

## PURE CULTIVATION OF SPIROCHÆTA REFRINGENS.\*

By HIDEYO NOGUCHI, M.D.

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

PLATE 69.

In 1905, in the article in which the discovery of *Treponema pallidum* was announced, Schaudinn and Hoffmann described a coarse spirochæta found in superficial lesions on the genitals. This organism was observed not only in syphilitic, but also in non-syphilitic lesions. It is differentiated from the pallidum by its larger dimensions, its plump shape, its comparatively few, irregular curves, and by its readiness to take various anilin dyes. The Giemsa solution stains it violet red. Schaudinn and Hoffmann named this organism *Spirochæta refringens*, and considered it non-pathogenic.

While the organism frequently comes under observation in examining scrapings for the diagnosis of syphilis, yet our exact knowledge of its biology has been fragmentary for the reason that no one had succeeded in isolating it in pure culture. Unquestionably, certain investigators have obtained a growth of this spirochæta in highly impure cultures, but a study based upon such contaminated material aided little in advancing our knowledge.

In my systematic attempt to secure pure cultures of various spirochætæ, I have succeeded in growing this organism in pure culture.

The strain of *Spirochæta refringens* that I cultivated was derived from the scrapings of a moist condyloma around the anus of a female patient. Under the dark-field microscope, the scrapings showed a moderate number of the refringens. The material was ground in a sterile mortar with sodium citrate solution, and the emulsion thus prepared was inoculated into several tubes of solid culture medium consisting of one part of ascitic fluid, two parts of slightly alkaline agar, and a piece of fresh rabbit kidney. The

\* Received for publication, March 4, 1912.

technique employed was the same as that for the cultivation of the pallidum<sup>1</sup> from a human being. After two weeks' incubation at 37° C. an impure culture was obtained. By the same process that was used for the pallidum and for the mouth spirochætæ, the refringens was finally purified.

*The Properties of the Pure Culture (Figures 1 to 5).*—*Spirochæta refringens* grows luxuriantly in the deeper part of a tube of ascitic tissue agar, and forms hazy colonies that gradually extend from the deeper parts of the tube to the upper. It is an anaerobic organism and no growth takes place within two centimeters of the surface of the medium. At 37° C., the growth becomes visible on about the fourth day after the inoculation, and proceeds for some weeks. It does not produce any visible changes in the medium, and the culture is free from any odor. Unlike the pallidum, a growth is obtainable in the medium without the addition of fresh tissue, although, like the microdentium, the growth is poorer when the tissue is absent.

In pure culture, *Spirochæta refringens* measures, on the average, about 0.5 to 0.75 of a micron in width, and about 6 to 24 microns in length. Chains of two, three, and four or more individuals may be seen. They are connected with one another by means of a thin filament. The number of curves varies with different individuals, but, as a rule, the middle part of the body is merely wavy, while the two extremities are more regularly and deeply curved. Not infrequently, however, the majority of the spirochætæ in certain cultures are fairly regularly curved throughout their entire length, the distance between two curves measuring about 2 microns. The ends of the organism are pointed, and to one or both of them is usually attached a fine, long, more or less stiff, but elastic projection, which has regular minute curves.

In a culture growing under optimum conditions, the spirochætæ are very motile. Under these conditions the curved flagella-like projections rotate with extraordinary rapidity around their point of attachment to the spirochætæ. While the flagella-like projection is rotating rapidly, the body of the spirochæta may remain motionless, or may be traversed by waves, convex curves becoming concave, and concave curves becoming convex. The waves begin at

<sup>1</sup> Noguchi, *Jour. Exper. Med.*, 1912, xv, 90.

one end and may pass along the entire body, or may stop after having passed over only a few curves. Whenever motility is observed in the body of the spirochæta, the movements are repeated at frequent intervals. In a dead specimen, the curves are much more irregular and are often almost straightened out.

The body of the refringens was thought by Schaudinn to be ribbon-like, but in pure culture I find it to be cylindrical. In the refringens cultivated by me, there is no undulating membrane.

Regarding the mode of multiplication, I am still unable to decide whether or not a longitudinal division occurs before the spirochætæ are found attached end to end in pairs or in chains of several individuals. The ultimate division of these chain forms is transverse.

*Pathogenicity*.—Several attempts were made to produce lesions in the testicles of rabbits, or in the skin of *Macacus rhesus* monkeys, but thus far without a positive result.

