SOME PROBLEMS IN THE IDENTIFICATION AND
CLASSIFICATION OF SPECIES OF SPIRILLUM
I. EARLIER TAXONOMY OF THE GENUS SPIRILLUM

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Spirillum Ehrenberg, 1830, is one of the oldest bacterial generic names now recognized. The genus was originally characterized by Ehrenberg (1838) as follows:

CHARACTER: Animal e familia Vibrioniorum, divisione spontanea imperfecta (et obliqua?) in catenam tortuosam s. cochleam rigidum et in cylindriformam extensam abiens.*

Ehrenberg recognized three species in the genus—Spirillum tenue, S. undula, and S. volutans. One species, S. tenue, was described as new, the other two had previously been included by Müller in his genus Vibrio. Ehrenberg distinguished the species on the basis of the diameter of the cells, the type of spiral curvature, and the type of motility. Essentially the same morphological characters were used throughout the next hundred years in the separation of the species. Physiological and biochemical characters have had little application in writing diagnoses of the species of Spirillum. Two reasons for this failure to use physiological characters are: (1) many of the species of the genus were named and described prior to the development of techniques for recognition of physiological differences in bacteria; (2) even more important is the fact that species of Spirillum as studied today show few if any distinctive physiological or biochemical characteristics such as those used in the identification and characterization of many other bacteria.

Species of bacteria are commonly characterized by physiological characters; morphological characters, as a rule, are used only in the higher taxa, such as family and order,

*An animal of the family Vibrionae with spontaneous incomplete (and oblique?) division into a crooked chain or a stiff spiral and assuming the form of an extended cylinder.

and are relatively unimportant in distinguishing related genera and species.

Differences in morphology are the only features which distinguish species of the genus Spirillum, the morphological characters first noted by Ehrenberg (1838) are still significant. Additional characters used today (Bergey's Manual, 1957) are the Gram-negative reaction; the possession of polar flagella; a chemoheterotrophic type of metabolism; and the absence of bacteriochlorophyll and sulfur inclusions. While the morphological characters (used by Ehrenberg) as the diameter of the cell and type of spiral curvature are sufficient for the separation of morphological types as species, these characters alone are seldom sufficiently distinctive to provide reliable means for identifying a new isolate with a previously described species. The species of Spirillum are, as a rule, so physiologically inert that the physiological characters commonly used are not distinctive. The introduction by Giesberger (1936) of the techniques of using known carbon compounds as the sole sources of energy in a mineral medium provided physiological characters by which a previously described species could be reidentified, but only when these characters are correlated with a distinctive morphology.

The descriptions of the species of Spirillum Ehrenberg given in Bergey's Manual of Determinative Bacteriology (7th ed. 1957) are quite inadequate for identification. They are composites of the characters assigned to the several species by Giesberger (1936) and those given to the same species by previous students, consequently it is difficult to identify an isolate on the basis of measurements alone. Measurements, unsupported by illustrations of the organism, may lead to errors in identification unless they record the differences in the type of spiral curvature. The uniqueness of a particular morphological type was shown by Williams and Rittenberg (1957) for three different species of Spirillum whose over-all measurements were essentially similar.

Characterization of the species of Spirillum, by comparing descriptions of morphologically similar strains assigned to the same species by various investigators with the physiological characters found for strains assigned the same species by Giesberger (1936), makes exact identification of an isolate of the genus difficult. Ostensibly the species names, Spirillum undula, S. tenue, and S. volutans have

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been used by many workers since the time of Ehrenberg (1838), but it is questionable whether the organisms have been correctly identified in most cases. However, at the present time it should be possible to reidentify a previously described species by correlating a unique morphology with the utilization of a specific set of carbon compounds. To identify an isolate of the genus Spirillum it is necessary to include in the comparison both morphological and physiological characters with those of cultures of adequately described species. Such direct comparisons have been sharply limited by the absence of suitable authentic type or standard cultures. If the isolate differs from any available culture of species of Spirillum, the only recourse is to compare its characters with those of other species so inadequately described in the literature, emend the description and designate type or neotype cultures to be deposited in suitable Type Culture Collections.

Much of the literature on the genus Spirillum Ehrenberg appeared prior to 1900, some of it is not readily available. A review shows that spirilla of the same morphologies have been described for a hundred and twenty years under the species names of Spirillum tenue, S. undula, S. volutans, and S. serpens. Some of the problems in the identification of species of Spirillum stem from differences in the morphological and physiological characters ascribed by various investigators to organisms bearing the same species name. The most concise, although not the most exhaustive, sources of information on the genus Spirillum are the records prepared by earlier investigators. Some (not all) of these taxonomic treatises are here considered.* Plates and figures from these earlier publications are reproduced here to illustrate the morphology considered typical of the species by the several authors.

