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CULTIVATION OF TREPONEMA CALLIGYRUM (NEW SPECIES) FROM CONDYLOMATA OF MAN.*

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PLATES 6 AND 7.

While engaged in the cultivation of various species of spirochætæ found in the oral cavity or about the lesions on the external genitals and the anus of human beings, I encountered on two occasions a spirochæta which morphologically occupies an intermediary position between *Treponema pallidum* and *Spirochæta refringens*. The material was taken in one instance from a condyloma latum in which both the pallidum and this organism were present. In another instance, it was found in a condyloma situated around the anus of a boy, nine years of age, in whom the existence of syphilis was finally ruled out by clinical and serological examinations.¹ In this case, the condylomatous tissue was excised and inoculated into the testicles of three rabbits by the usual method, but no lesion resulted during a period of four months. An emulsion of the tissue examined under the dark-field microscope showed some contaminating bacteria, but no other spiral organisms save the one under consideration. In the sections stained by the Levaditi silver method, the spirochætæ were found on the surface, but not within the tissue. No pallidum was detected. The spirochæta in question could hardly be mistaken for *Treponema pallidum* as it appeared decidedly thicker than the latter, although some specimens had quite deep and regular curves. The extremities were obtuse or sharply tapered, and some possessed delicate, finely curved projections at one or both ends. The movements were chiefly those of rotation around the axis, but there was also a change in the form of the curves. Neither

* Received for publication, October 1, 1912.

¹ A medico-legal case.

a vibratory nor lateral movement such as is common with *Treponema macrodentium* occurred. The spirochætæ measured about 0.35 to 0.4 of a micron in width, and ranged from a few microns to twenty microns in length. The number of curves was determined by the length of the organism, and in typical specimens the distance between the curves was usually about 1.6 microns. The curves were not as closely set as in the pallidum. Compared with *Spirochæta refringens* it was somewhat thinner, more regular, and possessed more numerous curves and showed less motility. Stained with Giemsa's solution, it was red with a faint violet tint.

Cultivation.—Emulsions of the condylomatous tissue were used in both cases. The method employed consisted in inoculating a series of tubes containing a mixture of ascitic fluid (one part) and slightly alkaline agar (two parts) containing a piece of fresh sterile tissue at the bottom of each tube. The introduction of the emulsion was made with the aid of a capillary pipette connected with a syringe. After inoculation the tubes were covered with a layer of sterile paraffin oil and placed in a thermostat at 37° C. At the end of ten days, a hazy zone radiating from the central stab canal, which was now densely filled with the growth of bacteria, appeared along the canal up to three centimeters of the surface.

Purification.—After two weeks' growth some of the best tubes were selected and the organisms in the hazy zone were examined under the dark-field microscope. The colonies from the first case (syphilitic) revealed the presence of two distinct varieties of spirochætæ; namely, a thin, regularly and deeply curved organism, and a thick but also regularly curved one. The thin type was undoubtedly the pallidum and the thick one the spirochæta in question. The colonies developing in the tubes inoculated with the material from the second case (non-syphilitic) showed the presence of only one type of spirochæta which was in all respects identical with the form found in the original material.

I was able to separate these organisms from the bacteria.² While colonies of the syphilitic material contained both the pallidum and the second organism in the first generation, the pallidum type finally disappeared in subsequent subculture, thus leaving the thicker type

² Noguchi, H., *Jour. Exper. Med.*, 1912, xv, 90.

in pure condition. The purified culture of the second or non-syphilitic material yielded solely colonies of this organism.

Properties of Pure Culture.—When transplanted into the ascitic agar tissue medium, it develops so far in three or four days as to show hazy, somewhat opalescent colonies about the tissue. During the following several days the colonies gradually spread towards the upper part of the medium, thus rendering the latter unevenly filled with hazy opalescent colonies. At the end of four weeks the growth usually reaches the greatest height that it ever attains,—about two centimeters from the surface. The colonies are diffuse and usually somewhat more dense than those of *Treponema pallidum*. The organism grows well in fluid medium containing ascitic fluid when cultivated by the special method described for *Treponema pallidum*.³ The growth of this spirochæta does not bring about any visible change in the medium and there is no perceptible odor produced. Like *Spirochæta refringens* it is capable of growing in ascitic agar without the addition of fresh sterile tissue, although the growth is far better in the presence of the tissue. The organism is a strict anaerobe. There is no growth at room temperature.