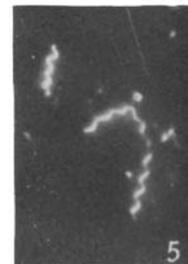
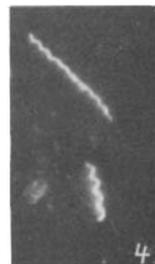
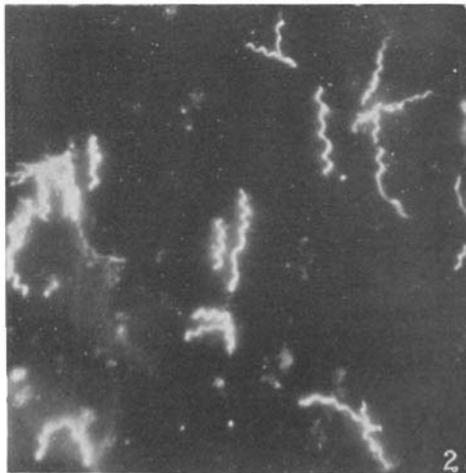
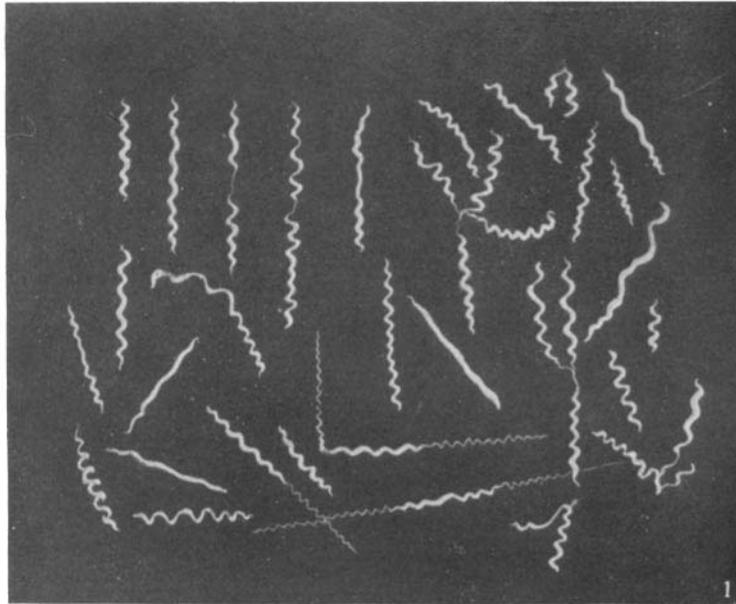
#### GENERAL CONSIDERATION.

That *Spirochæta refringens* constitutes an independent species, and not a form representing merely a certain stage of *Treponema pallidum*, is proved by the present study and by my published observation on different strains of the pallidum in pure culture. Neither the refringens nor the pallidum lose their characteristics at any stage of cultivation. As a consequence, they may always be distinguished even in cultures.

A few investigators in questioning the evidence for the specificity of *Treponema pallidum* for syphilis have argued that, if the refringens is a form of the pallidum, the latter cannot be specific for syphilis, because the former is found in so many non-syphilitic lesions. As the two organisms are now shown to be different, this argument can no longer be employed.

*Spirochæta refringens* belongs, doubtless, to the genus *Treponema*, but, like the other members of this genus, it is entirely distinct from the pallidum.

There are still some important problems that await solution by means of pure cultures. Balanitis erosiva circinata is a clinically well defined disease, and in the lesion a large spirochæta resembling the refringens is usually found. Von Prowazek and Hoffmann



considered this organism to be its causative agent. Mindful of the fact that the refringens has a wide distribution in the genital region, one naturally seeks a means by which the two morphologically allied forms, the refringens and balanitidis, can be definitely differentiated. This is possible only through the growth of both organisms in pure culture. Unfortunately, I have seen but few cases of this type of balanitis and for this reason I have been unable to attempt seriously its cultivation.

From the point of view of cultivation, another problem of interest is the relation between *Spirochæta buccalis* and the refringens. There is at present much controversy in regard to the identity of these spirochætæ. Some consider them identical, while others regard them as independent varieties. My numerous attempts to isolate the buccalis in pure culture have thus far been fruitless. There seems to be no doubt but that the buccalis is much more difficult to grow on artificial medium than the microdentium or macrodentium. In an impure culture of the buccalis, I was much impressed with the morphological similarity that it bears to the refringens, but the question of the identity of these two spirochætæ requires further study.

#### SUMMARY.

A strain of *Spirochæta refringens* has been obtained in pure culture, and its morphological and cultural characteristics have been studied. The strain possessed no pathogenicity for rabbits or monkeys. It probably belongs to the genus *Treponema*, but it is easily differentiated from *Treponema pallidum*, *Treponema microdentium*, and *Treponema macrodentium*. Its relation to *Spirochæta balanitidis* and *Spirochæta buccalis* is still undetermined.

#### EXPLANATION OF PLATE 69.

FIG. 1. A schematic drawing of *Spirochæta refringens* from pure cultures (dark-field).

FIG. 2. *Spirochæta refringens* from a three weeks' old pure culture in ascitic tissue agar, at 37° C. (dark-field).  $\times 1,100$ .

FIG. 3. *Spirochæta refringens* from a pure culture seven days old (dark-field).  $\times 1,100$ .

FIGS. 4 and 5. *Spirochæta refringens* from a pure culture two weeks old (dark-field).  $\times 1,100$ .