THE TREPONEMA PALLIDUM

OBSERVATIONS ON ITS OCCURRENCE AND DEMONSTRATION IN SYPHILITIC LESIONS*

BENJAMIN WHITE AND OSWALD T. AVERY
BROOKLYN, N. Y.

The discovery by Schaudinn and Hoffmann[1] in 1905 of a spirillum in various luetic lesions marked the beginning of a decided advance in our knowledge of the etiology of syphilis. The announcement of the discovery, while most conservative in its assertions, gave a fresh impetus to the study of the many phases presented in the parasitology and pathology of this disease. A wide-spread interest was awakened and many investigators directed their attention to the problems in this new field of research. From the results of many of their observations we have already gained a new conception of the cause, course and treatment of lues.

The organism found by Schaudinn and Hoffmann was first described by them under the name of *Spirochæta pallida,* but a more intimate study of its morphology led them to forsake its classification under the spirochætæ and to rename it the *Treponema pallidum.* Much careful study has been given to the biologic nature of this organism, its demonstration and its behavior toward staining reagents. No less attention has been directed to observations on its occurrence in specific and non-specific lesions, and to the effect produced by both local and general treatment on its presence in such lesions. The evidence thus far available seems to show that this spirillum differs in its characteristics from all other known spirilla and thus probably constitutes a new species. Its absence from non-specific lesions and its almost constant presence in specific lesions are being demonstrated repeatedly, while its general disappearance from these lesions under local and systemic treatment is an observation reported from many sides. These facts adduced from such evidence would seem to establish this organism as the actual infecting agent in syphilis. Owing

*From the Department of Bacteriology, Hoagland Laboratory, Brooklyn.

1. Schaudinn and Hoffmann: Vorläufiger Bericht über das Vorkommen von Spirochæten in syphilitischen Krankheitsprodukten und bei Papillomen. Arb. a. d. k. Gsndhtsamte, 1905, xxii, 527.——Ueber *Spirochæta pallida* bei Syphilis und die Unterschiede dieser Form gegenüber anderen Arten dieser Gattung. Berlklin. Wchnschr., 1905, No. 42, p. 673.

to the fact, however, that the Treponema fulfils only one of the conditions of Koch's law—since, so far, all attempts to cultivate it have proved futile—we can make no definite statement concerning the specificity of its rôle.

Although this further confirmation is lacking, yet in the clinics and among the private practitioners in Europe, and to a small but increasing extent in this country, the presence of the Treponema in a suspected lesion is held as establishing the specific nature of the infection, and therefore its demonstration in such cases is considered as a valuable diagnostic aid. With the older methods of observation, on the other hand, often a delay of several weeks was necessary, depending on the appearance of secondary symptoms, before a definite diagnosis could be made. An early diagnosis is now asserted to be of distinct advantage in the treatment of this disease.

I.—THE DEMONSTRATION OF THE TREPONEMA

The inaccessibility of much of the literature, combined with the more or less unsatisfactory results obtained with the intricate and time-consuming staining methods, may account for the lack of initiative on the part of many of the medical men of this country to avail themselves of this aid to diagnosis. It is therefore the purpose of this paper to give a critical review of the various methods recommended for staining the Treponema, to describe a simple and reliable procedure for its demonstration, and, further, to report a series of observations made in about one hundred cases, both syphilitic and non-syphilitic in character.

As a diagnostic measure, the recovery and demonstration of the *Treponema pallidum* from primary lesions is probably of greatest importance, and in this instance the attempt usually meets with a greater degree of success than is the case when later lesions are examined. Its presence in some of the secondary lesions is frequently less constant and its demonstration presents greater difficulties. Reports of positive findings in the pustules and ulcers of the tertiary stage are comparatively few in number. The recovery of the spirillum from the circulating blood in primary and secondary syphilis has been reported, but the nature of the technic renders any description unnecessary here.

The untreated chancres, the condylomata, the mucous patches and moist papules offer the most promising field for investigation, but the precise nature of the material to be obtained from these lesions is of primary importance. Many failures to demonstrate the Treponema are traceable to the faulty manner of preparing the specimen rather than

to the method of staining employed. Some authors advise that a small incision be made in the lesion with a lance or a Hagedorn needle, and the resulting drop of blood spread in a thin film on a cover glass or slide. This method is not always feasible or satisfactory. The method used in the present research is simple in execution and produces uniform and dependable results. Since this step constitutes so important a factor in making a successful preparation its detailed description may be justified here:

The lesion is washed when necessary, then thoroughly cleansed by wiping with gauze. The juncture of the necrotic with the sound tissue, and also a part of the floor of the sore, are then curetted with a small curette (the chalazion eye curette answers the purpose admirably), or scraped with a scalpel until the detritus and superficial tissue are removed and a slight flow of blood is produced. The lesion is then sponged with dry gauze until the blood has ceased to flow and clear serum is seen to ooze. A drop of this serum is then spread on a perfectly clean glass slide in the thinnest possible uniform film. The preparation, after being allowed to dry in the air, may be fixed either by carefully passing through the flame three times, by immersion in ethyl or methyl alcohol, by osmic acid or in the osmium tube of Hamm.

