THE SPIROCHETAL FLORA OF THE NORMAL MALE GENITALIA.

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Plates 30 to 32.

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An accurate knowledge of the varieties of spirochetal organisms which normally inhabit the smegma or the mucous membranes of the urogenital region has become imperative for the establishment of an etiological relationship between a spirochete and a disease in which the organism may be found in the urine.

The classic work of Inada, Ido, Hoki, and others on the presence of Leptospira icterohaemorrhagiae in the urine of convalescents from infectious jaundice has introduced a new procedure by which the disease may be easily diagnosed, and it is natural that a similar procedure should be followed in the search for an etiological agent in other diseases of infectious origin.

Attention has been directed by Martin, Nankivell and Sundell, and Patterson to the urine in cases of trench fevers. In fact, Nankivell and Sundell early demonstrated minute spirochetes in specimens of urine from soldiers suffering from so-called trench fever. Of 26 patients, most of them suffering from a "five-day fever," 99 specimens were examined, with 29 positive findings. Spirochetes were found in 12 out of 15 typical cases, while none of the 8 controls showed a spirochete (Fig. 32). The investigators considered the possibility of contamination of the urine from the smegma or from preputial sources, but it seemed to

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2 Martin, C. J., quoted by Nankivell and Sundell.
them improbable that the spirochetes came from these sources, since the urine usually contained the minute spirochetes unaccompanied by the coarse \textit{Spiro-
ema refringens}, and in case of contamination the presence of the large varie-
ties was to have been expected. Moreover, in some positive instances no spiro-
chetes could be discovered in the smegma. The occurrence of spirochetes in the 
urine was not constant, that is, not detectable on successive occasions, but was recurrent at irregular intervals. A certain relation seemed to exist between the 
appearance of spirochetes in the urine and the height of pyrexia, spirocheturia occurring usually 24 hours after the height of fever. In still other cases they 
appeared on the 14th to the 16th day. They were actively motile, averaged 8.15 \mu in length and 0.3 \mu in width, with an average of five curves; \textit{i.e.}, varying from two and a half curves in 5 \mu to ten curves in 12.5 \mu. The spirals varied in depth. The extremities tapered to sharp points with a flagellum at one or both 
ends. The organism differed from \textit{Treponema pallidum} in its shortness and its fewer spirals.

Patterson, using the Fontana, Wilmaers-Renaux, and India ink methods, ex-
amined specimens of urine from various groups of trench affections, 3 cases of trench nephritis, 1 case of pyelonephritis, with abscess of the lungs, 15 cases of 
relapsing type of pyrexia of unknown origin, 1 case of myalgia following pyrexia 
of unknown origin, and 5 cases of appendicitis (?) not yet diagnosed, finding 
spirochetes with the following features: They were about one to one and a half 
times the diameter of a red blood corpuscle, very thin, with tapering extremities, 
some having five to eight more or less regular curves, some being straight, some 
bowed, or lying in a semicircle (Fig. 33). The spirals were not so fine as those of 
\textit{Treponema pallidum} or so coarse as those of \textit{Spiro-
ema recurrentis}. The organism took Giemsa's, Leishman's, or Romanovsky's stain poorly but were easily demonstrated by the Indian ink method. Patterson depicts the spiro-
chetes found in the abdominal type of cases as rather closely wound, short, thick 
forms, and those found in the relapsing type as much more tightly coiled. Little 
attention was given to control cases.

The main objection to the work of the British investigators has been the pos-
sibility of an accidental contamination of the urine by unclean surroundings. 
Many have insisted upon the necessity of collecting specimens by catheteriza-
tion. The investigation of Stoddard\textsuperscript{6} brought out an unsuspected source of 
spirochetes in the periurethral as well as the intraurethral region of the male 
genitalia. After examining 50 healthy soldiers and 50 miscellaneous hospital 
patients without history or symptoms of relapsing fever (trench fever), Stoddard 
drew the conclusion that (1) spirochetes are not uncommon organisms in the 
urethra of men without history or symptoms of relapsing fever; (2) many dif-

