PURE CULTIVATION OF SPIROCHÆTA PHAGEDENIS (NEW SPECIES), A SPIRAL ORGANISM FOUND IN PHAGEDENIC LESIONS ON HUMAN EXTERNAL GENITALIA.*

By HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 32 AND 33.

In the ulcerative processes situated around the genital region of man one encounters, besides various forms of bacteria, a number of spiral microörganisms belonging to the genus Spirochæta or Treponema. Thus Schaudinn and Hoffmann[1] described *Spirochæta refringens;* von Prowazek and Hoffmann,[2] *Spirochæta balanitidis;* Róna,[3] *Spirochæta gangrænosa nosocomialis;* Corbus and Harris,[4] forms resembling Vincent's spirillum; Mulzer,[5] *Spirochæta pseudopallida;* and Polland,[6] a large spirochæta with five or six blunt curves.

While *Spirochæta refringens* is now known to be non-pathogenic, the etiological relation of *Spirochæta balanitidis* to balanitis erosiva circinata or that of *Spirochæta gangrænosa nosocomialis* to ulcus gangrænosum genitalicum has not been definitely established. Several difficulties must be overcome in order to settle this question.

First of all, it is not easy by morphology alone satisfactorily to identify a spiral organism found in certain lesions with the organisms previously described by these authorities. For example, there

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[1] Schaudinn, F., and Hoffmann, E., *Arb. a. d. k. Gsndhtsamte,* 1905, xxii, 527; *Berl. klin. Wchnschr.,* 1905, xlii, 673.

[2] Hoffmann, E., and von Prowazek, S., *Centralbl. f. Bakteriol., Orig.,* 1906, xli, 741, 817.

[3] Róna, S., *Verhandl. d. deutsch. dermat. Gesellsch.,* 1907, ix, 471.

[4] Corbus, B. C., and Harris, F. G., *Jour. Am. Med. Assn.,* 1909, lii, 1474.

[5] Mulzer, P., *Berl. klin. Wchnschr.,* 1905, xlii, 1144.

[6] Polland, R., *Wien. klin. Wchnschr.,* 1905, xviii, 1236.

are certain investigators[7] who fail to recognize any essential morphological differences between *Spirochæta refringens* and *Spirochæta balanitidis,* while others[8] hold that there are at least four distinct varieties which were indiscriminately described under the name of *Spirochæta refringens.* From this it is easily seen how difficult it is to establish the etiological relation of any one of those organisms to a given definite pathological condition with which it is associated.

The second difficulty is that up to the present we have been unable to isolate these organisms in pure culture and have, therefore, been unable to determine their pathogenicity. The same lack of clearness surrounds the organism of Róna and those of the others. It appears that only a systematic cultural study of all these organisms can clear up the situation and it is for this reason that I describe in this article a spiral organism that has been isolated and grown in pure culture from a rather slowly progressing phagedenic lesion on the external genitalia of a woman.

Material and Cultivation.—The entire left labium showed an enormous swelling with induration, redness, and slight edema. The surface was moist with a seropurulent discharge from the vagina. In the center of the indurated region was an ulcer two by four centimeters and this was covered along its thickened edge with whitish serofibrinous matter. When touched, the ulcer was painful and it bled readily. The lesion had been present for about ten days and was increasing slowly in area. Under local anesthesia a portion of the ulcerated tissue was removed and used for the cultivation experiments.

The tissue thus obtained was rinsed thoroughly in sodium citrate saline solution and then ground in a sterile mortar by adding a fresh lot of citrate solution. In the resulting emulsion, the dark-field microscope revealed numerous irregularly wavy organisms which had a motility of their own that was slow but unmistakable. The length of the spirochætæ varied between four and thirty

[7] Rille, *München. med. Wchnschr.,* 1905, lii, 1377; Kraus, A., *Arch. f. Dermat. u. Syph.,* 1906, lxxx, 255.

[8] Eitner, E., *München. med. Wchnschr.,* 1907, liv, 770; Richards, G. M. O., and Hunt, L., *Lancet,* 1906, i, 667.

microns, but was usually about fifteen microns; the width was about 0.75 of a micron. The number of waves varied greatly, some having as many as eight and others only one or two, and some were almost straight. The wave length also varied greatly in different spirochætæ. Very frequently the organism took the form of the interrogation point. The ends were definitely pointed although not sharply drawn out. The short spirochætæ bent their bodies very slowly almost to a semicircle and then stretched out suddenly with a whipping motion. Some forms moved the terminal portion of the body to the left and right very much as an earth worm moves its head. The organisms were stained by Giemsa's stain, but did not retain the Gram stain.