Müller's Taxonomy

The earliest attempt at the classification of microorganisms (animacules) was made by the zoologist, O. F. Müller, in two publications, Vermium terrestrium et fluviatilium, 1773 and Animacula infusoria fluviatilia et marina, 1786.

* For a list of earlier references see Buchanan (1918).

Müller did not distinguish between protozoa and bacteria; in fact this differentiation was not generally recognized until nearly a hundred years later. Müller considered his Vibrio undula to be the "little eel" described by Leeuwenhoek. He says

Leeuwenhoek points out that it is an animacule whose little body excels in slenderness the end of the tail of the seminal animacule and is ten-hundred-thousand thousand times smaller than a mustard seed. I agree with the given description, and continuing from that point, I gladly quote below from this author: 'Since the animacules contract their little tails when they move in circles and folds, we can rightly conclude that these little tails do not lack tendons, muscles and joints any more than do the tails of the dormouse and the mouse; nor does anyone doubt that these same animacules, which swim in water, are equipped with organs of locomotion just as are larger animals. Furthermore, how much equipment of visceral organs, or how great a lack of them is enclosed in such a small animal?' Likewise, in his Letter (Arcene Naturae 96) he calls it an eel, because when it swims with incredible speed, it bends its body in the manner of an eel.

Müller (1786) included the organisms now recognized as bacteria in two genera, Monas and Vibrio, the spiral forms were assigned to the new genus Vibrio. This genus contained eight species; two were probably not bacteria. Drawings of the six species recognizable as bacteria, are reproduced in Fig. 1. Three of these species can be recognized as spiral forms; Vibrio undula, V. serpens, and V. spirillum. The specific epithets of two of Müller's species are still in use in Spirillum undula and S. serpens. Because of its historical interest, Müller's description of V. serpens is quoted with a rather free translation of the Latin text as follows:

56. VIBRIO SERPENS

A thread-life Vibrio with its windings protracted at an obtuse angle. Table VI. Fig. 7,8.

Especially when extended it is longer than Vibrio

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undula by tenfold; it is seen to differ from Vibrio spirillum by its obtuse angles and is differentiated from both of these by its intestine which extends through its entire length, and by its greater size.

It is a gelatinous animacule, extremely slender, showing a linear serpent shape, with even and loose curves, but not in the least manner resembling the fabled river serpent of Norway. When it is extended it is straight, not spiral, it is loosely coiled with serpentine curves.

It is rarely found in river water.

Although it would be difficult at present to identify an isolate of the spiral organisms from the descriptions and drawings given by Müller, the drawings do show that his organisms were morphologically similar to those observed today. The present-day cultures recognized as those of Spirillum undula and S. serpens show only a slight morphological resemblance to their prototypes, Vibrio undula and V. serpens. Nevertheless, Müller's description of V. serpens lists one characteristic which is found in cultures of S. serpens today; namely the tendency of the organisms to appear as straight rods when extended or in rapid motion.

Ehrenberg's Taxonomy

Ehrenberg, like Müller, also included among the protozoa several organisms now placed with the bacteria. Ehrenberg increased the number of genera and species now included in the bacteria, retaining Müller's names Monas and Vibrio for two of his genera. In 1838, in his book Die Infusionsthierchen als vollkommene Organismen, Ehrenberg presented a classification of the microorganisms observed by him. He described many species now assigned to bacteria and improved the definitions and diagnoses of the genera as well as the species. In 1829 Ehrenberg had observed a large spiral organism in St. Petersburg which he described in 1830 as Spirillum volutans. Ehrenberg utilized the specific epithet Spirillum of Müller's species, Vibrio spirillum, as the generic name for this organism. In the descriptions of this species in 1838, Ehrenberg discusses his reasons for this change. According to Ehrenberg (1838) Kohler first observed this organism in 1777. He states:

Der Entdecker oder erste Verzeichner dieses Thierchen war 1777 Kohler in Leipzig, dann beschrieb es Hermann in Strassburg aus dem Aufguss von vegetabilischen Kuchen-Abfall und sagt 1784, dass es die Beobachtungen vor 18 Jahren gemacht. Er schickte seine Zeichnung an Müller zur Bestimmung und so erhielt sein Thierchen von Müller den namen Vibrio Undula. Die Zeichnung ist nach zu kleiner Vergrosserung, entworfen, zeigt aber deutlich die Form das Spirillum volutans durch mehrfache Schraubenwindungen. Müller selbst lernte diese Form schon 1782 in einen Aufguss von Sonchus arvensis kennen, hatte aber noch längere Schrauben angetroffen, die ihn bestimmten, jene kurzen von Hermann als V. Undula aufzuzeichnen. Auch erst in den spätem Infusionwerk ist bei V. Undula bemerkt, dass es Formen dieser Art mit mehrfachen Spiral windungen gebe.

