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MORPHOLOGICAL CHARACTERISTICS AND NOMENCLATURE OF LEPTOSPIRA (SPIROCHÆTA) ICTERO-HÆMORRHAGIÆ (INADA AND IDO).

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PLATES 25 TO 29.

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In a previous communication, the writer reported the presence in American wild rats of a spirochete morphologically and immunologically identical with the *Spirochæta icterohæmorrhagiæ* of Inada and his associates and also with the strain isolated by Stokes from cases of infectious jaundice among British soldiers in Flanders.[1] The European strains, which have now been isolated from cases on the British, French, and Italian fronts, as well as from wild rodents captured not only near the battle-lines but in regions remote from them, are undoubtedly strains of the same organism.[2] Jobling and Eggstein[3] have also found the same spirochete recently among wild rats caught in Tennessee.

Just how, in nature, a rat becomes a carrier of the spirochete is not at once apparent. It is not improbable that the contamination of a foodstuff by the urine of an infected rat may transmit the organism to other rats; or the animal may become infected by feeding upon an infected dead rat, since a rat may be experimentally infected by feeding it with an infected foodstuff or with an infected tissue or

[1] Noguchi, H., *J. Exp. Med.*, 1917, xxv, 755.

[2] Costa, S., and Troisier, J., *Presse méd.*, 1916, lxxx, 526, 565. Courmont, J., and Durand, P., *Bull. et mém. Soc. méd. hôp.*, 1917, xli, series 3, 115. Clément, P., and Fiessinger, N., *Presse méd.*, 1916, lxxx, 598. Garnier, M., *Compt. rend. Soc. biol.*, 1916, lxxix, 928. Manine, Cristau, and Plazy, *Compt. rend. Soc. biol.*, 1917, lxxx, 531. Wilmaers, L., and Renaux, E., *Arch. méd. Belges*, 1917, lxx, 115, 207. Dawson, B., and Hume, W. E., *Quart. J. Med.*, 1916–17, x, 90. Zironi, A., and Capone, G., *Sperimentale*, 1917, lxxi, 298. Ascoli, M., and Perrier, S., *Gazz. osp.*, 1916, xxxvii, 1618. Sisto, P., *Sperimentale*, 1917, lxxi, 361. Siccardi, P. D., and Bompiani, G., *Ann. ig.*, 1917, xxvii, 609. Moreschi, C., *Policlinico, Sez. Prat.*, 1917, xxiv, 265. Sampietro, G., *Ann. ig.*, 1917, xxvii, 23.

[3] Jobling, J. W., and Eggstein, A. A., *J. Am. Med. Assn.*, 1917, lxix, 1787.

organ. Whatever the mode of preservation in nature, *Spirochæta icterohæmorrhagiæ* is a common commensal among rodents.

Morphology.

The morphology of this organism has been the subject of much study by its discoverers and by others, but its distinctive feature does not seem to have been recognized. Inada and his associates described the organism as a spirochete with several irregular waves, the entire body being dotted with alternate bright and shadowy portions.[4] Hübener and Reiter, who described a similar picture, apparently believed that the organism had a series of minute knots, and hence gave it the name *Spirochæta nodosa.*[5]

That these investigators overlooked the true structure must have been due either to the difficulty of observing the organism, even under a powerful dark-field illumination, or to the indistinctness of the minute spirals in a stained preparation. It appears as an almost smooth bodied, wavy organism, not unlike *Spironema refringens* when fixed in methyl alcohol and stained with Giemsa's solution (Figs. 1, 2, and 3). As has been said in a previous paper,[1] the natural features of the organism can be well preserved when it is fixed in osmic vapor and then stained over night with Giemsa's solution. In such a preparation it is stained light purple and is seen to consist of a very tightly and regularly wound cylindrical filament tapering to sharply pointed extremities. The filament usually assumes a graceful hook at one or both ends, while the main portion may be straight or slightly bent (Figs. 4 and 5). The number of spirals (not waves) varies considerably according to the length of the specimen, which may be between 3 to 20, 30, or even 40 μ, but the distance between the apices of two spirals measures about 0.5 μ. For example, a specimen measuring 9 μ would have eighteen spirals. The thickness, or diameter of a cross-section, of the organism is nearly uniform until it approaches the terminal portion, which may be so conveniently designated because of its tapering points and its hooked attitude. The number of spirals in the terminal portion appears to

[4] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[5] Hübener and Reiter, *Deutsch. med. Woch.*, 1916, xlii, 1.

be about six in all specimens, and it is this portion which exhibits the greatest tendency to become bent to a semicircle. Unlike various spironemata or treponemata, the spiral amplitude near the extremities is not noticeably less than that of the middle portion of the organism.

In certain specimens the terminal portions are far less intensely stained than the main portion (Figs. 1, 2, and 3). In the majority of specimens, both terminal portions are bent to the same side (Figs. 5, 6, 13, 16, 17, 19, and 22), but in some they form hooks of opposite direction (Fig. 4), unipolar hooks (Figs. 8, 9, 15, and 20), or are not bent at all (Figs. 11 and 12); and some are contorted (Fig. 18). In the less well preserved specimens the spirals are no longer distinct but appear as somewhat more deeply stained dots (Fig. 21). As has already been pointed out, under a powerful dark-field illumination the organism in rapid rotary motion seems to be surrounded by a halo. This may be only an optical effect, but a similar clear zone has been noticed in the stained preparations of some specimens (Figs. 12, 15, 17, and 23).

The dark-field picture of the organism is such that one may mistake the minute spirals for refractive beads arranged diagonally or somewhat obliquely with respect to the axis of the organism (Figs. 24 and 27 to 33), as originally depicted by Inada and his associates and others. But, as has been stated before, with a favorable and powerful illumination, the real structure can be revealed (Figs. 24, 25, and 34).

Only a few of the photomicrographs represent the characteristically hooked forms (Figs. 26 and 28) as actually seen in active rapid rotary motion in a free space, because it was difficult to photograph the organisms in motion, and as soon as motion ceases many of them lose the typical hooks. The large wavy undulations, however, (not the elementary spirals), as assumed by the organisms when penetrating semifluid medium, are well shown in some of the specimens at rest (Figs. 27 and 29 to 33). The remarkable flexibility of the organism in a semisolid medium is also shown (Figs. 27, 32, and 33). These minute filamental organisms dart through the soft medium with great rapidity, first in one direction and then in another, searching for a loose spot which they can pierce through. When encountering

an impenetrable obstacle they reverse their progression and start anew. A striking sight is thus presented by these little vermicular organisms darting in all directions. A vibratory motion of the free portion of the organism results when it is extricating itself from an entanglement. In an emulsion of infected liver one may encounter a tangle of several actively motile organisms (Figs. 24 and 25), while in a culture several weeks old a mass of hundreds of motile spirochetes may be found (Fig. 35).

The European (Figs. 36 to 57) and Japanese (Figs. 58 to 68) strains have all the morphological features given for the American strain. It might be mentioned here that the elementary spirals in the terminal portion are much smaller in number and less regular in the stained specimens of the European strain, but this may be due to imperfect fixation of the organism, because under the dark-field microscope the spirals are equally close and regular.

Classification.

Characteristics of Different Genera of Spiral Organisms.

