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TREPONEMA MUCOSUM (NEW SPECIES), A MUCIN-
PRODUCING SPIROCHÆTA FROM PYORRHEA
ALVEOLARIS, GROWN IN PURE CULTURE.*

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PLATE 18.

Pyorrhœa alveolaris is generally regarded as the sequel to a pathological metabolism known as the uric acid diathesis. Whatever the primary predisposing factors may be, the characteristic fetid odor from the affected mouth betrays the presence of certain putrefactive microorganisms in the perialveolar tissues which surround the loosened roots of the diseased teeth. The bacteriological studies which, on account of the lack of cultural methods for most of the organisms concerned, were necessarily limited to their morphology, helped but little, for the varieties of the microbes therein found do not differ greatly morphologically from those commonly seen in the gingival deposit on unclean teeth of individuals who do not have pyorrhœa alveolaris. Most authorities mention the presence of *Spirochæta buccalis*, *Treponema macrodentium*, *Treponema microdentium*, fusiform bacilli, and, occasionally, a spirillum allied to that of Vincent. But which of these organisms is most responsible for the fetid odor could not be determined for the reason that until very recently neither the spirochætæ nor the other forms were isolated in pure cultures.

The fusiform bacilli and spirilla of Vincent's type have been obtained in pure culture by Ellermann,¹ Weaver and Tunncliff,² Mühlens,³ and others,⁴ who found that these organisms produce the peculiar fetid odor characteristic of pyorrhœa alveolaris.

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¹ Ellermann, V., *Centralbl. f. Bacteriol., Orig.*, 1904, xxxvii, 729.

² Weaver, G. H., and Tunncliff, R., *Jour. Infect. Dis.*, 1905, ii, 446.

³ Mühlens, P., *Deutsch. med. Wchschr.*, 1906, xxxii, 797.

⁴ Tunncliff, R., *Jour. Infect. Dis.*, 1906, iii, 148.

Of the spirochætæ, one medium type, *Treponema macrodentium*, and one small type, *Treponema microdentium*, have been cultivated by me from non-pyorrhæal cases,⁵ but the macrodentium produces no odor and the microdentium produces a putrefactive odor which is somewhat different in nature from that which comes from pyorrhæa alveolaris.

In the present communication I shall describe a small spirochæta which was isolated from the pus derived from a case of pyorrhæa alveolaris. Morphologically the organism resembles greatly *Treponema pallidum* and *Treponema microdentium*, but it differs from both in several principal biological properties. Its most striking feature is its capacity to produce in pure culture a mucin and a strong fetid odor. Since this species has, for the first time, been recognized and isolated from other varieties, I propose for it the name *Treponema mucosum*. At present I shall not discuss the question of how much this particular spirochæta contributes towards the production of the fetid odor in the pyorrhæal discharge, but I shall describe the method of cultivation and its morphology and biology in pure culture.

Method of Cultivation.—The purulent discharge of pyorrhæal teeth is collected by means of a sterile capillary pipette and then suspended in a few cubic centimeters of sterile citrate solution. By means of a capillary pipette this emulsion is inoculated into a number of tubes of solid culture media consisting of one part of ascitic fluid, two parts of ordinary agar, and having a piece of sterile fresh rabbit kidney at the bottom; the whole medium is covered with a layer of sterile paraffin oil. The technique is the same as that used for obtaining the pallidum or refringens from a contaminated chancre or condyloma. After inoculation the tubes are incubated at 37° C. for about ten days. The whitish semi-transparent growth may be seen to spread out from the stab canal which is now filled with a dense growth of various bacteria. The tube is cut open at the middle, the freshly exposed surface of the agar column is carefully sterilized with a sublimate alcohol solution, the moisture carefully wiped off and then the cloudy colony of the spirochætæ is reached by inserting a sterile capillary pipette through

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

the clean surface. A small portion of the colony is thus taken out and transferred to a new medium. By repeating this process of purification one finally obtains a pure culture.

Properties of the Pure Cultures.—In the ascitic agar medium, containing fresh sterile tissue, the colonies become visible within twenty-four to forty-eight hours, and with the exception of the upper 1.5 centimeters the entire agar column is rendered unevenly opalescent. The individual colonies are recognized as dense, whitish turbidities of varying sizes concentrically arranged but having no definite outer boundaries. In a rapidly growing culture the neighboring colonies unite with each other by their gradually thinning peripheries, thus making the whole tube quite opalescent, although the original colonies still stand out more or less distinctly.