\textsuperscript{5} Wilmaers, L., and Renaux, E., Quarante-sept cas de Spirochétose ictero-

ferent varieties are found; (3) some of the varieties seen are morphologically closely similar to pathogenic varieties; (4) the spirochetes occur so definitely within the urethra that they are an obvious source of contamination in uncatheterized specimens of urine; (5) they are a sufficiently dangerous source of error even in catheterized specimens to deserve attention in careful work; and that finally (6) it is possible that a staining reaction or some other morphological character may be discovered to differentiate microscopically the common and harmless from the pathogenic spirochetes. Of 50 hospital cases 56 per cent showed spirochetes, of which 46 per cent were not \textit{Spirocomma refringens}. Of 50 American soldiers spirochetes in the urethra occurred in 22 per cent, 2 per cent of which showed \textit{Spirocomma refringens} also. Films from periurethral parts contained more of the coarse \textit{refringens} type.

The spirochetes found by Stoddard measured from 3.75 to 22 \(\mu\), more commonly 6.75 to 9 \(\mu\), but 11 \(\mu\) was not uncommon. They were either moderately thick or extremely slender. The ends tapered or were blunt and formed a hook. The spiral length varied from 0.5 to 1 \(\mu\). Flattened and longer spirals also occurred, averaging 1.5 to 3.3 \(\mu\) and as long sometimes as 4 \(\mu\). Stoddard states that a type with about eleven curves in 7.5 \(\mu\) occurs frequently, but I have not been able to verify this finding. In some cases the spirals were exceedingly close and fine and almost impossible to count. They were often irregular, the deep narrow, deep wide, flat narrow, and flat wide types of spirals sometimes occurring simultaneously in the same organism. The middle portion sometimes had looser coils or none at all. In one film many different varieties were often present, including frequently organisms similar to \textit{Leptospira icterohaemorrhagiae}.\footnote{Noguchi, H., \textit{Spirocheta icterohaemorrhagiae} in American wild rats and its relation to the Japanese and European strains. First paper, \textit{J. Exp. Med.}, 1917, xxv, 755.}

Obviously it is not simple to interpret what one sees in the rich spirochetal material as described by Stoddard, who sees in it many different varieties, including the leptospira type. But, as this article is intended to show, a critical analysis of the spirochetal flora reduces the number of varieties to not more than three, or at most four; namely, \textit{Spirocomma refringens}, \textit{Treponema calligyrum}, and \textit{Treponema minutum}, n. \textit{sp.}

Since the time of Schaudinn and Hoffmann a coarse spirochete designated by them \textit{Spirocheta refringens} has been known to inhabit the genital region, but no particular attention has been given to the possibility of the existence of other varieties. It was not until the subject was taken up not only from the morphological but also from the cultural standpoint that some interest came to be attached to these
spirochetes. In the present work a strain of *Spironema refringens* was isolated and its morphological and cultural features studied, thus establishing its entity as a species. Later a strain of spirochete (*Treponema calligyrum*) was obtained from a condyloma, which resembled *Treponema pallidum* on the one hand and *Spironema refringens* on the other, being an intermediary organism in its morphological and cultural characteristics. Subsequent observations have led me to regard this particular species as one of the most common varieties that are found in the flora of the smegma or of the urethral region. In fact, *Treponema calligyrum* is more frequently met with than the better known coarse *Spironema refringens*. There is, in addition to these two varieties, another, much smaller spirochete in the genital flora, which will be described in a subsequent paragraph. These three, the minute, medium, and coarse types, constitute the spirochetal flora which at first glance present such a complex aspect.