Morphology.—(Figures 1, 2, 6, 12, 13, and 14.) In solid cultures three or four days old the majority of the organisms are short and vigorously motile, while in cultures which have been growing for about two weeks they are longer and less active. In fluid medium long specimens as well as short ones are present from the beginning. The length as well as the width of the organisms is more variable in the fluid cultures.

The spirochæta in pure culture measures about 0.35 to 0.4 of a micron in width and 6 to 14 microns in length. Forms as short as 4 microns or as long as 30 microns, according to the age and nature of cultures, may occur. In old fluid cultures, individuals measuring in width as little as 0.3 or as much as 0.45 of a micron are apt to appear. The curves are very regular and quite deep. The distance between the curves is about 1.6⁴ microns and the depth of a curve is about 1 to 1.5 microns. The apex of curves is not angular, but

³ Noguchi, *Jour. Exper. Med.*, 1912, xvi, 211.

⁴ The wave length of *Treponema pallidum* measures about 1.2 microns and that of *Treponema microdentium* about 1 micron.

more or less rounded. In the short specimens the curves are less constant and often obliterated through movements of the body. While the curves of less motile specimens remain unmodified during movement, those of vigorously active specimens change their form in an undulatory manner. A small number of individuals have shallower curves or become almost straight, especially near the middle portion of the body; but I regard these as the irregularities due to the abnormal effect of artificial cultivation. The body has a uniform width until toward the terminal portion which ends in a sharp point. In this respect it resembles *Spirochæta refringens* in pure culture, and not *Treponema macrodentium*, whose body gradually thins out towards the ends (figure 8).

The spirochæta shows often a delicate, regularly curved, rather stiff projection attached to one or both ends. The length of this appendage varies in different specimens, but usually measures about six microns. The presence or absence of this projection seems to depend greatly upon cultural conditions and the age of the cultures. It has appeared oftenest in fluid cultures about two weeks old.

The varieties of movements are chiefly rotatory, sometimes undulating, and seldom lashing. I have never observed lateral vibration such as is commonly seen in active specimens of *Treponema macrodentium*,⁵ *Spirochæta obermeieri*,⁶ and *Spirochæta gallinarum*,⁷ in pure cultures.

The organism stains reddish violet by Giemsa's solution, faintly red by carbol fuchsin, but not by Gram's method.

Division.—Longitudinal division has been observed, but transverse division may also occur.

Pathogenicity.—Repeated attempts to produce lesions with pure cultures of this organism in the skin of monkeys (*Macacus rhesus*, *Cercopithecus callitrichus*, *Papio babuan*), and in the testicle or cornea of rabbits resulted negatively.

Differentiation from Allied Species.—There is no difficulty in differentiating this organism from *Treponema microdentium*⁸ and

⁵ Noguchi, *Jour. Exper. Med.*, 1912, xv, 81.

⁶ Noguchi, *idem*, 1912, xvi, 199.

⁷ Noguchi, *idem*, 1912, xvi, 620.

⁸ Noguchi, *idem*, 1912, xv, 81.

Treponema mucosum,⁹ as both the latter are much smaller and produce an unpleasant odor in culture (figures 10, 11, and 18.) Differentiation from *Treponema pallidum* in culture offers no difficulty when one is dealing with the average and thinner types of the pallidum,¹⁰ because this organism is altogether too thick to be confused with the average specimens of pallidum. On the other hand, a certain precaution is required when one is dealing with a strain of pallidum belonging to the thicker type.¹¹ In this instance, especially when one lacks means of comparing both species in pure culture under exact and parallel cultural conditions, the two are apt to be confused, for it is not impossible that, by providing one species with one condition and the other with another, the morphological features will approach each other so nearly that considerable resemblance may result. In my cultivation work this difficulty did not arise, because most of my strains of the cultivated pallidum belonged to the average type.¹² Besides, in cases when the heavier pallidum strains were obtained from the testicular lesions of rabbits, there was no possibility of obtaining the above species, and when a dubious strain was isolated directly from human chancres or condylomata, only that which produced characteristic lesions in animals (monkeys) was designated as the pallidum.¹³

Since obtaining two strains of this organism in pure cultures I have compared it with the thicker type of the pallidum under parallel cultural conditions. The study showed that under the same conditions even the thick type of pallidum is still decidedly thinner than the specimens here described. Under unfavorable conditions, especially in fluid medium, the width of the pallidum (thick type) may reach in some specimens that of the thinnest of the spirochætæ in question, but the majority of pallida have a width of about 0.3 of a micron, while in the latter 0.4 to 0.45 is the average. In suitable solid medium, the thick pallidum never exceeds 0.3 of a micron, while the other specimens measure usually 0.35 to 0.4 of a micron, with few exceptions. Moreover, the curves of the pallidum are

⁹ Noguchi, *Jour. Exper. Med.*, 1912, xvi, 194.

