. W., a man, aged 24, gave a history of a primary sore on the penis two years ago. He had taken three injections of arsphenamin at the time. The blood Wassermann reaction was + + + +.

F. B., a woman, gave no history of infection. She had had two recent miscarriages. Her husband had been taking antisyphilitic treatment. The blood Wassermann reaction was + + + + in the cholesterin antigen and + + in the noncholesterin antigen.

N. N., a woman, gave no history of infection or miscarriages. She had been "feeling ill" for the past two years coincident with her husband's infection which dated back to that time. He had been taking treatment for syphilis during the past year. The patient's Wassermann reaction was + + + + in both antigens.

This series illustrates that there may be a complete correlation between the presence of protective substances in the serum and the stage of the infection. Where true latency has been attained by the individual, the serum exerts a spirocheticidal effect on *Spirochaeta pallida*. On the other hand, where the disease has not yet become latent and where an active focus appears to exist, the serum seems to be devoid of spirocheticidal properties. In one instance an acute infection coexisted with syphilis, so that it is difficult to interpret the result. An intercurrent infection may vitiate the effective functioning of any existing serum properties even in those persons who may be "latent" cases.

PROTECTIVE PROPERTY OF SERUM IN RELATION TO THE WASSERMANN REACTION OF THE BLOOD

No attempt will be made to discuss the matter of antisyphilitic treatment and the measure of its efficiency in terms of the Wassermann reaction, or to evaluate the possible effects which any treatment may have on the natural immunity response to the invading *Spirochaeta pallida*. It is clear from the foregoing experimental study that a negative Wassermann reaction following treatment for syphilis may or may not go hand in hand with spirocheticidal activity of the serum in question. It is equally clear that continued treatment which renders a Wassermann test repeatedly negative does not nullify any protective property which may be present in the serum. The important point is that spirocheticidal activity of serum, irrespective of the Wassermann reaction, is essentially a function of time and depends on the degree to which the individual has elaborated and distributed the slowly accumulating antibodies. A negative blood Wassermann reaction attending treatment of a recent infection is not comparable to a similar result in the case of a person whose infection is of older origin. In the one instance we may find no spirocheticidal serum properties and in the other we may. A temporary negative Wassermann reaction must not be confused with one which is repeatedly negative and in

accordance with which a treated patient may be classified as "serologically well." These points are illustrated in Experiment 2 (M. W.) and in Experiment 5 (E. L. and C. W.).

SPIROCHETICIDAL SERUM AND ITS EFFECT ON THE
DISSEMINATION OF *SPIROCHAETA PALLIDA*

Spirochaetae pallidae are discharged into the blood stream early in the course of infection from a primary focus. The organisms may be recovered from the regional lymph glands or the blood stream as early as forty-eight hours after experimental inoculation and may be present for an indefinite period up to and including the first signs of infection (Brown and Pearce,¹¹ Levaditi¹²).

An experiment was planned with a view to determine the effect of a possible spirocheticidal reaction within the rabbit testicle on the dissemination of *Spirochaeta pallida*. The animals which were used in this group were selected at random from one of the series at a time

TABLE 6.—SUMMARY OF EXPERIMENT 6

Aug. 14, 1920 Series A Rabbit		Sept. 4, 1920 Series B Rabbit
336	2 c.c. blood →	353 and 354
337	2 c.c. blood →	355 and 356
338	2 c.c. blood →	357 and 358
339	2 c.c. blood →	359 and 360

when the outcome as to spirocheticidal activity of the serums under test was not known. The results were decisive, although the series was not large.

Experiment 6.—Rabbits were injected intratesticularly with mixtures of serums and virulent *Spirochaeta pallida* to determine the presence of protective substances in the specimens. Twenty-one days later, these animals were bled from the heart under ether anesthesia and 2 c.c. of defibrinated blood injected immediately into the testicles of normal rabbits in duplicate series. The sub-inoculated animals were examined regularly over a period of six months, during which time repeated testicular punctures were made in order to check up the examination. Table 6 is a summary of the experiment.

Results of Inoculation.—Oct. 18, 1920, Rabbit 339 presented a small bean-sized nodule in the testicle. Dark-field examination showed enormous numbers of *Spirochaetae pallidae*. Jan. 6, 1921, rabbits 359 and 360 developed pea-sized nodules in the upper pole of the testicle. Dark-field examination showed enormous numbers of *Spirochaetae pallidae*.