In order to determine the systematic position of the organism of infectious jaundice, it may be well to review here the characteristics of various genera of spiral organisms. Through the recent investigations of Gross,[6] Zuelzer,[7] Dobell,[8] Gonder,[9] Swellengrebel,[10] and others, the organism for which Ehrenberg created the term *Spirochæta* in 1838 is now known to be distinct from the majority of so called spirochetes. It consists of a long, highly flexible, central axial filament surrounded by a regularly wound layer of protoplasm, usually of great length (200 to 500 μ), and is free living in fresh or marine water (Fig. 108). Neither a membrane nor a flagellum is present. Multiplication takes place by transverse fission. The organism

[6] Gross, J., *Centr. Bakteriol., 1te Abt., Orig.*, 1912, lxv, 83.

[7] Zuelzer, M., *Arch. Protistenk.*, 1912, xxiv, 1.

[8] Dobell, C., *Proc. Roy. Soc. London, Series B*, 1912, lxxxv, 186.

[9] Gonder, R., Spironemacea (Spirochaeten), in von Prowazek, S., Handbuch der pathogenen Protozoen, Leipsic, Liefg. 6, 1914, 671.

[10] Swellengrebel, N. H., *Ann. Inst. Pasteur*, 1907, xxi, 448; *Compt. rend. Soc. biol.*, 1907, lxii, 213.

creeps along the surface of an object but does not swim. Only four species belonging to this genus have been described. The organism under discussion does not belong to it.

Cristispira and Saprospira.—For a limited variety of coarse, actively motile spiral organisms infesting the crystalline styles of certain mollusca, the genus *Cristispira* was proposed by Gross in 1910.[11] The characteristic features are: the presence of a membranous structure running spirally from one end of the body to the other, assuming the aspect of a crista or ridge; the chambered structure of the body; the absence of a terminal filament; and the existence of a strong, flexible membrane (Figs. 104 to 106). According to Gross, reproduction may be effected by multiple transverse fission or sporulation, though I have failed to confirm the occurrence of sporulation. More than a dozen species have been described, but from personal observations I doubt whether these so called species are sufficiently characteristic to be so distinguished. The type organism was first described by Certes in 1882[12] as found in oysters, and was known as *Spirochæta* or *Trypanosoma balbianii*. Another genus, *Saprospira*, was proposed by Gross in 1912[13] for a few varieties of spiral organisms in mussels which differed from the cristispiræ in not having a crista (Fig. 107). The organism in question, however, belongs to neither of these genera.

Spironema and Treponema.—Next in order is the large group of small parasitic spiral organisms commonly called spirochetes. Among them are the causative agents of syphilis and yaws (Figs. 69 to 72 and 103) and of relapsing fevers in man and animals (Figs. 94 to 100), non-pathogenic parasites in certain rodents, and various saprophytic types on or about the oral, alimentary, or genital mucous membranes (Figs. 73 to 93). Their essential feature is a spiral flexible body with terminal filaments, but no undulating membrane. They seem to multiply by transverse as well as longitudinal fission. The rigidity of the curves differs greatly in different organisms, some becoming almost flat at death or constantly changing the waves by oscillatory undulation, others retaining their regular curves even during motion or after death. The whole group has been called *Spirochætæ* or *Spirilla*, in spite of the

[11] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 41.

[12] Certes, A., *Bull. Soc. zool. franc.*, 1882, vii, 347; 1891, xvi, 95.

[13] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 188.

fact that they have no affinity with the real spirochete or non-flexible spirillum. Gross includes them in the genus *Spironema*, a term introduced by Vuillemin[14] in 1905 to distinguish Schaudinn's organism of syphilis from those with less rigid spirals. Dobell,[8] however, believes that the term *Treponema*, as proposed by Schaudinn[15] himself in 1905 for his organism, should be employed to designate all these minute parasitic varieties. Gonder[9] takes a more conservative stand and upholds the distinction made by Schaudinn between the treponema type and that with less constant curves. For example, Gonder retains the genus *Spironema* for the latter and *Treponema* for the former type. I agree with Gonder in this respect, as the general features are sufficiently distinct to enable one to differentiate the two groups.

Nomenclature of Leptospira (Spirochæta) icterohæmorrhagiæ.

The striking differences between the organism of infectious jaundice and all the other so called spirochetes, or rather spironemata and treponemata, are apparent at a glance. The closely set, regular spirals of the organism of Inada and Ido remain unmodified during its rotary, spinning motions in a free space and when it is piercing a semisolid medium. While in motion in a free space, the whole body appears tightly drawn into a straight line, except for the usual hook formation of one or both terminal portions. When one end is extended and straight and the other semicircularly hooked, the organism usually progresses in the direction of the straight portion and seems to be propelled from the rear by the rotating hook (Figs. 8, 9, 15, and 20). A specimen with both ends hooked remains stationary in spite of its rapid rotary motions (Figs. 13, 16, and 19). By straightening one end or the other alternately, the organism changes its progression from one direction to the opposite one. When the organism penetrates a soft medium, changing direction very rapidly, it seldom shows hooked ends (Figs. 11, 12, 29, 30, 32, and 33). In this sort of movement the body assumes wide wavy undulations such as are seen in an active specimen of *Spironema refringens*. The behavior of the organism in semisolid medium is different from that

[14] Vuillemin, P., *Compt. rend. Acad.*, 1905, cxl, 1567.
[15] Schaudinn, F., *Deutsch. med. Woch.*, 1905, xliii, 1728.

in a free space. The persistence of the minute elementary spirals at all times is a feature which distinguishes this organism from any treponema or spironema. The depth of the spirals does not exceed the diameter of the body, a fact unknown among other so called spirochetes. A diligent search by means of various staining methods, as well as by dark-field illumination applied to cultures of different ages, has not demonstrated a terminal flagellum or peritrichal flagella or membranes. It is well to recall at this point that in old cultures of all the spironemata or treponemata I have isolated I have been able to demonstrate the presence of a terminal filament, even when it was observed with difficulty in uncultivated specimens. This organism, however, as far as we know at present, moves by means of its terminal portions. Moreover, unlike spironemata and treponemata, it withstands the action of 10 per cent saponin. Clearly it does not belong to either of these genera, but must remain in a class by itself until other similar organisms come to our observation. The nearest approach to it in morphological and biological respects is *Spirochæta biflexa*, which was isolated by Wolbach and Binger[16] in 1914 from a filtrate of stagnant water taken from the shore of a fresh water pond near Boston. There is a great similarity between the two organisms. Both are filterable through Berkefeld filters. Wolbach and Binger did not succeed in obtaining a second generation in culture, and no tests of pathogenicity for experimental animals were made.

For the reasons which have been discussed, it seems justifiable to include the type of organism in question under *Leptospira* (λεπτός fine, + σπεῖρα, coil), as has already been proposed.[1]

The genera with their type organisms are presented below. The measurements of each of these representative members and the characteristic features used for identification of the genera are considered. There is little difficulty in distinguishing *Spirochæta*, *Saprospira*, *Cristispira*, and *Leptospira* from one another. But the distinction between *Spironema* and *Treponema* depends chiefly upon the rigidity and regularity of the spirals which are characteristic of the treponemata. Under natural conditions this difference is so marked that there should be no confusion in classification, but under cultural

[16] Wolbach, S. B., and Binger, C. A. L., *J. Med. Research*, 1914, xxx, 23.