The smegma and urethral films from six soldiers who were admitted to the Hospital of The Rockefeller Institute for treatment for pneumonia have been examined. These specimens were examined in fresh condition under the dark-field microscope and also as stained preparations. For staining methods Giemsa’s stain, Fontana’s silver impregnation, and occasionally Benians’ Congo red negative im-

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(a) Fix the air-dried film in Solution 1, which consists of

- Glacial acetic acid .................................................. 8 cc.
- Formalin ............................................................ 20 cc.
- Distilled water ..................................................... 100 cc.

for 1 minute and wash well with water. (b) Mordant with Solution 2, which consists of

- Tannin ............................................................... 5 gm.
- Phenol ............................................................... 1 cc.
- Water ................................................................. 100 cc.

for 1 minute over a gentle flame to the point of steaming, then wash thoroughly in water. (c) Treat in a 0.25 per cent silver nitrate solution to which one drop of ammonia is added to 40 cc. of the solution. The film turns brown in a few minutes. Wash in water and then (d) cover with the mordant and warm it over a flame until it begins to steam. Then wash the film in water and dry.
pression method were employed; also a mordant staining recommended by me for various spirochetes, including *Treponema pallidum*. The method is similar to that advanced by Wilmaers and Renaux, but seems to give a better color value on account of the use of gentian violet instead of fuchsin. The film is fixed in methyl alcohol for 15 minutes, then after being washed in water is covered with a solution of mordant (5 per cent tannin plus 1 per cent phenol) and held over a gentle flame for 1 minute, during which time it begins to steam. It is again washed in running water, covered with a strong aqueous solution of gentian violet to which 1 per cent phenol has been added, and steamed briefly over a flame, then washed well in water, air-dried, and examined. This method gives excellent results also with the *pallidum*. Care must be taken not to make too thick a film.

The number of cases examined was small, but the finding was such that it was sufficient to determine the average flora in male genitalia. The varieties of spirochetes encountered in most of the smegma were the same as those found in one. All contained *Treponema calligyrum* and *Treponema minutum, n. sp.*, and most of them *Spiroinema refringens*, although the latter was absent in some cases.

No spirochetes were found in the films made from the urethral mucosa by means of a platinum loop. Just where the fault in the technique lay I am unable to explain. The finding was uniformly negative also with the specimens of urine from ten soldiers. With the idea that in nephritis cases there might be more possibility of encountering spirochetes in the urine, ten different specimens from acute as well as chronic cases of nephritis were subjected to a careful examination, but with no positive finding as yet.

10 A few drops of a 2 per cent Congo red solution (filtered) are mixed with a drop of the material suspected of containing a spirochete and spread over a clean slide to form a film. The slide after being air-dried is immersed in a jar of absolute alcohol containing 1 per cent hydrochloric acid. In a few minutes the red color of the film turns to a bluish tint. The slide is then removed from the acid alcohol and air-dried.

11 Noguchi, H., Morphological characteristics and nomenclature of *Leptospira (Spirocheta) icterohaemorrhagiae* (Inada and Ido), *J. Exp. Med.*, 1918, xxvii, 575.

12 The specimens used in these tests were obtained through the courtesy of Dr. W. W. Palmer of the Presbyterian Hospital.
are recorded some of the results obtained in the present study. There are at least three different varieties distinguishable in the photomicrographs or under the dark-field microscope, a minute (minutum), a medium (calligryrum), and a large (refringens) type. Their biometric characteristics, as encountered in twenty-five specimens of each type, are given in Table I.