The inability of early investigators to demonstrate the existence of the Treponema in syphilitic lesions may be ascribed in part to the inadequacy of the optical appliances then available and to the difficulties presented in impregnating its cell substance with any of the usual staining reagents. The perfecting of apparatus for the production of dark-ground illumination has made it possible to render these poorly refracting cell bodies visible, and to enable one to observe them in a living condition. This method of direct examination is by far the most satisfactory, not only for making a rapid diagnosis of suspected material, but for an intimate study of the morphology and manner of reproduction of these organisms. The employment of this method is, however, limited by the nature of the apparatus required.[2]

The simplest, and for general purposes, the most satisfactory method of demonstrating the Treponema is, therefore, by means of some appropriate staining procedure. Such a vast array of staining methods has been advocated that a search for one which involves no elaborate technic, which is rapid in execution and which gives satisfactory preparations leads only to bewilderment. A great majority of these

2. Recently this method has been described in detail by Harris and Corbus: The clinical value of the spirochæta pallida in the diagnosis and treatment of syphilis. Jour. Am. Med. Assn., 1908, li, 1928.

methods, when attempted by one not skilled in laboratory manipulations, yield discouraging results, and this means of diagnosis is then abandoned or intrusted to those who have given more or less study to the technic involved.

The difficulty in demonstrating the syphilis organism by any of the usual laboratory methods is largely due to the fact that the protoplasm of the Treponema exhibits only a slight affinity for the majority of the anilin stains. Schaudinn and Hoffmann[1] were the first to succeed in producing stained preparations of these spirilla. They found that in smears, fixed with osmic acid and allowed to remain immersed in Giemsa solution for twenty-four hours, the Treponema acquired a delicate rose color, while other spirilla which might be confused with the Treponema were more intensely stained. Since the publication of this method, the Giemsa solution, particularly the preparation of Grübler, has been widely employed for this purpose. Many modifications in its use have been advocated, each being supported by claims as possessing distinct advantages over the original procedure. For the most part these variations yield no more satisfactory results than the method of Schaudinn and Hoffmann. One modification, however, that described by Schereschewsky,[3] is so simple in detail and produces such excellent results that it has been almost exclusively employed in the present investigation. This method will be fully described in another part of this paper.

When it was learned that a demonstrable micro-organism was present in the lesions of syphilis many bacteriologists resorted to the common staining reagents in the hope of discovering a method which would render the use of the more delicate eosin and azure solutions unnecessary. Methylene blue, either in watery or alcoholic solution or prepared according to the method of Loeffler, has been employed by Borrel and Burnet,[4] Weitlaner[5] and others who have found that it renders the Treponema visible and imparts to it a distinct blue color. Others, among them, Bandi and Simonelli,[6] Ehrlich and Lenartowicz[7]

3. Schereschewsky: Das Verhalten der *Spirochæta pallida* (Schaudinn) bei der Giemsafärburg. Centralbl. f. Bakteriol., 1908, No. 45, p. 91.

4. Borrel and Burnet: Procédé de diagnostic rapide des lésions syphilitiques, Compt. rend. Soc. de biol., 1906, No. 49, 212.

5. Weitlaner: Noch einiges über *Spirochæta pallida*. Klin. therap. Wchnschr., Vienna, 1905, No. 12, p. 1124.

6. Bandi and Simonelli: Ueber die Anwesenheit der *Spirochæta pallida* in sekundär-syphilitischen Manifestationen und über die zu ihrem Nachweis angewendeten Färbungsmethoden. München. med. Wchnschr., 1905, No. 52, 1668.