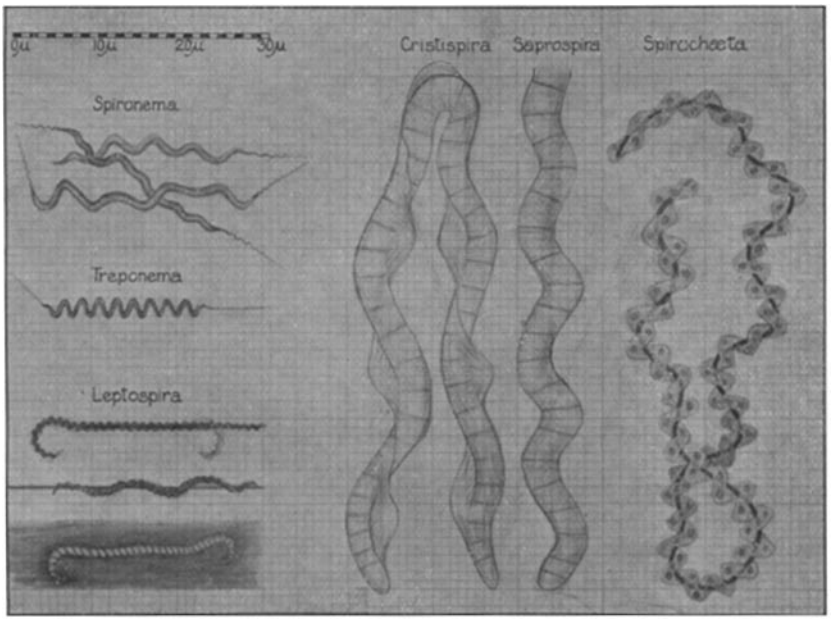
conditions the spirals of the spironemata acquire such rigidity and regularity that they, too, may be called treponemata. Dobell[8] and Gross,[6] independently of each other, and without any knowledge as to the morphological modifications due to cultivation, regarded the distinction between *Treponema* and *Spironema* as insufficient to maintain two separate genera, and Dobell chose the term *Treponema* and Gross *Spironema* for the same group of organisms. In my opinion the characteristics of *Treponema* and *Spironema*, under natural conditions, are sufficiently pronounced to justify retaining the two terms in classification. Neither *Treponema* nor *Spironema* has any feature which is likely to be confused with those of the other four genera referred to above. Text-fig. 1 shows the types mentioned below.

Genus.—Spirochæta (Ehrenberg, 1838). *Type Organism.—Spirochæta plicatilis* (Ehrenberg, 1838) (Fig. 108). *Measurements.*—Length, 100 to 500 μ; blunt end. Diameter, 0.5 to 0.75μ; cylindrical. Spiral amplitude, 2 μ; regular. Spiral depth, 1.5 μ; regular. Waves, several, large, inconstant, irregular. *Axial Filament.*—Distinct in stained specimens; flexible; elastic. *Chambered Structure.*—Absent. *Membrane.*—Absent. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in fresh or marine water. *Other Species.—Plicatilis marina, plicatilis eustrepta, stenostrepta, daxensis. Staining Properties of Axial Filament and Cell Membrane.*—Axial filament consists of chitin or cutin-like substance. Stains violet by Giemsa's solution and gray by iron-hematoxylin. *Staining Properties of Body.*—Plasmic spirals of the body stain with eosin, rubin, etc. Contain volutin granules. *Trypsin Digestion.*—Axial filament resistant. *Bile Salts (10 Per Cent).*—Becomes shadowy pale but is not dissolved. *Saponin (10 Per Cent).*—Lives 30 minutes. Later becomes shadowy, but is not dissolved.

Genus.—Saprospira (Gross, 1911). *Type Organism.—Saprospira grandis* (Gross, 1911). *Measurements.*—Length, 100 to 120 μ; obtuse end. Diameter, ?μ; cylindrical. Waves, large, inconstant, shallow, irregular, 3 to 5 in number. Sometimes almost straight. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.*—Distinct, flexible, elastic. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in foraminiferous sand. *Other Species.—Nana.*

Genus.—Cristispira (Gross, 1910). *Type Organism.—Cristispira balbianii* (Certes, 1882) (Figs. 104 and 105). *Measurements.*—Length, 45 to 90 μ; obtuse end. In stained preparations the end may be sharply pointed, but this is due

to shrinkage by fixing reagents. Diameter, 1 to 1.5 μ; cylindrical. Waves, 2 to 5, sometimes more, large, irregular, shallow. In a dying specimen the waves may be more numerous and regular. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.*—Distinct, flexible, elastic. *Crista.*—Present, a ridge-like membrane. Spirally wound body. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Parasitic in the alimentary canals of shell-fish. *Other Species.*—*Ostræ, anodontæ, modiolæ, veneris, tapetos, chamæ,* etc. *Staining Prop-*



TEXT-FIG. 1. Diagram contrasting the characteristic features and relative proportions of *Spironema, Treponema, Cristispira, Saprospira, Spirochæta,* and *Leptospira.* The scale in microns is given in the upper left-hand corner of the figure.

erties of Axial Filament and Cell Membrane.—Membrane behaves like chitin or cutin substance. Stains violet by Giemsa's solution and light gray by iron-hematoxylin. *Staining Properties of Body.*—The body is alternately stained red and bluish violet and the crista red by Giemsa's solution. Iron-hematoxylin brings out sharp septa and a layer of chromatin granules. *Trypsin Digestion.*—Membrane resistant. Crista and chambers disappear. *Bile Salts (10 Per Cent).*—Crista quickly destroyed. Body not attacked. *Saponin (10 Per Cent).*—Crista becomes fibrillar, then indistinct. Body not affected.

Genus.—Spironema (Vuillemin, 1905). *Type Organism.—Spironema recurrentis* (Lebert, 1874[17]) (Figs. 94 to 96). *Measurements.*—Length, 8 to 16 μ; pointed ends. Diameter, 0.35 to 0.5 μ; cylindrical or slightly flattened. Spirals, large, wavy, inconstant, about five in number. Closer and more regular in cultures. *Axial Filament.*—Probably present. *Chambered Structure.*—Absent. *Membrane.*—Delicate, flexible, double contoured. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present, easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse, possibly also longitudinal. *Habitat of Genus.*—Numerous pathogenic and non-pathogenic varieties. *Other Species.—Carteri, kochi, novyi, duttoni, berbera, aegyptica, gallinarum, anserina, theileri, equi, muris, eugyratum, microgyratum, buccalis, refringens,* etc. *Staining Properties of Axial Filament and Cell Membrane.*—Membrane difficult to differentiate. *Staining Properties of Body.*—Stains violet by Giemsa's solution. *Bile Salts (10 Per Cent).*—Disintegration complete. *Saponin (10 Per Cent).*—Immobilized in 30 minutes then broken up in a few hours. In some there is an axial filament laid bare.

Genus.—Treponema (Schaudinn, 1905). *Type Organism.—Treponema pallidum* (Schaudinn and Hoffmann, 1905[18]) (Figs. 69 to 72 and 103). *Measurements.*—Length, 6 to 14 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 1 μ; regular, rigid. Spiral depth, 0.8 to 1 μ; very constant. Waves, one or more slight undulating curves may be present. *Axial Filament.*—Doubtful. The whole seems to consist of a spirally wound axial filament. *Chambered Structure.*—Absent. *Membrane.*—Doubtful; if there is one it must be flexible. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present. Easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse or possibly also longitudinal. *Habitat of Genus.*—Two pathogenic and several harmless parasites. *Other Species.—Pertenue, microdentium, macrodentium, mucosum, calligyrum, minutum. Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains pink by Giemsa's solution. *Trypsin Digestion.*—Resists digestion for many days. *Bile Salts (10 Per Cent).*—Disintegration complete. *Saponin (10 Per Cent).*—Broken up in time.