**TABLE I.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Length</th>
<th>Thickness</th>
<th>Spiral amplitude and intervals</th>
<th>Spiral depth</th>
<th>No. of spirals or waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minute type</td>
<td>7-10μ</td>
<td>3-14μ</td>
<td>0.25-0.3μ</td>
<td>0.2-0.5μ</td>
<td>7-10 spirals; vary according to length</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.9-1μ Fairly regular intervals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium type</td>
<td>9-12μ</td>
<td>4-14μ</td>
<td>0.35-0.4μ</td>
<td>0.5-1μ</td>
<td>5-8, varying according to the spiral amplitude; some only 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.75μ. Usually fairly regular; that is, a given amplitude is well maintained in a specimen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large type</td>
<td>12-16μ</td>
<td>7-22μ</td>
<td>0.7μ</td>
<td>0.5-1.5μ</td>
<td>3-5; quite variable; exceptionally 8 in a very long specimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-3μ. Usually more or less regular.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. pallidum</td>
<td>8-14μ</td>
<td>6-18μ</td>
<td>0.25-0.3μ</td>
<td>0.8-1μ</td>
<td>8-14; some 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1μ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some of the dark-field, as well as the ordinary photomicrographs, representing the minute, the medium, and the large types are shown in Figs. 1 to 14. The minute type is decidedly smaller than *Treponema pallidum* and has a larger number of shallower spirals in proportion to its length (Figs. 1 and 5). There are also many short specimens such as are never found among the *pallida*. The medium type has an aspect like that of atypical specimens of the *pallidum*. The spirals are fairly deep but not so deep as those of a typical *pallidum*, while the intervals between them are wider (Figs. 2, 5, 6, 9, and 10). All appear somewhat thicker than the *pallidum* (Fig. 21) when seen under the dark-field microscope. This does not apply to the specimens stained by mordanting techniques (Fontana’s and the writer’s), in which there occurs often an uncontrollable uneven heavy deposit of the dyes, due to various external factors (Figs. 5 to 14, 17, 20, and 22). Among organisms of the medium type are noticed two forms, one with more closely set spirals and the other with wider ones, but this is due to certain temporary conditions and may be made to disappear or reappear by regulation of cultural conditions. For example, there will be more of the wide, flat spiral forms when the medium is more fluid. The large type is much heavier, comparatively short, with few spirals, and constantly changes its curves (Figs. 4 and 8). The spirals of the minute type become readily obliterated after the death of the organism (upper organism in Fig. 8).

When fresh they all exhibit moderately active movements, rotary, lashing, and forward and backward locomotion. The large type is the most energetic and the minute variety the least so. In many of the large type there is a distinct double contour effect upon examination under the dark-field microscope. All are provided with a terminal filament or flagellum at one or both ends.

As has been noted before, not all smegmata contain a spirochete, and the varieties present may all belong to one or two of the three groups. As a rule, however, all three types are present, the medium type usually predominating.
Cultural Characteristics.

By selecting the smegma specimens which were rich in the type desired, a culture of each of the three types described was obtained. The technique employed was similar to that previously used for the cultivation of *Spirocheta refringens* and *Treponema calligyrum*. All require strict anaerobiosis (addition of fresh tissue to the media), the presence of suitable body fluid (ascitic fluid), and an optimal temperature (37°C). The growth in the fluid medium, consisting of ascitic fluid and a piece of fresh rabbit kidney and a layer of paraffin oil, is invisible, while in a solid medium, consisting of 2 parts of the neutral agar and 1 part of ascitic fluid with a piece of the fresh rabbit kidney at the bottom, a faint haze appears to develop near the tissue, gradually extending upward within a fortnight. No discrete, circumscribed, sharp colonies have so far been observed. In this respect all the strains obtained are analogous to the cultures of various anaerobic treponemata and spironemata. None produced a putrefactive or offensive odor, the absence of odor from the culture of the minute type serving to distinguish it from either *Treponema microdenticium* or *Treponema mucosum*. Carbohydrates added to the culture media exert neither a favorable nor a retarding influence upon growth, and no visible alterations of the media result from their presence.

In young fluid cultures, whether of the minute, medium, or large type, the organisms are short and active, but as they grow older (2 weeks) the longer forms, some in chains, and some in tangled masses, predominate, their motility meanwhile being considerably reduced. The spirals are quite regular (Figs. 23 to 31). Very short forms do

12 Noguchi, Pure cultivation of *Spirocheta refringens*, *J. Exp. Med.*, 1912, xv, 466.
14 Noguchi, Cultivation of *Treponema calligyrum* (new species) from condylomata of man, *J. Exp. Med.*, 1913, xvii, 89.
16 Noguchi, Cultural studies on mouth spirochetes, *Treponema microdenticium* and *macrodentium*, *J. Exp. Med.*, 1912, xv, 81.
not appear in the solid media, the organisms appearing to attain average length within a short time. The spirals are remarkably regular in solid media and so deep, in the case of the medium type, as to simulate a *pallidum* (Fig. 29). In older cultures two, three, and four individuals in chains have occasionally been encountered (Fig. 27). Division in all three types is brought about by transverse and perhaps also by longitudinal fission.