7. Ehrlich and Lenartowicz: Ueber Färbungen der *Spirochæta pallida* für diagnostische Zwecke. Wien. med. Wchnschr., 1908, No. 58, p. 1018.

have obtained satisfactory results with Ziehl's solution of carbol fuchsin, while Gonder and Hoffmann, Ploeger,[8] Herxheimer,[9] Oppenheimer and Sachs,[10] and Scholtz[11] advocate the use of gentian violet in a solution of anilin water, or better in dilute carbolic acid. The intensity of the stain produced by the various anilins seems to be markedly increased by the use of phenol, either in dilute solution as a solvent for the stain or in combination with other substances as a mordant. Proca and Vasilescu[12] first mordant the preparation with a solution of tannic acid in 5 per cent. carbolic acid and then stain with carbol gentian violet. Flexner[13] has reported favorable results obtained with this method.

Quite different from any of the above methods is that described by Stern.[14] The air-dried film is fixed by heating at a temperature of 37.5 C. for several hours. The slide is then immersed in a colorless glass vessel containing a 10 per cent. watery solution of silver nitrate. The vessel is allowed to stand in diffuse daylight until the film appears brown with a metallic luster. If the action of the light has not been too intense, after thoroughly washing in water, the Treponema should appear evenly stained a deep brown with little precipitate on the slide. Flexner[13] advises that the reduction be allowed to take place slowly in weakly diffuse light, as otherwise the protoplasm appears coarse and the contour is more or less broken and uneven. With this stain the cell body is somewhat swollen and does not show the delicate appearance produced by eosin-azure stains. This has little significance from a diagnostic standpoint, as none of the other characteristics are affected. This method has been repeatedly tried in this laboratory and has given positive results when it was impossible to demonstrate the presence of the Treponema with many of the other stains. The Treponema impregnated by the above method is shown in *b-b* in the accompanying illustration.

---

8. Ploeger: Die Spirochäten bei Syphilis. München. med. Wchnschr., 1905, No. 52, p. 1381.

9. Herxheimer: Zur Kenntnis der *Spirochæta pallida*. München. med. Wchnschr., 1905, No. 52, p. 1861.

10. Oppenheim and Sachs: Eine einfache und schnelle Methode zur deutlichen Darstellung der *Spirochæta pallida*. Deutsch. med. Wchnschr., 1905, No. 31, p. 1156.

11. Scholz: Ueber den Spirochätennachweis bei Syphilis. Deutsch. med. Wchnschr., 1905, No. 31, p. 1467.

12. Proca and Vasilescu: Sur un procédé de coloration rapide du *Spirochæta pallida*. Compt rend. Soc. de biol., 1905, No. 57, p. 1044.

13. Flexner: *Spirochæta* (*Treponema*) *pallida* and syphilis. Jour. Exper. Med., 1907, No. 9, p. 464.

14. Stern: Ueber den Nachweis der *Spirochæta pallida* im Ausstrich mittelst der Silbermethode. Barl. klin. Wchnschr., 1907, No. 44, p. 400.

In this laboratory all of the above mentioned methods, and further those advocated by Goldhorn,[15] Wood, Hastings,[16] and Gradle[17] have been thoroughly tried out on material known to contain the Treponema in large numbers. In addition to the testing of these methods original attempts were also made to stain this organism with certain anilin colors, among them thionin, pyronin, safrinin and the three-solution diptheria stain of Neisser. To summarize briefly the present observations made on the respective merits of these various methods it may be stated that: (a) of the simple anilin colors, fuchsin, methylene blue and gentian violet, when they do not yield entirely negative results, impregnate the protoplasm of the Treponema only to a slight degree; (b) the action of these anilins is intensified by the use of phenol or tannin as mordants, but at the best their employment leads to unsatisfactory preparations; (c) the silver impregnation method of Stern is thoroughly dependable, but the length of time required for its execution constitutes more or less of a disadvantage; (d) the Treponema is stained more intensely by solutions containing eosin and azure; (e) the Giemsa solution as used by Schaudinn and Hoffmann, and in many of its later modifications, may usually be depended on to produce well stained preparations, but with its employment are associated certain unsatisfactory features in the matter of time consumption, etc.

The results obtained by the method as modified by Schereschewsky have proven uniformly satisfactory. This fact, combined with its rapidity of execution and simplicity of technic, has led to its adoption in preference to all others for the purposes of the present investigation. The technic in detail is as follows:

The smears are obtained in the manner already described and after being allowed to dry in the air are carefully passed through the flame three times. The staining mixture is freshly prepared by adding thirteen drops (from a dropping bottle) of Giemsa solution (Grübler) to 10 c.c. of a 0.5 per cent. watery glycerin solution. The mixture is then heated to boiling, immediately poured on the slide and allowed to remain for three to five minutes. The stain is then poured off and the slide washed with neutral distilled water. The slide is dried by rapidly shaking it in the air and a second application is made for the same length of time. As a rule two applications suffice to impart to the