¹⁰ Noguchi, *idem*, 1912, xv, 201.

¹¹ Noguchi, *idem*, 1912, xv, 201.

¹² Noguchi, *idem*, 1912, xv, 201.

¹³ Noguchi, *idem*, 1912, xv, 90.

more closely set and more rigid than those of the present organism. Another point of difference between the two species is that in ascitic agar the pallidum never grows without fresh sterile tissue, while the other can grow without tissue.¹⁴

The next species entering into consideration are *Spirochæta balanitidis*¹⁵ and *refringens* and *Treponema macrodentium*. *Spirochæta balanitidis* is described as having a width of 0.5 to 7.5 microns and a length of 10 to 12 microns. Its curves are rather regular and quite deep. Besides, the balanitidis is described as having a band-like body, an undulating membrane, and a periblastic projection. The present species is decidedly thinner, has a cylindrical body, and no undulating membrane. *Spirochæta refringens*¹⁶ is much coarser and has wavy, irregular curves, although in pure cultures the curves may be quite regular in some specimens (figure 9). The appearance of the growth and the cultural conditions required are similar for the refringens and the present species. *Treponema macrodentium*¹⁷ resembles the present organism in length, but differs from it because of slightly less width, tapering body, irregular curves, creeping and lateral vibratory movements (figure 8). When viewed under the dark-field microscope there is no difficulty in distinguishing them. Further, the macrodentium in pure culture never grows without the tissue.

Identification.—In the foregoing pages I have distinguished this organism from *Treponemata pallidum*, *microdentium*, *mucosum*, and *macrodentium*, and from *Spirochæta refringens* by means of morphological and biological properties present in pure cultures. It

¹⁴ Whether or not a strain of pallidum which has suffered a marked morphological and biological deviation from the original culture, such as appears to be the case with the strain I sent to Paris, will grow in a medium without tissue, I cannot say. This strain in my possession still remains unmodified since its isolation from a condyloma latum a little over a year ago. The preservation of morphological and biological characteristics in my hands is undoubtedly due to the more strictly parasitic condition provided in my culture medium, while the deviation of the same strain from the original type in the hands of Levaditi and Danulesco (*Compt. rend. Soc. de biol.*, 1912, lxxiii, 256) can easily be explained by the modification of cultural methods and media adopted by these authors.

¹⁵ Hoffmann, E., and von Prowazek, S., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1906, xli, 741.

¹⁶ Noguchi, *Jour. Exper. Med.*, 1912, xv, 466.

¹⁷ Noguchi, *idem*, 1912, xv, 81.

was further differentiated from *Spirochæta balanitidis* from the description and photographs given by Hoffmann and von Prowazek. The question now arises whether or not the present species corresponds with some other organisms described by previous investigators.

Mulzer in 1905 described a small spirochæta resembling the pallidum occurring in cases of ulcerating carcinoma of the uterus or breast and proposed the name of *Spirochæta pseudopallida*.¹⁸ This organism may have been identical with the variety found in cases of carcinoma by Hoffmann,¹⁹ Krienitz,²⁰ Loewenthal,²¹ Kiolemenoglou and von Cube,²² Scholtz,²³ Loewy,²⁴ and Schmorl.²⁵ Krienitz's organism is called *Spirochæta microgyrata*, while that of Hoffmann is called *Spirochæta gangrænæ carcinomatosæ*. The lack of the descriptions regarding their behavior in the living state and of other biological properties renders it impossible to identify the present organism with any of these. In 1907 Veszprémi²⁶ proposed the name *Spirochæta gracilis* for a small organism found in an ulcerating neoplasm of the jaw which communicated with the buccal cavity. The description of the morphology and the figures of the organism make it probable that the organism described by him belongs to the dentium varieties and is especially allied to *Treponema macrodentium*. At all events, *Spirochæta gracilis* cannot be identified with the present organism. In 1909 Levaditi and Stanesco²⁷ obtained in mixed cultures a spirochæta from syphilitic as well as non-syphilitic lesions and pus occurring on the human genitalia.

¹⁸ Mulzer, P., *Berl. klin. Wchnschr.*, 1905, xlii, 1144.