Genus.—Leptospira (Noguchi, 1917). *Type Organism.—Leptospira ictero hæmorrhagiæ* (Inada and Ido, 1914) (Figs. 1 to 68, 101, and 102). *Measurements.*—Length, 7 to 9 to 14 μ; exceptionally 30 to 40 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 0.45 to 0.5 μ; regular, rigid. Spiral depth, 0.3 μ; regular. Waves, one or more gentle wavy curves throughout the entire length. When in a free space, one or both ends may be semicircularly hooked, while in semisolid media the organism appears serpentine, waved, or bent. Its flexibility is most striking. *Axial Filament.*—Not recognized. *Chambered Struc-*

[17] Lebert, H., Rückfallstyphus, Flecktyphus und Cholera, in von Ziemssen, H., Handbuch der speciellen Pathologie und Therapie, Leipsic, 1874, ii, 267.

[18] Schaudinn, F., and Hoffmann, E., *Arb. k. Gsndhtsamte.*, 1905, xxii, 527.

ture.—Absent. *Membrane.*—Not recognized. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Not recognized. *Flagella.*—Absent. *Highly Motile End Portion.*—Well developed in the last six to eight spirals. *Division.*—Transverse. *Habitat of Genus.*—One pathogenic and one possibly non-pathogenic variety known. *Other Species.—Biflexa* (Wolbach and Binger). *Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains reddish violet by Giemsa's solution. *Bile Salts (10 Per Cent).*—Easily dissolved. *Saponin (10 Per Cent).*—Completely resistant.

The comparative dimensions of these representative organisms may be shown by putting side by side the diameter, spiral amplitude, spiral depth, and length of each, taking the diameter of the finest member, *Leptospira icterohæmorrhagiæ*, as a unit of comparison (Table I).

TABLE I.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1.2 (0.3 μ)	56 (14 μ)
Treponema pallidum.....	1.2 (0.3 μ)	4 (1 μ)	3.6 (0.9 μ)	48 (12 μ)
Spironema obermeieri.....	2 (0.5 μ)	12 (3 μ)	6 (1.5 μ)	32 (8 μ)
Cristispira balbianii.....	5 (1.2 μ)	60 (15 μ)	24 (6 μ)	200 (50 μ)
Saprospira grandis.....	5 (1.2 μ)	32 (8 μ)	8 (2 μ)	400 (100 μ)
Spirochæta plicatilis.....	3 (0.75 μ)	18 (4.5 μ)	6 (1.5 μ)	600 (150 μ)

One may obtain the comparative proportions for each genus by using the diameter of its representative member as a unit of comparison, as in Table II.

TABLE II.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Spirochæta plicatilis.....	1 (0.75 μ)	6 (4.5 μ)	2 (1.5 μ)	200 (150 μ)
Saprospira grandis.....	1 (1.2 μ)	7 (8 μ)	1.8 (2 μ)	83 (100 μ)
Cristispira balbianii.....	1 (1.2 μ)	13 (15 μ)	5 (6 μ)	41 (50 μ)
Spironema obermeieri.....	1 (0.5 μ)	6 (3 μ)	3 (1.5 μ)	16 (8 μ)
Treponema pallidum.....	1 (0.3 μ)	3.3 (1 μ)	3 (0.9 μ)	40 (12 μ)
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1 (0.25 μ)	56 (14 μ)

The proportions are distinctive for each genus, and form, with other differentiating features already discussed, a fairly well established basis for the classification of these spiral organisms, hitherto so indis-

criminately called by the general name of spirochetes. It would be desirable, in describing a new spiral organism, to place it in one of the six classes discussed, since under the vague name of spirochete no one can visualize the actual features of the organism in question, while if it is called *Leptospira*, for example, certain definite features are connoted, and confusion with other so called spirochetes is avoided. This is particularly important when one is examining specimens of urine such as those from certain cases in which a *Leptospira* or a *Treponema* may be present alone or together, as in a study of trench infections. Patterson[19] and Nankivell and Sundell[20] discovered the latter type in cases of trench fever of unknown origin, while the former has been found responsible for a number of cases of various trench affections.[21, 22]

A brief note may be made of the relation of *Leptospira* to a comparatively minute species of spirochete, *Spirochæta stenostrepta*, described by Zuelzer[7] (Figs. 109 and 110). The organism was found in stagnant water with *Spirochæta plicatilis*. It has a diameter of 0.25 μ and a length of 20 to 60 μ, seldom reaching a length of 200 μ. In a short specimen which measured 13 μ there were eleven spirals. In life an axial filament was recognized. Here the leptospira can be distinguished by its lack of an axial filament and its closer spirals. In the latter respect certain oscillatorial organisms such as *Spirulina vesicolor* (Figs. 111 and 112), or *Spirulina tenuissima* have a superficial resemblance to leptospira, but their multicellular structure, which can be demonstrated by subjecting them to a preliminary treatment with trypsin solution before staining, shows them to be very different. Each coil here represents an individual cell separated from the adjoining cells by walls. The spirulina has blunt ends and does not exhibit the active, brusque movements characteristic of leptospira.

[19] Patterson, S. W., *J. Roy. Army Med. Corps*, 1917, xxix, 503.
[20] Nankivell, A. T., and Sundell, C. E., *Lancet*, 1917, ii, 672, 836.
[21] Couvy, L., and Dujarric, R., *Compt. rend. Soc. biol.*, 1918, lxxxi, 22.
[22] Dudgeon, L. S., *Lancet*, 1917, ii, 823.

SUMMARY.

The present study deals with the morphology and systematic position of the causative agent of infectious jaundice. There are several features which are not found in any of the hitherto known genera of Spirochætoidea which led me to give this organism an independent generic name, *Leptospira*, denoting the peculiar minute elementary spirals running throughout the body. The absence of a definite terminal flagellum or any flagella, and the remarkable flexibility of the terminal or caudal portion of the organism are other distinguishing features. Unlike all other so called spirochetes the present organism resists the destructive action of 10 per cent saponin.

A detailed comparative study of related genera, including *Spirochæta*, *Saprospira*, *Cristispira*, *Spironema*, and *Treponema*, has been given with the view of bringing out more strongly the contrast between them and the new genus.

A study has been made to discover whether any differential features exist among the strains of *Leptospira icterohæmorrhagiæ* derived from the American, Japanese, and European sources, but none has been found.

It is hoped that the creation of a new genus may facilitate a more exact morphological description than has hitherto been possible, due to the vague use of the term *Spirochæta* which indiscriminately covered at least six large genera of spiral organisms.

EXPLANATION OF PLATES.

PLATE 25.

Figs. 1 to 23 show the morphological features of the American strain of *Leptospira icterohæmorrhagiæ* in stained preparations.

FIG. 1. *Leptospira icterohæmorrhagiæ* in the blood of an experimentally infected guinea pig, showing irregular refringent waves, but no minute elementary spirals. Methyl alcohol fixation and Giemsa's solution. × 1,000.

FIG. 2. The same in a liver emulsion from a similar animal. Except for the few moderate undulations of the body, there is no indication here that these are spiral organisms. Methyl alcohol fixation and Giemsa's solution. × 1,000.