15. Goldhorn: A rapid and certain method of staining *Spirochæta pallida*. Proc. New York Path. Soc., 1905, No. 5, 169.

16. This stain has recently been recommended by Geraghty: Johns Hopkins Hosp. Bull., 1908, XIX, 364.

17. Gradle (H. S.): A clinical stain for the *Spirochæta pallida*. Jour. Am. Med. Assn., 1908, l, 1265.

smear a distinct pinkish tinge. Should the pink be too faint a third application is made. When the desired shade is reached the slide is washed as above and dried by shaking or with fine blotting paper. The preparation is then examined with a one-twelfth oil-immersion lens. In order to obtain ideal results certain precautions must be heeded. All vessels with which the stain comes in contact must be perfectly clean. Before mixing each fresh lot of stain the test tube used should be cleansed by scrubbing with clean cotton and alcohol, then rinsed with distilled water. Any deposit of stain about the neck and lip of the dropping bottle should be removed by carefully wiping with filter paper. The water used in making the glycerin solution and for washing the slide should be neutral, as the least degree of acidity causes the formation of a precipitate. Should any of these precautions be neglected it will be found that a heavy bluish precipitate forms in the mixture on boiling and its staining ability is thus impaired. The exact tint of the smear to be attained is a matter of no great importance and is easily determined. After a few trials in which the importance of the above details is realized one may expect to produce satisfactory preparations.

In the preparations thus stained the Treponema should appear a deep pink with the background pale in comparison. Fixation by heat has a tendency to straighten the convolutions, but this may be easily overcome with the exercise of a little care. The Schereschewsky stain, on account of the glycerin it contains, produces a slight swelling of the cell body. With this stain, however, a sufficiently accurate exposition of the morphologic features of the Treponema is produced. The *Treponema pallidum* is a delicate spirillum varying in length from 4 to 14 microns, and having a breadth which is generally less than 0.25 microns. The ends are somewhat pointed. The convolutions, according to Hoffmann and Halle,[18] have a width of 1 to 1.2 microns, a depth of 1 to 1.5 microns, and are usually 6 to 14 in number, although at times longer forms are seen which exhibit as many as 20 to 24 convolutions. The angularity and the regularity of these windings are markedly characteristic of this particular organism. A careful microscopic examination renders the differentiation of the Treponema from all other spirilla certain. The majority of spirochetes, on account of their coarser form, their irregular and broader convolutions and their greater affinity for stains, can scarcely be confused with the syphilis spirochete. The *Spirochæta refringens,* frequently found in suspicious lesions, is

18. Hoffman and Halle: Ueber eine bessere Darstellungsart der *Spirochæta pallida* im Ausstrich. München med. Wchnschr., 1906, No. 53, p. 1516.

easily distinguished by its greater width and its more wavy form. Should doubt arise concerning the specific nature of the organism under examination the material should be examined in the fresh state best by means of the dark field illumination. When this is impossible the preparation may be fixed rapidly with osmic acid and then stained for several hours—preferably twenty-four—with a mixture containing one drop of the Giemsa solution in 10 c.c. of neutral distilled water. In all of the examinations made in this laboratory no difficulty has been experienced in establishing a diagnosis by means of the Schereschewsky method.

II.—THE OCCURRENCE OF THE TREPONEMA IN SYPHILITIC LESIONS[19]

It seems unnecessary to review here the large number of reported observations on the occurrence of the Treponema in syphilitic lesions. The results of these observations may be briefly summarized thus:

1. The Treponema has been found in the great majority of untreated primary and secondary lesions. Negative findings in such specific lesions are probably attributable to faulty technic.

2. The examination of material from tertiary lesions usually yields negative results. A few positive findings have been reported, however.

3. The Treponema has not been found in non-specific lesions.

4. Both local and general mercurial treatment tend first to make these spirochetes degenerate and later to cause their disappearance from the lesion.

With a view toward gaining further evidence in this direction a series of observations on 101 cases were made extending over a period of one year. A certain routine procedure was established which consisted in obtaining the material from the various lesions directly upon the admission of the patient to the clinic.[20] In many instances subsequent smears were made in order to verify the initial findings and without exception these were substantiated. Before publishing the results of these observations the appearance of the secondary symptoms was awaited—when the examination was made on the primary lesion—so

19. A review of the literature extending through the year 1906 may be found in Kolle-Wassermann's Handbuch der pathogenen Organismen, Supplementary Volume I, 544; also in Mutzer: Arch. f. Dermat. u. Syph., 1906, lxxix.