¹⁹ Hoffmann, E., *Berl. klin. Wchnschr.*, 1905, xlii, 880; *Deutsch. med. Wchnschr.*, 1906, xxxii, 967; *Atlas der ätiologischen und experimentellen Syphilisforschung*, Berlin, 1908.

²⁰ Krienitz, W., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1906, xlii, 43.

²¹ Loewenthal, W., *Berl. klin. Wchnschr.*, 1906, xliii, 283.

²² Kiolemenoglou, B., and von Cube, F., *München. med. Wchnschr.*, 1905, lii, 1275.

²³ Scholtz, W., *Deutsch. med. Wchnschr.*, 1905, xxxi, 1487; 1910, xxxvi, 215.

²⁴ Loewy, K., *Arch. f. Dermat. u. Syph.*, 1906, lxxxii, 107.

²⁵ Schmorl, G., *München. med. Wchnschr.*, 1907, liv, 188; *Deutsch. med. Wchnschr.*, 1907, xxxiii, 876.

²⁶ Veszprémi, D., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1907, xlv, 332, 408, 515, 648.

²⁷ Levaditi, C., and Stanesco, V., *Compt. rend. Soc. de biol.*, 1909, lxxvii, 188.

This organism was described as having a width of 0.33 to 0.5 of a micron, a length of 3.5 to 15.5 microns, as presenting fairly regular curves, and as being more actively motile than the pallidum and staining bluish red with Giemsa's solution. As regards pathogenicity no conclusion could be drawn, as they used mixed cultures to produce an acute inflammation of the prepuce of a chimpanzee, and did not rule out the part played by contaminating bacteria in the process. They ascribe the inflammatory process to their spirochæta, which they call *Spirochæta gracilis*. They did not identify their organism with Veszprémi's, previously so named, or make any reference to his previous work. Thus if Levaditi and Stanesco applied this name to their organism without knowing that it was already used by Veszprémi, and if their organism was not identical with that described by this author, they must seek a new appellation. On the other hand, if they had identified their organism with that of Veszprémi, the species we are describing is clearly different from theirs. Judging from the description and also the source from which Levaditi and Stanesco obtained their mixed cultures, I am, however, inclined to think that their organism was probably identical with that described by me in the present article.²⁸ Even if their organism were the same as mine, it would be impossible to accept the name *Spirochæta gracilis* for this species as, in doing so, it would be erroneously identified with that of Veszprémi. On this account, and since I have obtained the new species in pure culture and studied its properties systematically, I feel justified in proposing as its name *Treponema calligyrum*.²⁹

CONCLUSIONS.

I. On the surface of genital or anal lesions, either syphilitic or non-syphilitic, there may be found occasionally a spirochæta resem-

²⁸ E. Hoffmann, in his latest publication (Finger, E., Handbuch der Geschlechtskrankheiten, Vienna, 1912, 828), remarks that while *Spirochæta balanitidis* and *Spirochæta refringens* are much coarser organisms than *Treponema pallidum*, yet there may be found some specimens which are quite thin and may cause difficulty in being distinguished from the pallidum. He does not state, however, whether the thin forms are to be considered as an independent variety or only atypical forms of the refringens or balanitidis.

²⁹ I take pleasure in acknowledging my indebtedness to Professor Gonzalez Lodge of Columbia University for selecting a suitable adjective.

bling *Treponema pallidum*, but somewhat thicker than the latter. In general characteristics, this organism occupies an intermediary position between *Treponema pallidum* and *Spirochæta refringens*.

2. The treponema was obtained in pure culture from two cases. For the organism the name of *Treponema calligyrum* is proposed.

3. *Treponema calligyrum* is non-pathogenic for monkeys and rabbits.

4. *Treponema calligyrum* can be distinguished from *Treponema pallidum*, *Treponema microdentium*, *Treponema macrodentium*, *Treponema mucosum*, and *Spirochæta refringens* either by the morphology, cultural and biological properties, or all these conditions in combination.

5. Under certain cultural conditions *Treponema calligyrum* becomes thinner and may cause some difficulty of differentiation from a strain of the thick type of *Treponema pallidum*, especially when the latter is grown under conditions which tend to render it thicker. There is no such difficulty when the average and thin types of *Treponema pallidum* are concerned. On the other hand, by providing the calligyrum with conditions which lead to the appearance of thicker specimens and by supplying *Spirochæta refringens* with conditions of growth that favor the development of individuals that are thinner and more regularly curved, the refringens may be made to resemble the calligyrum in general appearance; but under identical cultural conditions they can be readily distinguished.