**Identification.**

The morphological and cultural characteristics of the large type show it clearly to be a *Spironema refringens*, those of the medium type identify it with *Treponema calligyrum*. The latter type may be the same organism as that described by Levaditi and Stanesco in 1909 as *Spirocheta gracilis*, found in a case of balanitis, but, as pointed out previously, these authors used a name already designating another spironema from an ulcerating jaw, which is very different from the present medium type. The name *Treponema calligyrum* was given to a non-pathogenic spirochete cultivated from the surface of a condyloma, but subsequent studies on the spirochetal flora of the genitalia have convinced me that this type is one of the most commonly met inhabitants of the genital region.

The minute type is not unlike the minute spirochete of the mouth, *Treponema microdentium*, but its cultural characteristics differentiate it from the latter. *Treponema microdentium* produces a peculiar odor, especially when freshly isolated, and in a fluid medium the color of the fresh tissue is made grayish within about 10 days and the fluid somewhat faintly opalescent. The *minutum* produces no odor and remains without any perceptible action upon the culture medium, though in dimension there is a general resemblance.

In order to determine whether these two closely similar organisms are immunologically related to each other, agglutination tests were undertaken in which the action of a *microdentium* antiserum (rabbit) was tested on both types. It was found that the serum caused a marked agglutination of *Treponema microdentium* in 1:500 dilution but only a slight one with two different strains of the *minutum*, even

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in a dilution of 1:20. In this connection it may be mentioned that a *calligyrum* serum (rabbit) gave a copious agglutination with the cultures of the medium type in a 1:200 dilution, but only a slight one with those of the minute type in 1:20. There was a partial reaction, but not marked enough to render the differentiation of the two types difficult.

In all probability the minute smegma spirochete has been repeatedly observed by investigators, but no special attention seems to have been given to its identity. I have been accustomed to pass it over as probably identical with *Treponema microdentium*. Now that this type has been found to constitute an independent group, differentiated by several well defined features, it may well be known under a separate name, *Treponema minutum*.

In the spirochetal flora of male smegma, therefore, only the three forms, *Spironema refringens*, *Treponema calligyrum*, and *Treponema minutum*, were recognized.

**DISCUSSION AND SUMMARY.**

The varieties of spirochetes enumerated and photomicrographed from the male smegma flora represent practically every form hitherto described by Nankivell and Sundell and by Patterson in the specimens of urine from trench fever cases (Figs. 32 and 33). The urethral flora, as studied by Stoddard, seem to contain more varieties, but, except those of his more detailed morphological descriptions, every form observed by him is among those found in the smegma. Stoddard saw certain forms with hooked ends suggestive of the *Leptospira icterohaemorrhagiae* of infectious jaundice, but the resemblance ends with this one feature, and differentiation should always be possible under the dark-field microscope, by means of which the leptospira reveals its highly characteristic minute elementary spirals, presenting the appearance of a chain of dots (Fig. 18). Fig. 19 shows that a very favorable fixation with the osmic acid vapor followed by Giemsa's staining may also bring out the elementary spirals. Of all the spirochetes, none has so closely set spirals as the jaundice leptospira, the distance between two spirals being only 0.5 μ. Various methods, including Fontana's, Benians', the mordant gentian violet stain, or Burri's India ink method, are inadequate
to differentiate the leptospira from other spirochetes (Figs. 12, 14, 15, 16, 17).

Why a positive spirochete finding with the films from the urethra and in the specimens of urine was not obtained, is difficult to explain, except on the grounds of the paucity of specimens examined. At all events, the recent negative results reported by Fiessinger with French soldiers and invalids after cleansing of the urethra and glans seem to be in harmony with my results.

In conclusion it may be stated that *Spironema refringens*, *Treponema calligyrum*, and *Treponema minutum* represent practically all the spirochetal forms observed in the male smegma flora. A leptospira has never been conclusively shown to be present in the specimens of normal urine or smegma. For the satisfactory microscopic demonstration of a leptospira a dark-field illuminator is indispensable.