FIG. 3. The same in a kidney emulsion. Fixation and staining the same as above. × 1,000.

FIG. 4. The same in a blood specimen of an infected guinea pig. Fixation and staining the same as above. × 1,000.

Figs. 1 to 4 are intended to show the appearance of the leptospiræ in an air-dried specimen, fixed with methyl alcohol, and stained with Giemsa's solution. They do not show any elementary spirals and appear as smooth, somewhat wavy filaments.

FIGS. 5 to 11. *Leptospira icterohæmorrhagiæ* in stained preparations from a culture in its first generation on the 5th day. They were fixed when moist by osmic acid vapor for 2 minutes, then hardened in absolute alcohol for 30 minutes, and after being thoroughly washed in distilled water, were stained over night with Giemsa's solution (1:20 dilution). In these preparations there were many instances where the fixation and staining were not so satisfactory as in the specimens shown in these photomicrographs. A careful examination makes possible recognition of the closely set, minute, regular spirals throughout the entire length of the organism. With a magnification of 1,000 they are almost too minute to enable one to count the number of the spirals. × 1,000.

FIGS. 12 to 23. *Leptospira icterohæmorrhagiæ* magnified 3,000 times, which brings out the features more distinctly. All except Figs. 21 to 23 show the elementary spirals well. There are ten to twelve spirals to every 5 μ, making the distance between the apex of one spiral to that of the next about 0.5 μ. The terminal portions of the organisms are recognized by the gradually decreasing diameter and the coloration, which is lighter than that of the main portion of the body. These end portions seem to possess about six elementary spirals and measure about 3μ in length. They exhibit remarkable activity and flexibility and serve as propellers in progression in free space and as feelers in guiding the organism through a semisolid medium. Note Fig. 12.

Fig. 18 shows a specimen fixed probably during a somersault movement. The elementary spirals appear as dimly stained cross bars (imperfect fixation).

Fig. 21 shows three organisms attaching themselves to a red corpuscle. The spirals are not distinctly brought out, but one recognizes them as more intensely stained dots, arranged obliquely with respect to the optical axis of the organism.

Fig. 22 (also Figs. 13 and 19) shows a specimen fixed while rotating on its axis in a free space. The organism was otherwise stationary, as shown by its symmetrically bent hooks. Compare with Fig. 20, which has one hook, and therefore must be proceeding in the direction of the straight end.

The two specimens in Fig. 23 show no definite direction of progression. The spirals, though not well fixed, are fixed sufficiently for recognition.

In Figs. 12, 15, 17, 21, and 23, there is a clear space, or halo (about 0.15 μ wide) about the organisms along the entire length. Whether this clear zone, or halo, indicates the presence of a less chromatic membrane enveloping the organisms or is merely due to the dispersion of particles (culture media) from their immediate neighborhood by their rotary movements cannot yet be determined.

PLATE 26.

FIGS. 24 to 35. Specimens of the American strain of *Leptospira icterohæmorrhagiæ* as seen under the dark-field microscope.

Fig. 24. The organisms in a liver emulsion of an experimentally infected guinea pig. They are in resting position and show no characteristic hooked ends. One isolated leptospira has both ends hooked, but not typically, as it would be while actively rotating or progressing in a free space. The spirals appear as regularly set cross bands. × 1,000.

Figs. 25 and 26. A higher magnification of the same specimens. The finely set regular spirals are distinctly shown at the right in Fig. 24, and the cross-barred or dotted aspect of the spirals is shown in the other two of the same figure and also in Fig. 26. × 3,000.

Fig. 27 (× 1,000) and Figs. 28, 29, and 30 (× 3,000) show the leptospiræ in the kidney emulsion of an infected guinea pig. Except for the specimen at the center of Fig. 27, the organisms are in undulatory positions, with gracefully wound, rather loose waves. This position almost always indicates that the organisms are in a semisolid medium, which they are penetrating by means of spiral propulsion. They often remain in the same position for some time before renewing their efforts to extricate themselves. Their dotted or cross-barred appearance remains unmodified under these circumstances.

Fig. 31 (× 1,000) and Fig. 32 (× 3,000) show similar but more pronounced characteristic features.

Fig. 34. The minute elementary spirals are plainly seen in the three entangled leptospiræ in the right upper corner, while in three organisms of Fig. 33 they are recognizable only as dots or bars. × 3,000.

Fig. 35. A large mass of leptospiræ in a fluid culture 3 weeks old. They grow considerably longer in such a medium and form a mass of entangled organisms having the same minute elementary spirals as uncultivated specimens. × 1,000.

PLATE 27.

Figs. 36 to 57 represent the British strain (Stokes) of *Leptospira icterohæmorrhagiæ*.

FIG. 36. A Fontana preparation of the leptospiræ in the liver emulsion of an infected guinea pig. The elementary spirals can hardly be distinguished. × 1,000.

FIG. 37. A badly fixed osmic acid-Giemsa preparation, in which one of the organisms on the extreme right appears as a negative image with minute elementary spirals well brought out. The dye settled about the leptospira without staining the organism itself. × 1,000.

FIGS. 38 to 43. A preparation better fixed with osmic acid vapor and well stained with Giemsa's solution. The leptospiræ were cultivated 7 days at 28°C. In none of them is there any difficulty in discerning the individual elementary

spirals throughout the entire length of the organism. Perhaps owing to imperfect fixation, the elementary spirals in the terminal portions are less numerous and the spiral depth is shallower than in the main portion, which also takes on a more intense stain. In the majority of specimens the spiral amplitude of the main portion is about the same as that of the American or the Japanese strain (0.5 μ). There are a few specimens, however, which measure 0.6 μ from one spiral to the next. × 1,000.

FIGS. 44 to 52. The same. × 3,000.

FIGS. 53 to 57. Dark-field views of the leptospira. Figs. 53, 56, and 57 are from a fluid medium, and Figs. 54 and 55 from a semisolid medium. × 1,000.

PLATE 28.

FIGS. 58 to 68. Dark-field views of the Japanese strain of *Leptospira icterohæmorrhagiæ* from a 7 day culture on semisolid medium. Figs. 58 to 62 are magnified 1,000 times and Figs. 63 to 68, 3,000 times. These photographs show the remarkable flexibility of the tight, elementary spirals of the organisms. The numerous circularly coiled specimens suggest the peculiar hoop-like coiling form of some specimens of *Cristispira balbianii* in the crystalline styles of oysters.

FIGS. 69 to 72. Dark-field views of *Treponema pallidum* which are given here for comparison with the leptospiræ. Their larger spiral amplitude and spiral depth, and their rigidity are sufficiently differentiating. Figs. 69 and 71 are magnified 1,000 times, and Figs. 70 and 72, 3,000 times.

PLATE 29.

Some of these photomicrographs are from stained and some from dark-field preparations. They are reproduced here to illustrate the differential characteristics of several constituent genera of the family of Spirochætoidea (Dobell). × 1,000.

Treponema Group.

FIGS. 73 to 76. Dark-field views of a minute treponema (*Treponema minutum, n. sp.*) found in a smegma. Their average spiral amplitude is 0.9 to 1 μ, spiral depth, 0.2 to 0.5 μ, average number of spirals, eight to ten in 7 to 9 μ, and thickness, 0.3 μ.