The other animals in both series remained uniformly and persistently negative. When injection with a mixture of spirocheticidal serum and spirochetes resulted in negative inoculations, the blood from these rabbits proved to be free from *Spirochaeta pallida*. When protective action was not manifested by the serum, dissemination occurred in the usual manner. The conclusion, therefore, is that spirocheticidal activity of serum in latent syphilis is of such a character as to prevent the normal dissemination of spirochetes from the primary focus.

THE BLOOD SERUM IN EXPERIMENTALLY INFECTED RABBITS

The rabbit serums which were studied for spirocheticidal properties were obtained from experimentally infected animals at different times after inoculation. Four strains of *Spirochaeta pallida* were used in these experiments. Two were isolated originally from two cases of latent syphilis—from the inguinal gland and semen, respectively—and two were isolated from penile chancres. The strains from latent sources are designated as No. 137 and No. 117, respectively, and the chancre strains as No. 169 and No. 170.

Serums containing suspensions of *Spirochaeta pallida* were injected into a series of normal rabbits in accordance with a combined in vitro and in vivo method which has already been described. Homologous and heterologous strains of spirochetes were used in making spirocheticidal tests. For controls, normal rabbit serums were used and also a number of test serums to which no *Spirochaeta pallida* had been added. The results with the different serums are grouped according to the time after infection when specimens were taken. There is indicated also the number of days which elapsed between the last positive dark-field examination of such infected rabbits and the date when serum was obtained. All subinoculated series of rabbits were kept under observation for from three to four months, during which time repeated testicular punctures and dark-field examinations were made, irrespective of the absence of any lesions.

The experiments are summarized in the following manner: Spirocheticidal activity was developed in the rabbit in the course of from six months to one year after the original infection. During the time that spirochetes were found in lesions and for a certain period during which dark-field examinations yielded persistently negative results, the serum was devoid of protective elements. Strains of *Spirochaeta pallida* from latent sources appeared to develop an immune serum having a wider range of protective action. Whereas such serums exerted spirocheticidal action toward heterologous as well as homologous strains of spirochetes, the serums obtained from rabbits which were inoculated with chancre strains failed to protect against inoculation with heterologous strains, even when the time which had elapsed since infection was comparable to that in the other series of animals (Experiment 3).

Experiment 7.—In this series, the strain of *Spirochaeta pallida* used was isolated from the inguinal gland of a person having latent syphilis (Strain 137). From one to three months after infection, no protective substances were found in the serum. Spirocheticidal substances were found after five months.

TABLE 7.—RESULTS IN EXPERIMENT 7

Serum	Test Strain of <i>S. pallida</i>	Days Positive for <i>S. pallida</i>	Days Negative When Bled	Days Since Infection	Result
(I)137*	137	26	69	144	Protection; animals well
(II)137	137	21	40	98	Positive lesions, 42-45 days
(II)137	(Control)				Negative; animals well
(III)137	137	14	28	67	Positive lesions after 38 to 42 days
(III)137	(Control)				Negative; animals remained well
(IV)137	137	9	10	40	Positive lesions after 42 to 45 days
(IV)137	(Control)				Negative; animals remained well
Normal rabbit	137				Positive lesions after 38 to 44 days

*In all of these protocols, the Roman numeral enclosed in brackets refers to the generation of the given strain of *Spirochaeta pallida* which was employed for experimental inoculation.

Experiment 8.—A series of rabbits was inoculated with Strain 117 which was isolated from the semen of a case of latent syphilis. The serums from a number of these animals were tested against a heterologous strain, No. 169. Protective substances were found in serums from six to fourteen months after infection, for homologous and heterologous *Spirochaeta pallida*.

TABLE 8.—RESULTS IN EXPERIMENT 8

Serum	Test Strain of <i>S. pallida</i>	Days Positive for <i>S. pallida</i>	Days Negative When Bled	Days Since Infection	Result
(II)117	117	91	117	402	Protection; animals remained well
(III)117	117	Spirochetes could not be found in dark-field examinations			
(III)117	117, 169	25	146	270	Protection; animals remained well
(III)117	117	18	156	197	Protection; animals remained well
(IV)117	117, 169	12	123	160	Protection; animals remained well
Normal rabbit	117				Positive lesions after 28 to 34 days

The next experiment shows in a more definite manner the difference between serums from animals in a state of infection approximating latency and those that have not yet reached that condition. There is, moreover, a distinct difference

seen in the outcome of the test when a heterologous strain of spirochetes is used, thus contrasting with the serums from rabbits which had been inoculated with latent strains.