The cultivation experiments with the material described above were carried out by means of a special medium and procedure described by me[9] for obtaining pure cultures of *Treponema pallidum, Treponema mucosum,* and *Spirochæta refringens* directly from contaminated human materials. Briefly stated, a number of ascitic agar tissue tubes were inoculated with the emulsion by means of a sterile capillary pipette. After inoculation the tubes were covered with sterile paraffin oil and incubated at 37° C. After a few days the stab canals of these tubes were filled up with the dense whitish colonies of the bacteria (chiefly staphylococci), but the rest of the agar column remained perfectly clear. At the end of one week some tubes commenced to show a very faint haze at various points along the stab canal, suggesting a colony of the pallidum. The intensity and size of the haze gradually increased, until, after about two weeks, there was an unmistakable growth of some organism. I left the tubes undisturbed for four weeks and then examined them to determine the cause of this hazy appearance. When the hazy portion of the media was taken out with a capillary pipette and examined under the dark-field microscope it showed a mass of rather heavy spiral organisms and a large number of round refractive bodies. Some of the organisms still showed distinct, apparently normal outlines, while others were granular and partly disintegrated. The round bodies were doubtless derived from the breaking up of these partly disintegrated

[9] Noguchi, H., *Jour. Exper. Med.,* 1912, xv, 90, 466.

forms. Transplants were made at once to a series of fresh culture tubes (ascitic agar tissue medium) in order to purify the culture. After several successive transplants a pure culture of the organism was finally obtained, and during the ten months that have elapsed since its purification the culture has passed through twenty subcultures.

Properties of Pure Cultures.—In the ascitic agar tissue medium a faint haze appears around the tissue within forty-eight hours and gradually extends upwards. The density of the growth increases, and within ten days it can be recognized without difficulty (figure 1). The organisms grow at 37° C., but not at 15° C. The strain is still transplantable after having been cultivated for three months at 37° C.

The length of different organisms varies considerably. In young cultures the majority of the spirochætæ measure ten to fifteen microns and show only one or two waves (figures 2, 3, 4, and 5). In old cultures many organisms attain a length of twenty to thirty microns, and the waves are more distinct and numerous (figure 6). The curvature and alternation of the waves are very irregular, and the changes in form are very gradual (figures 7, 8, and 9). The width of the body measures about 0.7 to 0.8 of a micron and is not always uniform throughout the entire length. In a long form there may be seen a nodular swelling and constriction at the middle or at each third of the body. The organisms usually have ends that are fairly pointed, although they may end obtusely. Young forms are frequently straight, but they may bend their bodies into parabolic curves, and then suddenly straighten out again. They also have a sluggish forward movement like that of a creeping worm. The terminal portion of the body is quite flexible and is moved like a feeler. Longer individuals are less motile. There is no flagellum, terminal projection, or undulating membrane. In a very old culture the majority of the organisms become granular and finally disintegrate into small fragments. At the same time there appear a large number of spherical bodies measuring 1.5 microns in diameter. These round bodies are often attached to the ends or the sides of short forms that are still well preserved (figures 10 and 11). When stained with Giemsa's solution these bodies show a small dot

of chromatic material at one pole, but do not take the ordinary bacterial spore stains. Another interesting feature of the organisms is that, under certain undetermined cultural conditions, they produce during disintegration numerous round or ovoid bodies of varying sizes, some as large as three microns in diameter, scattered singly, in pairs, or in clusters (figure 12). At one side of the protoplasmic mass of these bodies a careful examination reveals the presence of a highly refractive spot. In some of the larger forms there are two of these spots. In certain cultures there were transitions between the spherical bodies and those that contain one or two highly refractive spots in their more solid appearing protoplasm. The highly refractive points of the latter take a deep red stain with Giemsa's solution. Forms were also observed which showed more or less irregular refraction in different parts of the spiral body. This was possibly due to cross-bar concentration of the chromatin before the granular segmentation of the organism. In a stained preparation these refractive portions take a deep nuclear stain.

The organism is difficult to stain with most anilin dyes and does not retain the Gram stain. With Giemsa's solution it stains red.

It is a strict anaerobe and requires for its growth the presence of fresh sterile tissue in the ascitic fluid agar (the serum of rabbit or sheep is unsuitable). The protein constituents of the tissue and ascitic fluid are not visibly altered through its growth, but a distinct putrefactive sour odor somewhat resembling butyric acid develops in the culture.

Pathogenicity.—When introduced intradermically into a *Macacus rhesus* monkey and into rabbits an acute inflammation follows within twenty-four hours, but the reaction subsides in about three days without causing an ulcerative process. In the testicles of rabbits it causes a temporary induration which disappears completely within forty-eight hours.

Identification of the Organism.—Since the recent investigations of Gonder,[10] Gross,[11] Schellack,[12] Novy,[13] von Prowazek,[14] Keys-

[10] Gonder, R., *Centralbl. f. Bakteriol., Orig.*, 1909, xlix, 190.
[11] Gross, J., *Mitt. a. d. zoöl. Station z. Napel*, 1910, xx, 41.
[12] Schellack, C., *Arb. a. d. k. Gsndhtsamte*, 1908, xxvii, 364; 1909, xxx, 351.
[13] Novy, F. G., and Knapp, R. E., *Jour. Infect. Dis.*, 1906, iii, 291.
[14] von Prowazek, S., *Centralbl. f. Bakteriol., Orig.*, 1908, xlvi, 229.

selitz,[15] Swellengrebel,[16] and others, the classification of a spiral organism has become considerably more difficult. These authorities have shown that typical spirochætæ may fail to possess all the characteristics formerly regarded as differentiating the spirochætæ from the spirilla; *e. g.,* longitudinal fission, presence of an undulating membrane, absence of flagellum, etc.