FIGS. 80 to 83. *Treponema calligyrum* in smegma. Spiral amplitude, 1.75 μ, depth, 0.5 to 1 μ, four to seven spirals in 7 to 12 μ, thickness, 0.4 to 0.5 μ.

FIG. 84. The same from a culture.

FIG. 85. *Treponema microdentium* from the mouth.

FIG. 86. The same from a culture.

FIG. 88. *Treponema macrodentium* from a culture.

FIGS. 92 and 93. A treponema from the urine of a child, resembling the smallest smegma treponema (Figs. 73 to 76).

Spironema Group.

FIGS. 77 and 78. *Spironema refringens* from smegma. Spiral amplitude, 2 to 2.75 μ; spiral depth, 0.5 to 1.5 μ; four to eight spirals in 11 to 16 μ; thickness, 0.7 μ.

FIG. 79. The same from a culture.

FIG. 87. *Spironema vincenti* from the mouth.

FIGS. 89 to 91. *Spironema buccalis* from the mouth. Spiral amplitude, 2.75 to 3.7 μ; spiral depth, 0.7 to 1 μ; four to seven and one-half spirals in 11 to 17 μ; thickness, 0.5 to 1 μ.

FIGS. 94 and 96. *Spironema recurrentis* in a culture.

FIG. 95. The same in the blood of an infected mouse.

FIG. 97. *Spironema duttoni* in a culture.

FIG. 98. *Spironema kochi* in a culture.

FIG. 99. *Spironema gallinarum* in a culture.

FIG. 100. *Spironema novyi* in the blood of an infected rat.

Leptospira Group.

FIG. 101. *Leptospira icterohæmorrhagiæ*, American strain. $\times$ 1,000.

FIG. 102. *Leptospira icterohæmorrhagiæ*, Japanese strain. $\times$ 1,000.

FIG. 103. *Treponema pallidum* for comparison. Same magnification.

Cristispira Group.

FIG. 104. Dark-field view of *Cristispira balbianii* from oysters obtained near Woods Hole.

FIG. 105. The same. Osmic acid fixation. Stained with Giemsa's solution.

FIG. 106. *Cristispira veneris* (?) from clams obtained near Long Island Sound. Sublimate alcohol fixation and Heidenhain's iron-hematoxylin.

Saprospira Group.

FIG. 107. An organism possibly belonging to this genus. It was cultivated by me from oysters obtained near New York.

Spirochæta Group.

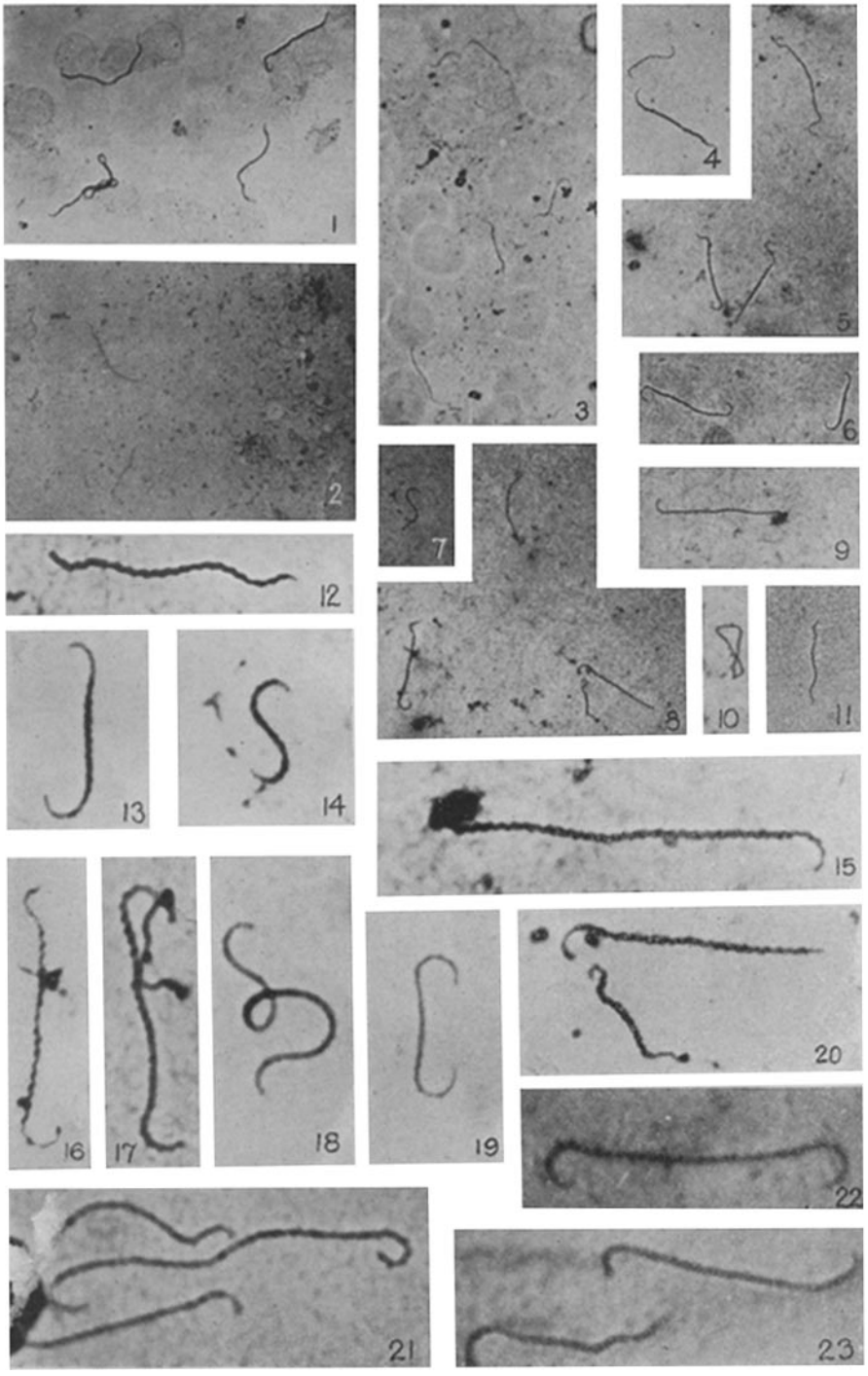
FIG. 108. *Spirochæta plicatilis*. Sublimate acetic-acid-alcohol fixation and iron-hematoxylin (after Zuelzer).

FIGS. 109 and 110. *Spirochæta stenostrepta* (after Zuelzer).