TABLE 9.—RESULTS IN EXPERIMENT 9

Serum	Test Strain of <i>S. pallida</i>	Days Positive for <i>S. pallida</i>	Days Negative When Bled	Days Since Infection	Result
(I)170	170	42	61	143	Positive lesions after 20 to 25 days
(I)170	(Control)				Negative; animals remained well
(I)170	170	42	138	220	Protection; animals remained well
(II)170	170	7	36	77	Positive lesions after 34 days
(II)170	(Control)				Negative; animals remained well
(III)170	170	15	47	92	Positive lesions, 50 days
(III)170	170	15	200	245	Protection; animals well
(III)170	169	15	200	245	Positive lesions; 36 days
(IV)170	170	13	0	33	Positive lesions after 27 days;
(IV)170	(Control)				Negative; animals remained well
(IV)170	170	13	166	199	Protection; animals remained well
(IV)170	169	42	109	171	Positive lesions after 36 days
(VIII)170	170	6	198	276	Protection; animals remained well
(X)170	170	24	121	172	Protection; animals remained well
(VI)169	169	24	152	199	Protection; animals remained well
Normal rabbit	169, 170				Positive lesions after 29 to 31 days

The conclusion which may be drawn from these results is that serums from rabbits experimentally infected with syphilis are uniformly negative for spirocheticidal properties except in those instances in which the infection has attained a relatively latent state which corresponds roughly to conditions in human syphilis.

DISCUSSION

The foregoing experiments have shown that latent syphilis in man is associated with a spirocheticidal property in the serum. This specificity is manifested at a time when the individual is outwardly free from the disease and appears to be well. When the infection is still active or is of recent origin, the protective element is not present in the serum. Substantially the same results are obtained with experimentally infected rabbits.

These observations would tend to confirm the belief that a true immunity does obtain at some time in the course of syphilitic infection. A state of latency would imply a balance that has been struck between the antibodies in an infected person and the invading spirochetes. The presence of *Spirochaeta pallida* in latent syphilitic persons⁹ is additional evidence in support of this idea. During that stage the spirochetes are innocuous for the host.

Immunity, in any sense, need not imply a condition which is incompatible with the life of a parasite. Resistance on the part of the host may be nicely balanced against that of the organism which continues to exist in certain surroundings without giving rise to manifestations characteristic of the disease. There are striking analogies in trypanosome infections, spirillary diseases and the carrier state in well-known diseases, such as typhoid and malaria.

The cyclic changes in syphilis are associated with reactions which point to a progressively increasing immunity on the part of the tissues. In the tertiary stage there is evidence of immunity in the paucity of *Spirochaeta pallida* in gummatous lesions and in the manifestations which conform to characteristic sensitization phenomena. A counterpart may be found also in tuberculosis and glanders. The mechanism by which immunity in syphilis develops would seem to be an elaboration of antibodies which is progressive and commences at the time when initial lesions are present. This begins as a local immunity reaction which extends from one group of tissues to another with a corresponding elaboration of specific substances into the blood. There is considerable evidence to support this view in some recent work¹¹ which has shown that a definite effect is exerted by one group of tissues on another in the production of clinical types of syphilis in the rabbit. The inhibitory effect of one local lesion on the development of lesions elsewhere has been mentioned by Nichols⁷ and more recently by Brown and Pearce.⁸

Failure to reinoculate with syphilis has been taken to mean existing disease rather than a state of immunity. This view, which has gained credence through Neisser and his school, can be interpreted from a different angle by supplementing the older facts with newer ones. The presence of *Spirochaetae pallidae* in organs and tissues of the body appears to be coexistent with immunity to reinfection in exactly the same sense as this occurs in piroplasmoses and trypanosome diseases. The immunity in these infections persists as long as the parasites are present, and the serum in some degree is known to have protective antibodies. In a similar manner, the presence in the human or animal body of *Spirochaetae pallidae* possessing full virulence when inoculated into new soil need not signify disease, but rather a latent stage in which the spirochetes are able to survive in the immunized body. The reaction which takes place is a reversible one, and it is likely that failure to reinfect with syphilis means simply the entry of spirochetes into surroundings which favor lodgment of organisms without the setting up of visible lesions or manifestations of a definite kind.