In differentiating spirochætæ from spirilla, the importance once attributed to the mode of division has been still further decreased by the discovery of Schmeidlechner,[17] that a group of bacilli multiplies by longitudinal division.

The flexibility of the body was also once considered to be characteristic of spirochætæ, but the organisms of the relapsing fevers (hitherto called spirochætæ or spirilla indiscriminately) seem to have as much flexibility as any of the spirochætæ, and yet on account of the fact that they are supposed to divide transversely, they have been called spirilla by certain authorities (Novy,[18] Fraenkel,[19] Borrel,[20] Levaditi,[21] etc.).

It seems that spirochætæ, and some varieties of spirilla, have certain features that are possessed by the protozoa, and others that are characteristic of the bacteria. They occupy, therefore, an intermediary position between the protozoa and bacteria.

All the various spirochætæ that I have studied have shown features which are more highly differentiated than those seen in bacteria. For example, in most of the spirochætæ we observe during certain periods of their life the secretion of a small round body that stains like chromatin. The organisms often concentrate the chromatin material at one part of the body and then undergo a peculiar segmentation. The granules thus liberated seem to remain alive and at certain periods develop into spiral forms. Again, at

[15] Keysselitz, G., *Arb. a. d. k. Gsndhtsamte,* 1906, xxiii, 566.

[16] Swellengrebel, N. H., *Compt. rend. Soc. de biol.,* 1907, lxii, 213; *Ann. de l'Inst. Pasteur,* 1907, xxi, 448, 562; *Centralbl. f. Bakteriol., Orig.,* 1909, xlix, 529.

[17] Schmeidlechner, K., *Ztschr. f. Geburtsh. u. Gynäk.,* 1905, lvi, 291.

[18] Novy, F. G., and Knapp, R. E., *loc. cit.*

[19] Fraenkel, C., *Berl. klin. Wchnschr.,* 1907, xliv, 681; *Centralbl. f. Bakteriol., Orig.,* 1908, xlvii, 471; *München. med. Wchnschr.,* 1907, liv, 201.

[20] Borrel, A., *Compt. rend. Soc. de biol.,* 1906, lviii, 138.

[21] Levaditi, C., *Ann. de l'Inst. Pasteur,* 1906, xx, 593, 924.

certain stages of their life some of the spirochætæ become banded or dotted with the chromatin substance.

These primitive nuclear phenomena and also the distinct differentiation of the cytoplasm of some species into ectoplasm and entoplasm appear to me sufficiently characteristic to hold these organisms together under the group of spirochætæ without classifying them either with the bacteria or with the protozoa. Upon the basis of the conception just outlined I place my organism among the spirochætæ, but should further investigation make it possible to distinguish between spirochætæ and spirilla, and should it be evident that the organism is wrongly classified I should like to be permitted to correct the error.

Returning now to the question of whether the organism here described has been previously found by other investigators, it appears that the spirochætæ reported by Róna and Polland bear a certain resemblance to my organism and may possibly be identical with it, but I am not justified in concluding that this is the case, and to do so might be misleading; for if my spirochæta is different, I should be giving wrong cultural and biological properties to their organism. In order to avoid such confusion I propose to call my organism *Spirochæta phagedenis,* a conventional name merely indicating its source, but not necessarily showing its etiological relation to the lesion. In the event that this particular organism is found constantly in the phagedenic ulcers on the external genitalia and not in other conditions, its etiological significance would be established. Through lack of material I have up to the present been unable to pursue this subject further.

CONCLUSIONS.

1. A hitherto undescribed spiral organism has been isolated in pure culture from a case of mild phagedenic ulcer on the external genitalia of a woman. For this organism the name *Spirochæta phagedenis* is proposed.

2. *Spirochæta phagedenis* is a strict anaerobe and grows in the presence of fresh tissue in ascitic agar. It produces no apparent change in the media, but a somewhat offensive odor develops in the culture tube.

3. *Spirochæta phagedenis* incites a slight inflammatory reaction in the skin of a *Macacus rhesus* monkey and in the skin and testicles of rabbits.

4. Its etiological relation to the phagedenic lesions on the external genitalia has not yet been determined.

EXPLANATION OF PLATES.

PLATE 32.

FIG. 1. A stab culture of *Spirochæta phagedenis* in ascitic agar tissue medium, three weeks old, at 37° C.

FIGS. 2 and 3. Smears from a pure culture, three weeks old, stained with Giemsa's solution.

PLATE 33.

The dark-field appearance of *Spirochæta phagedenis* from pure cultures.

FIGS. 4 and 5. Young forms with few waves.

FIG. 6. An average form with shallow, irregular curves.

FIGS. 7, 8, and 9. An entangled mass of organisms, with occasional spore-like spherical bodies.

FIGS. 10 and 11. Formation of spherical bodies which are seen still attached to the organisms or already in the free state.

FIG. 12. Plasma granules in an old culture.

FIG. 13. *Spirochæta refringens* from a pure culture (for comparison).