EXPLANATION OF PLATES.

PLATE 6.

FIGS. 1 and 2. *Treponema calligyrum* in pure culture, ten days old, in solid medium. Giemsa stain. $\times 1,100$.

FIGS. 3 and 4. *Treponema pallidum* in pure culture, four weeks old, in fluid medium. Giemsa stain. $\times 1,100$.

FIG. 5. *Treponema pallidum* from orchitis of the rabbit. Giemsa stain. $\times 1,100$.

PLATE 7.

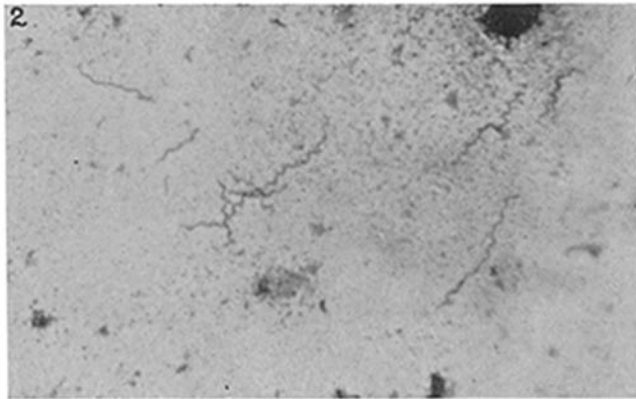
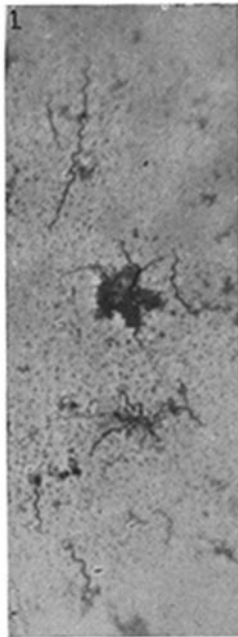
FIGS. 6 to 11. Schematic reproduction of dark-field views of various species of *Treponema* in pure culture. FIG. 6. *Treponema calligyrum*; FIG. 7. *Treponema pallidum*; FIG. 8. *Treponema macrodentium*; FIG. 9. *Spirochæta refringens*; FIG. 10. *Treponema microdentium*; FIG. 11. *Treponema mucosum*.

FIGS. 12 to 14. *Treponema calligyrum* in pure culture, ten days old, in solid medium. Dark-field view. $\times 1,100$.

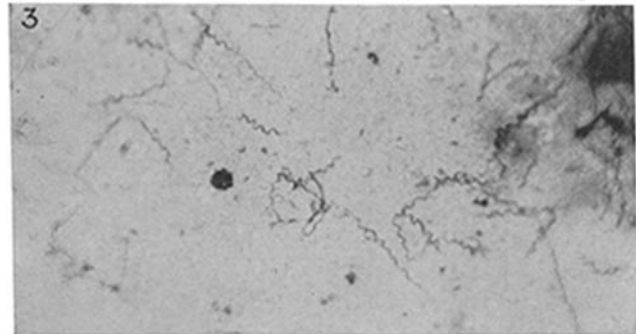
FIGS. 15 to 17. *Treponema pallidum* in pure culture, four weeks old, showing morphological variations among different strains. Dark-field view. $\times 1,100$.

FIG. 18. *Treponema microdentium* in pure culture, ten days old, in solid medium. Dark-field view. $\times 1,100$.