No gas is formed. The culture gives off a strong fetid odor that is easily recognized at a distance. After growing for two or three weeks the tissue in the culture becomes at first greyish and then quite dark (similar changes were found in the microdentium culture). The culture now contains a varying amount of mucin. When the media is broken up this is seen as an extremely fine string stretching from one piece of agar to another. The mucin string is not sticky, but draws out as a fine filament. I have seen no bacteria which produce a similar mucin.

The organisms remain alive for several weeks in the same culture at 37° C. The mucin-producing faculty has apparently suffered through repeated transplantations and at present, in its sixteenth generation within 160 days, the culture has almost lost this quality. On the other hand, its capacity to produce a bad odor has in no wise diminished.

Like other treponemata, the mucosum is an anaerobe and requires the presence of serum constituents for its growth. It grows well without the presence of fresh tissue. The fluid medium is made markedly opalescent without forming a definite coagulum. In this fluid medium the growth produces a very strong odor.

Morphologically *Treponema mucosum* resembles both the pallidum and the microdentium, measuring on the average 8 to 12 microns by 0.25 to 0.3 of a micron (figures 1 and 2, *Treponema mucosum*; figures 3 and 4, *Treponema microdentium*; figures 5 and

6, *Treponema pallidum*). The number of curves varies from six to eight. These curves are remarkably regular and are often quite deep. Both extremities are sharply pointed and often possess at one or both ends a fine, minutely curved projection that varies in length in different organisms. The length of this projection may reach eight to ten microns. The organisms show a graceful rotating movement, and are often seen joined to each other by a thin filament forming a pair, or chain of three, four, or more individuals. Under certain cultural conditions forms suggestive of a longitudinal division are observed.

When cultivated under unfavorable conditions a large number of irregular forms appear. Some of these are almost straight and some are slightly or irregularly curved. There are also many granular particles. These particles may be merely degenerative products or they may be segments which under favorable conditions are capable of reproducing the spirochætæ. These segments or granules take the chromatin stain and vary in size. Not infrequently a long spirochæta is found undergoing a granular segmentation (degeneration?), or a small spirochæta is seen attached to a round body as if it had just sprouted out of the latter. In its staining reactions the mucosum behaves as the microdentium does and takes the red in the Giemsa stain.

Pathogenicity.—When a large quantity of active fluid culture is inoculated into the cutaneous tissue of the skin of a *Macacus rhesus* monkey, it produces an acute inflammation and the tissues remain indurated for one week or ten days. There is no tendency to supuration, and after twenty-four hours the tissue does not contain living spirochætæ. In the testicular tissue of rabbits the inoculated organisms produce also a marked induration (acute inflammatory) which may last for one week, but no abscess formation was observed. The testicle returns to its normal appearance in about two weeks.

When a culture in ascitic agar (as an emulsion) is inoculated, the testicle becomes intensely inflamed within twenty-four hours and remains so for nearly ten days. The inflammation then gradually subsides, but the hard circumscribed nodule persists for many weeks. When the testicle is punctured with a capillary pipette a yellowish thick pus flows out. This pus has a fetid odor and in it a small

number of the spirochætæ are still to be seen. By transplanting the pus into a new culture medium a pure growth of this organism can be obtained. From this fact it seems that the mucosum can survive in the testicle of rabbits when there is simultaneously introduced a foreign substance (agar), but not without the aid of the latter; hence it is not an independent parasite.

Differentiation from the Allied Species.—The mucosum differs from the microdentium in producing a mucin, a stronger fetid odor, a denser growth, and in surviving in the rabbit testicle when introduced with agar. Morphologically it is almost impossible to differentiate these two species. The pallidum differs from this organism by its pathogenicity, by producing no odor or mucin, by its fainter diffuse growth, and by requiring for its growth the presence of fresh tissue in the media.

CONCLUSIONS.

1. A mucin-producing spirochæta has been obtained in pure culture from a case of pyorrhæa alveolaris. This organism is an independent species of the genus *Treponema* and, as it is recognized for the first time, I propose for it the name *Treponema mucosum*.
2. Morphologically the mucosum is difficult to separate from the pallidum and microdentium, but, through its biological properties and animal reactions it is easily differentiated from all the rest of the spirochætæ.
3. The mucosum is not parasitic in the strict sense of the term, but exerts a certain pyogenous action when the tissue has been so injured by foreign substances as to enable it to survive.
4. The strong fetid odor in the discharge from pyorrhæa alveolaris is due, at least in part, to the presence of the mucosum in the affected tissue.

EXPLANATION OF PLATE 18.

Dark-field views of the organisms. $\times 1,100$.

FIGS. 1 and 2. *Treponema mucosum* from pure cultures ten days old.

FIGS. 3 and 4. *Treponema microdentium* from pure cultures ten days old.

FIGS. 5 and 6. *Treponema pallidum* from pure cultures two weeks old.