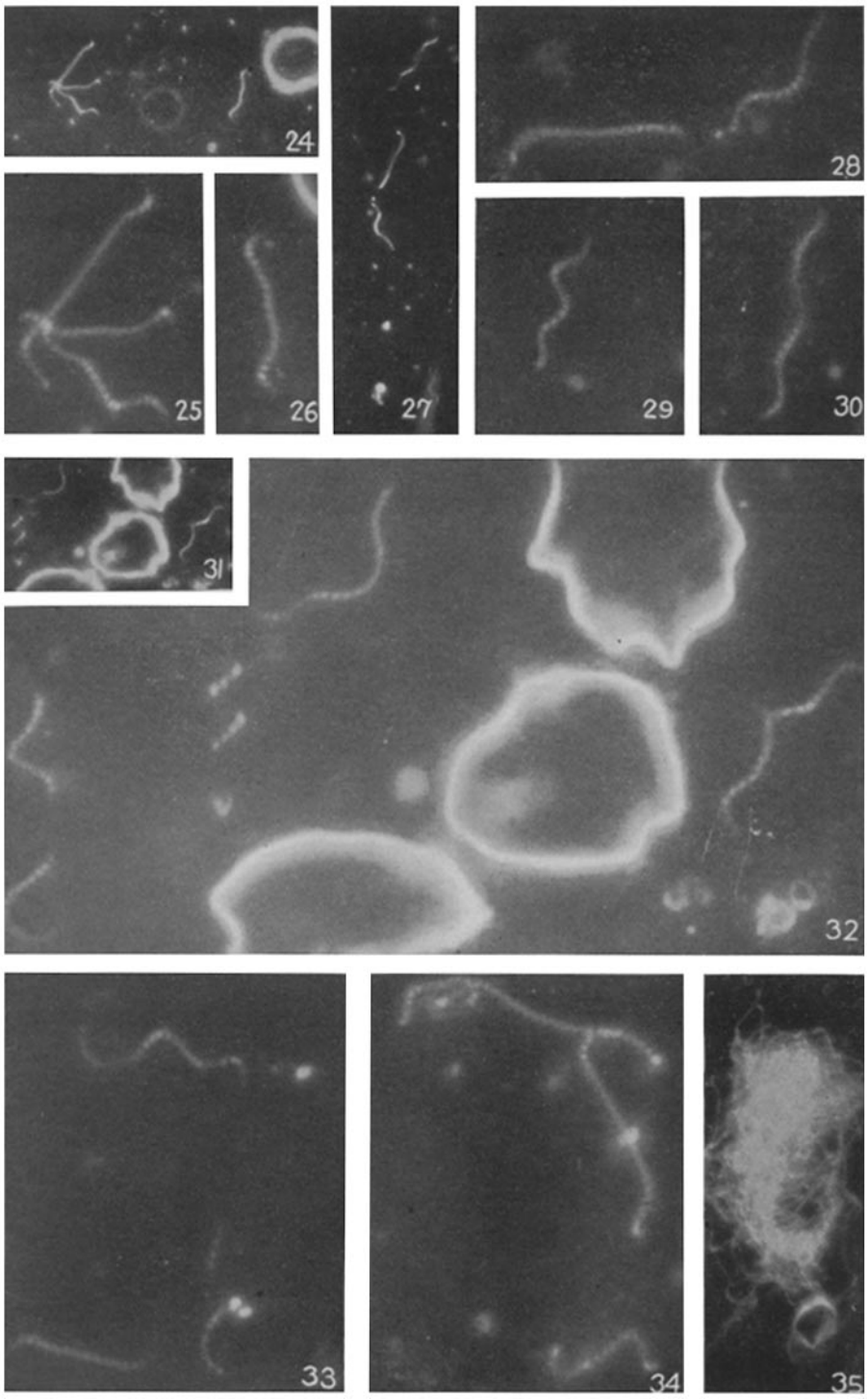
Spirulina Group.

FIG. 111. *Spirulina vesicolor.* This organism does not belong to the family of Spirochætoidea, but on account of its close spirals it is shown here. Iodine-alcohol and Delafield hematoxylin (after Zuelzer).

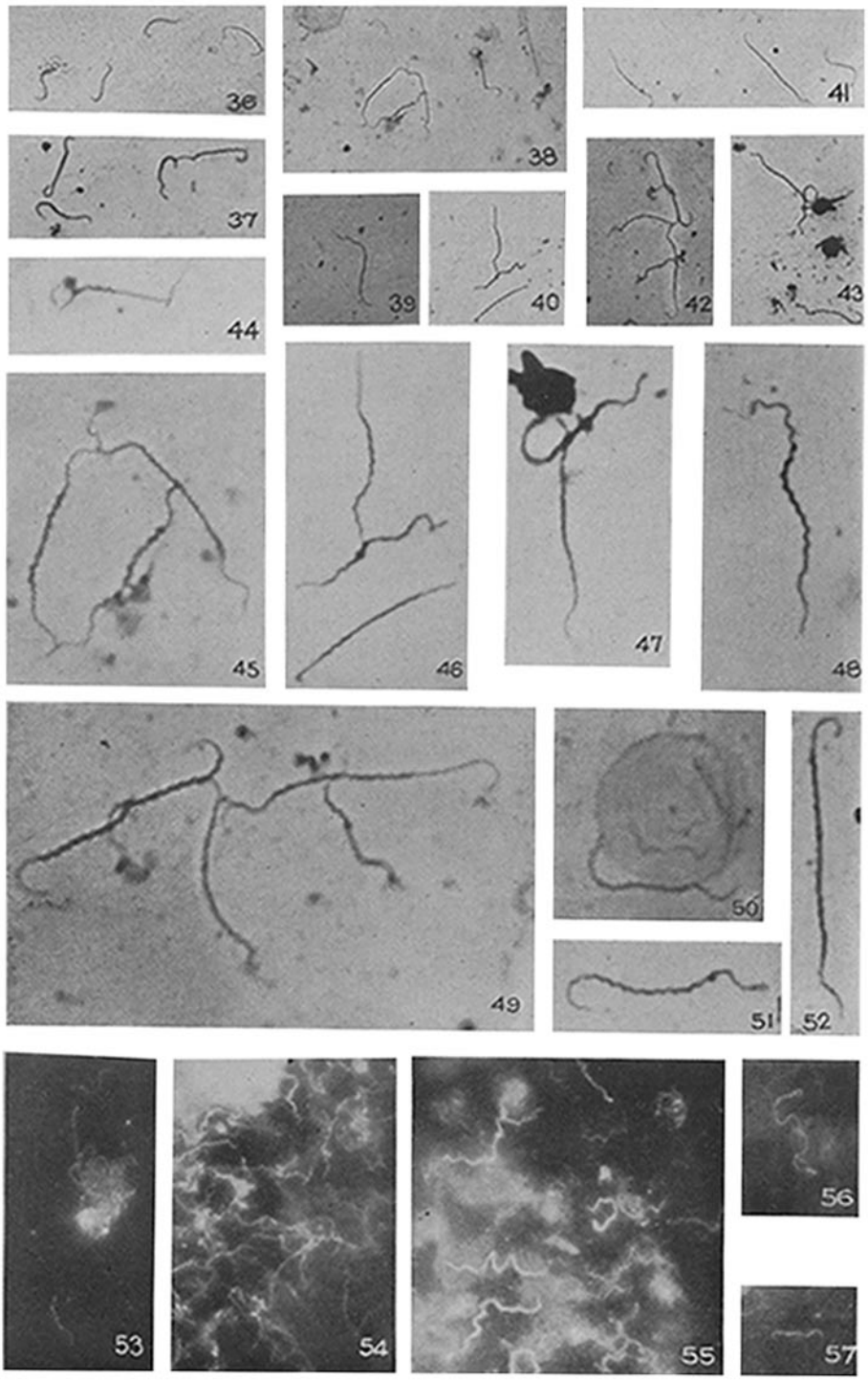
FIG. 112. The same, at another plane of focus, where the innermost structure is not brought out as in Fig. 111.