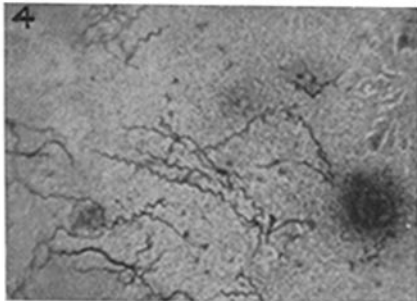
Calligyrum - Pure Culture



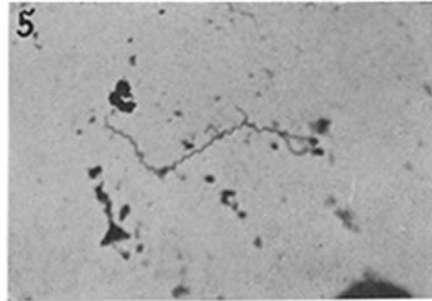
Pallidum - Pure Culture



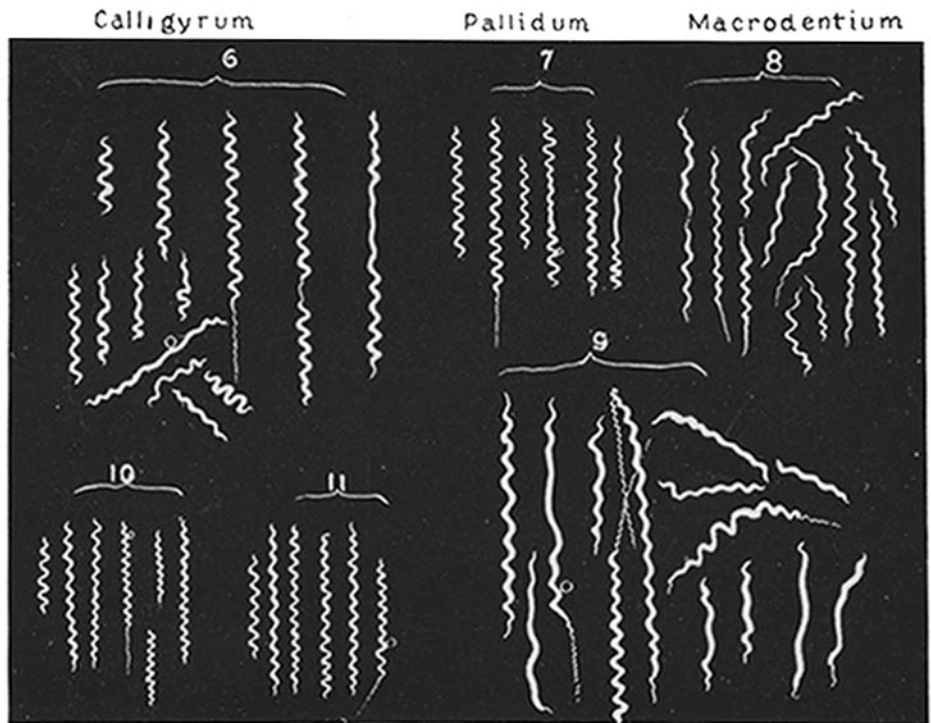
Pallidum-Pure Culture



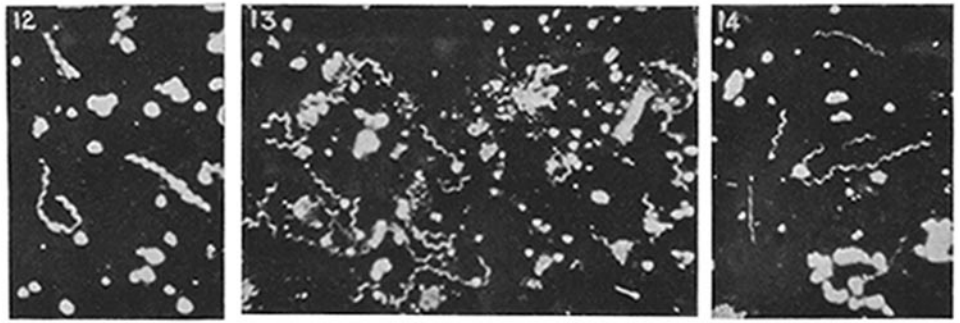
Pallidum in Tissue



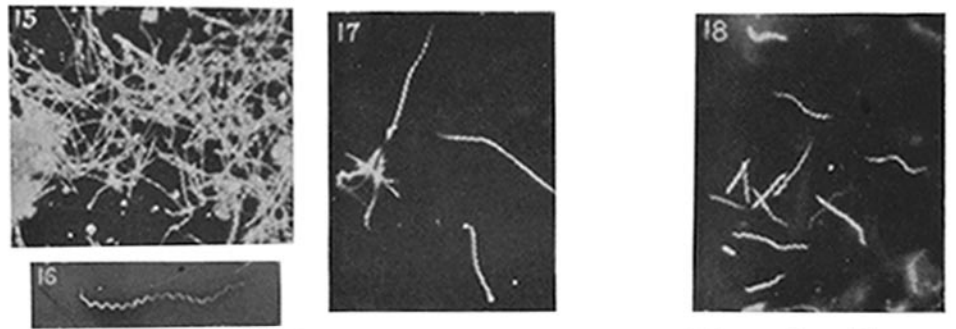
(NOGUCHI: Cultivation of *Treponema calligyrum*.)



Microdentium Microsum Refringens



Calligyrum



Pallidum

Microdentium

(NOGUCHI: Cultivation of *Treponema calligyrum*.)