**EXPLANATION OF PLATES.**

**PLATE 30.**

**Magnification, × 1,000.**

Figs. 1 to 4. Dark-field views of the spirochetes in a male smegma. Fig. 1 represents *Treponema minutum*, Fig. 2 *Treponema calligyrum*, Fig. 3 *Spironema refringens*, and Fig. 4 a *Spironema refringens* (below) and a *Treponema minutum*.

Figs. 5 to 11. Various types of spirochetes in smegma, stained by Fontana's method.

**Fig. 5.** Two specimens of *Treponema minutum*.

**Fig. 6.** A specimen of *Treponema minutum* and two of *Treponema calligyrum*, of varying lengths.

**Fig. 7.** A group of *Treponema calligyrum*, with two specimens of *Treponema minutum*.

**Fig. 8.** A group of *Spironema refringens* from a sample of male smegma.

**Figs. 9 to 11.** *Treponema calligyrum* from two different specimens of male smegma.

Figs. 12 and 13. *Treponema calligyrum* in preparation stained by the mordant gentian violet method. In Fig. 12 there are two specimens without distinct spirals which closely resemble *Leptospira icterohemorrhagiae* in similar stained preparations. Further study by means of a dark-field microscope is necessary to determine whether they are leptospira or *calligyrum*.

**Fig. 14.** A group of *Treponema calligyrum* type from a specimen of male smegma, stained by Giemsa's method. The organisms appear much thinner here than in specimens stained by other methods. A hooked spirochete resembling strongly the leptospira is seen near the left upper corner.

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Figs. 15 to 19. *Leptospira icterohemorrhagiae* under various conditions (for comparison).

Fig. 15. Four specimens of *Leptospira icterohemorrhagiae* stained by the mordant gentian violet method. They appear blunt and curved and without any indication of the minute elementary spirals which are the characteristic feature of this genus. As they appear here they are indistinguishable from the stretched forms of the *calligyrum* type.

Fig. 16. A few leptospires as demonstrated by Benians' Congo red method. Here, too, they do not show their elementary spirals.

Fig. 17. A group of *Leptospira icterohemorrhagiae* from a culture, stained by Fontana's method. They fail to show their elementary spirals by this staining.

Fig. 18. A leptospira viewed under the dark-field microscope, showing its minute elementary spirals.

Fig. 19. A number of *Leptospira icterohemorrhagiae*, fixed with osmic acid vapor and stained by Giemsa's stain, showing the elementary spirals.

Figs. 20 to 22. *Treponema pallidum* under different conditions (for comparison).

Fig. 20. *Treponema pallidum* when stained by the mordant gentian violet method.

Fig. 21. *Treponema pallidum* under the dark-field microscope.

Fig. 22. *Treponema pallidum* as stained by Fontana's silver impregnation method.

Plate 31.

Magnification, × 1,000.

Figs. 23 and 24. Dark-field view of a culture of *Treponema minutum* from a male smegma.

Fig. 25. *Treponema minutum* from a culture. Stained by the mordant gentian violet method.

Fig. 26. Similar specimens stained by Fontana's method.

Fig. 27. Dark-field view of a culture of *Treponema calligyrum* from a male smegma.

Fig. 28. A culture of *Treponema calligyrum* stained by the mordant gentian violet method.

Fig. 29. Similar specimens stained by Fontana's method.

Fig. 30. Dark-field view of a culture of *Spiroplasma refringens* from a male smegma.

Fig. 31. Similar specimens stained by Fontana's method.

Plate 32.

Fig. 32. Photographic reproduction of the photomicrographs of spirochetes in Nankivell and Sundell's article on the spirochetes in the urine in trench fever cases.

Fig. 33. Photographic reproduction of the schematic drawings by Patterson in his article.
(Noguchi: Spirochetal flora of normal male genitalia.)
FIG. 32.
I. Spirochetes of Type 1, abdominal P. U. O.
II. Type 2, relapsing P. U. O.
III. Spirillar form from urethra.

FIG. 33.
(Noguchi: Spirochetal flora of normal male genitalia.)