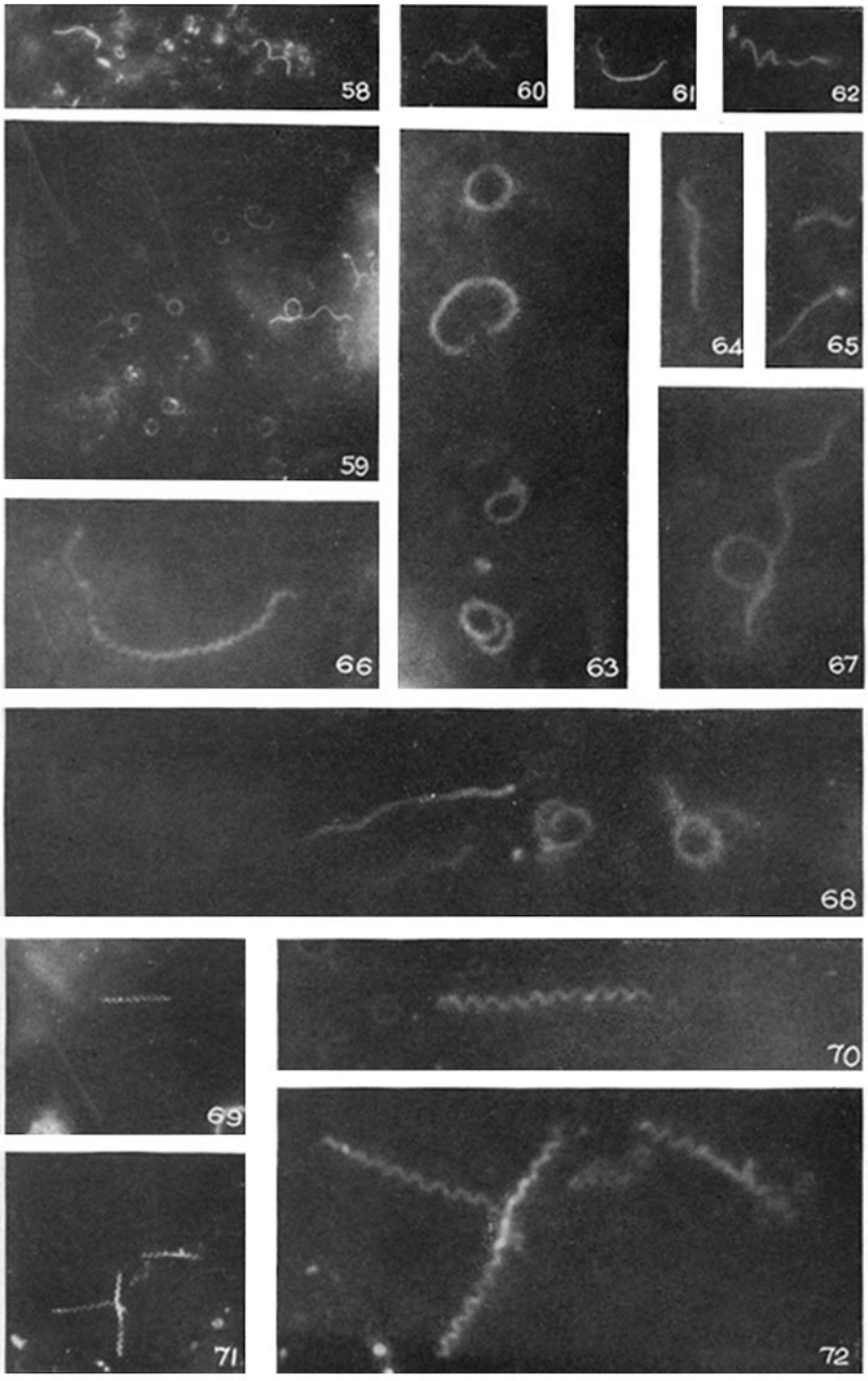
(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



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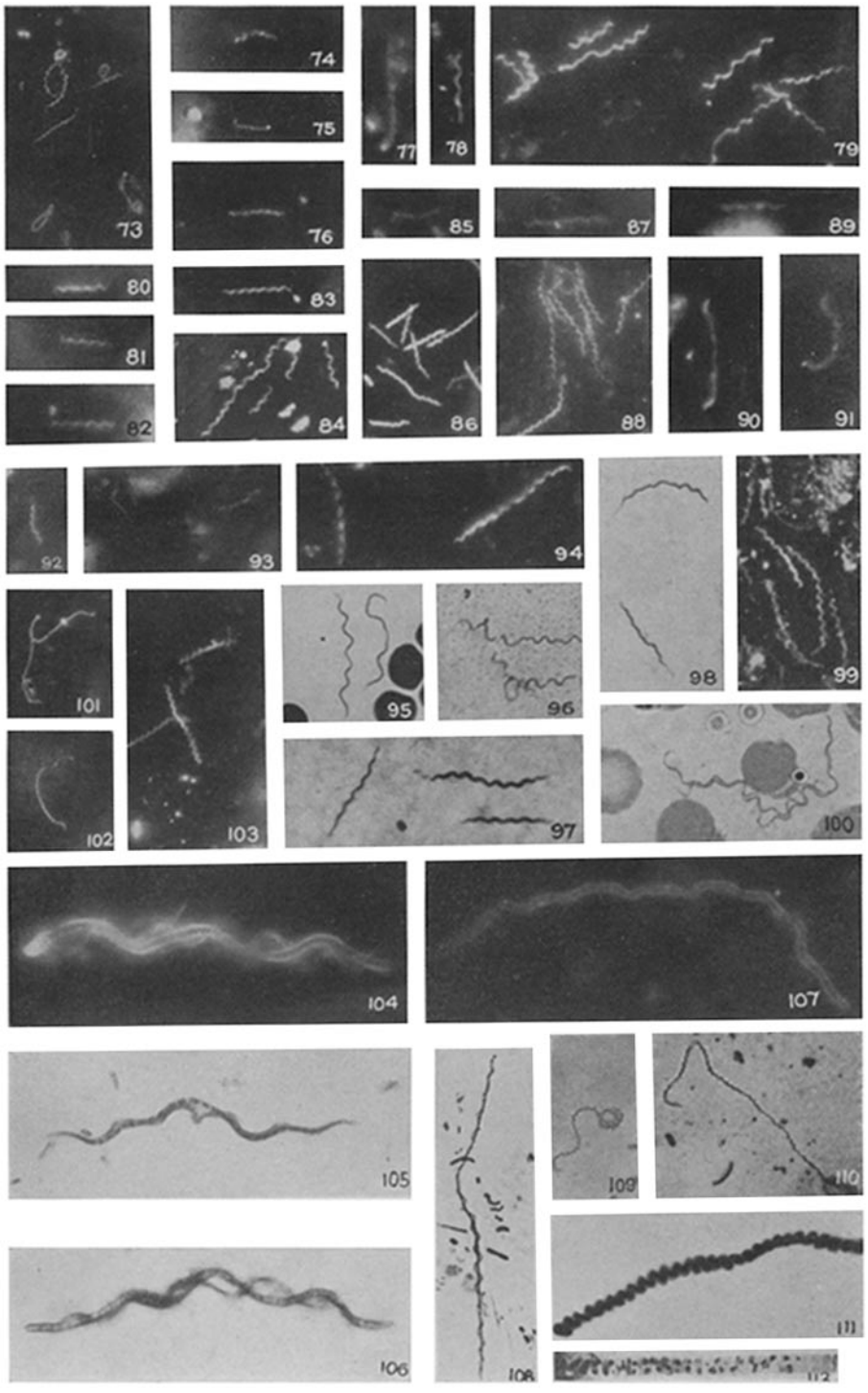

(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)