The old conception of immunity in syphilis was based on meager if not erroneous knowledge as to the mechanism of infection, the

dissemination of the spirochetes, and what was meant by generalization of the disease. More recent experimental study along these lines (Eberson,⁹ Brown and Pearce¹⁰) has given a different cast to immunity phenomena. Early dissemination of *Spirochaeta pallida* makes possible an early absorption of antibodies, until with the lapse of sufficient time, these accumulated products are discharged into the blood stream from different parts of the body where the spirochetes may have lodged. The blood immunity, which appears to prevail when syphilis is unequivocally latent, is a progressive development of an extensive tissue immunity which has been produced gradually in the infected person.

SUMMARY AND CONCLUSIONS

The blood serum from persons having latent syphilis was found to have spirocheticidal properties. Rabbits were protected uniformly against infection with virulent *Spirochaeta pallida* in combination with such serums.

Protective properties were found in the serums of asymptomatic persons with latent syphilis with the following histories:

Infection with syphilis dating back from three to twenty-five years.

Patients who had received treatment until the Wassermann reaction had become negative.

A number of patients who had no history of infection, who had taken no treatment, and who had a slightly positive Wassermann reaction usually in the cholesterin antigen.

A group of patients in whom the Wassermann reaction was slightly positive in the cholesterin and noncholesterin antigens, or strongly positive in either one, in inverse relationship.

An infant whose mother's serum was found to contain spirocheticidal properties.

Spirocheticidal activity of serums in latent syphilis is of such a character as to prevent the normal dissemination of *Spirochaeta pallida* from a primary focus. Failure to inoculate rabbits with mixtures of serums and spirochetes was correlated with negative inoculations with the blood from such animals.

In the experimental animal, spirochetolytic serum may be developed in the course of six months to one year after the infection. In the rabbit, as in man, protective substances are found at a time when the infection has attained a relatively latent state.

The presence of these substances in given serums apparently depends on the stage of infection. When definite latency has been established, the serum appears to protect against experimental inoculation, whereas the serum from cases of early syphilis or those in which true latency has not been attained is not spirocheticidal.

Spirocheticidal activity is essentially a function of time and depends on the degree to which the individual has elaborated and distributed the slowly accumulating antibodies.

Serums which were developed in rabbits by strains of *Spirochaeta pallida* from latent sources manifested a wider range of protective properties, as shown by the inhibitory effect on heterologous as well as homologous strains. Serums from latent cases behaved similarly. Chancre strains when used for experimental infection were not capable of developing spirocheticidal serums for heterologous organisms, in the few experiments which were attempted.

A negative Wassermann reaction following antisyphilitic treatment may or may not go hand in hand with spirocheticidal activity of serums. Continued treatment which renders a Wassermann reaction negative does not appear to nullify any existing protective property of the given serum.

By analogy with trypanosome and spirillary diseases and the carrier state of certain well-known infections, syphilis offers immunity phenomena which tend to explain latency on the basis of a blood immunity which is developed progressively from tissue immunity.

The mechanism by which immunity in syphilis develops would seem to be an elaboration of antibodies commencing at the time when initial lesions are present and continuing as a progressive extension of local immunity from one group of tissues to another until the immune substances are absorbed by the blood stream. Latent *Spirochaeta pallida* thus become innocuous for the host.

The presence of *Spirochaeta pallida* at certain times in the human or animal body need not imply disease but rather a latent stage in which the spirochetes are able to survive in the immunized body. Failure to reinfect with syphilis means, from this point of view, the entry of spirochetes into surroundings which favor lodgment without setting up of visible lesions or manifestations. Immunity need not imply a condition which is incompatible with the life of a parasite. Latency, then, connotes a balance that has been struck in the individual between the antibodies and the invading parasites.

The results of the experiments reported in this paper suggest that the serum from definitely established latent cases of syphilis may prove of therapeutic value.³¹

31. In addition to the references given, the following may be of interest:

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