20. We wish to express here our sincere thanks to Doctors Pedersen, States and other members of the staff of the Genito-Urinary Clinic of the House of Relief, New York City, and to Doctors Morton, Read and others of the Genito-Urinary Clinic of the Polhemus Memorial Clinic, Brooklyn, N. Y., for their many courtesies and hearty cooperation.

that no doubt might exist concerning the specific nature of the case. These results may perhaps be best exhibited in tabular form.

The findings in the cases of untreated primary lesions are confirmatory of the results of others regarding the presence of the Treponema solely in specific lesions. Local treatment with the various preparations of mercury, such as calomel powder, blue ointment and black-

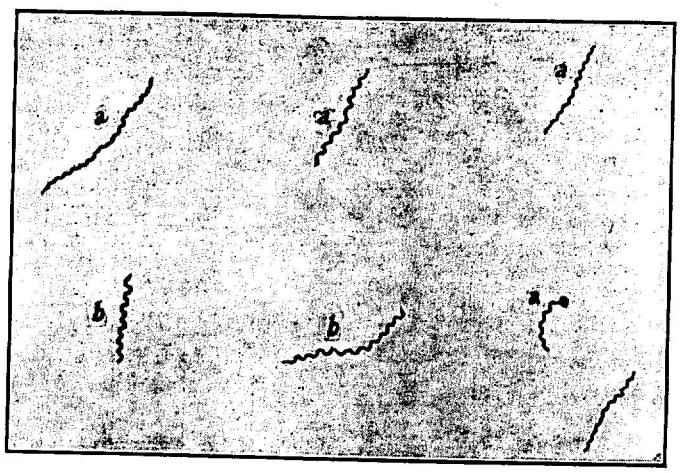
FINDINGS OF THE TREPONEMA PALLIDUM IN SPECIFIC AND NON-SPECIFIC LESIONS*

Nature of Lesion.	Total Cases.	Positive Findings.	Negative Findings.	Per cent. Positive Findings.
Primary lesions, untreated	33	33	0	100
Primary lesions, local treatment	13	7	6	53
Primary lesions, general treatment	1	0	1	00
Secondary lesions, untreated—				
Macular syphiloderms	1	0	1	00
Maculopapular syphiloderms	1	1	0	100
Condylomata	3	3	0	100
Mucous patches	1	1	0	100
Moist papules	3	3	0	100
Secondary lesions, treated—				
Maculopapular syphiloderms	3	1	2	33
Pustular syphiloderms	2	0	2	00
Mucous patches	6	3	3	50
Moist papules	1	1	0	100
Tertiary lesions	3	0	3	00
Non-specific lesions	30	0	30	00

* These results agree closely with the observations of Harris and Corbus (Jour. Am. Med. Assn., 1908, li, 1928), which have appeared since the completion of this paper and also with those of Geraghty (Johns Hopkins Hosp. Bull., 1908, xix, 364).

wash, may cause a disappearance of the Treponema from the superficial tissues, although it undoubtedly persists for a time in the deeper lymphatics of the lesions. Preparations made from mucous patches and moist papules, with the exception of three cases affected by treatment, were found to be unusually rich in spirochetes. Some of the prepara-

tions were taken directly from the surface of the lesion without previous curetting. This substantiates the earlier view of the highly infective nature of these particular lesions. The greater proportion of negative results in the secondary lesions after general treatment as compared to those before this treatment is indicative of the effects of the systemic administration of mercury on the presence of the Treponema. The absence of the Treponema in the cases of tertiary syphilis, while being in agreement with previous findings and with the usually accepted idea concerning the non-infective nature of these lesions, might be explained by some fault in our present method of examination.



Treponema pallidum, under magnification × 1200; *a-a* were stained with the Schereschewsky stain; *b-b* with the Stern silver stain.

From the review of the staining methods above recorded and from the observations made in the bacteriologic examination of syphilitic and non-syphilitic lesions the following conclusions may be reached:

1. The staining method as described by Schereschewsky appears to be the most satisfactory one for general use.

2. The presence of the *Treponema pallidum* in a suspected lesion may undoubtedly be considered as establishing the specific nature of the infection.

3. A negative result does not necessarily exclude the presence of syphilis. In this event further examinations should be made, and, when these lead to negative results a proper period of time should be allowed

to elapse in order that the appearance of the secondary symptoms may establish the diagnosis.

In the clinic of the House of Relief the finding of the Treponema has been considered as affording a positive diagnosis. The treatment when begun immediately on the report of a positive bacteriologic diagnosis has led to most gratifying results.