For these reasons, Ehrenberg (1838) assumed that Hermann's organism and the one which Müller had observed in 1782 were identical with his species, Spirillum volutans. Since the organism observed in 1829 had the many spiral windings depicted by Müller (1786) for Vibrio spirillum, Ehrenberg (1830) used Müller's specific epithet of Spirillum to name the new genus. In the legend to the various drawings of S. volutans, Ehrenberg (1838) admits the possibility that more than one species may have been observed. Although Ehrenberg (1838) appears to have made a large number of assumptions in regard to his species, S. volutans, he states that Vibrio spirillum is the prototype of S. volutans in the following sentence: "Müller's Spezial-Name ist zum Genus-Namen erhoben worden." In his description of S. undula, Ehrenberg (1838) says that this organism is the same as the one observed by Kohler in 1777. It is possible that Müller and the other authors mentioned by Ehrenberg observed different organisms, it being difficult at the present time to reach a decision from the data given. It should be noted, however, that Müller's description of Vibrio undula, given in the 1786 work, is essentially the same as that published in 1773.

In addition to Spirillum, Ehrenberg named three other genera of spiral organisms. One contained a species, Spirochaeta plicatilis, with flexible cells in contrast to the rigid

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spirals of Spirillum. Spirodiscus fulvus, a second species had the shape of a coiled watch spring. Several other species are placed in another genus of rigid spiral forms, Ophidomonas. Ehrenberg (1838) differentiated Spirillum and Ophidomonas on the basis of the presence of a motility organ and complete division in the latter, but not in the former. He also states that the genus Ophidomonas has pigmented species (O. jenensis olive-brown; O. sanguinea blood red). Ehrenberg (1838) did not include Müller's species Vibrio serpens in the genus Spirillum because, as he states in an addendum to the description of the genus, it was obviously a species of the algal genus Oscillatoria.

A portion of Ehrenberg's Plate V (1838), in which the species of Spirillum are illustrated, is reproduced as Fig. 2. A direct comparison of Müller's drawing of Vibrio spirillum (Fig. 1, no. 9) with Ehrenberg's drawing of S. volutans (Fig. 2, no. XIII) leaves little doubt that the two organisms were not the same. Müller (1786) described V. spirillum as being thread-like while the dimensions* given by Ehrenberg (1838) for S. volutans could never be so construed. They agree well with the dimensions of the cells found in the Pringsheim culture of this organism. The cells of the Pringsheim culture often show the tight spiral windings, depicted by Ehrenberg for the organisms observed in 1829 (Fig. 2, no. XIII (2)), particularly when the organisms are growing in a mixed culture containing many protozoa; i.e., conditions similar to those under which Ehrenberg observed his species. Migula (1900) points out that Müller's illustration of V. spirillum resembles Ehrenberg's drawings of Spirochaeta plicatilis more than it does his (Ehrenberg's) drawing of S. volutans. The descriptions given by the two authors for Vibrio undula, V. spirillum (Müller, 1773; 1838) do not permit a decision as to whether Ehrenberg was justified in his revision of nomenclature. The Müller and Ehrenberg illustrations, however, do show the various morphologies of spiral organisms.

* (Dimensions of S. volutans, Ehrenberg, 1838). Lange der Spirale $1/192$ bis $1/48$ Linie. Dicke etwa $1/1200$ Linie. (A Linie is equivalent to $1/12$ of an inch.)

Cohn's Taxonomy

Much of Müller's (1773, 1786) taxonomy of the organisms now included with the bacteria may be dismissed today as of "historical interest" only. Cohn's system of classification (1872) was the beginning of modern bacterial taxonomy (see Bergey's Manual, 1957, p. 6). Of the many systems of classification of the "bacteria," proposed by his predecessors, Cohn considered only those of Ehrenberg (1838) and Dujardin (1841) to have merit and took their publications as the starting point for his own system. Cohn's comments on the difficulties of classifying the bacteria have a familiar ring and are sufficiently relevant to be quoted in translation.

Whoever studies the pertinent recent literature knows that, to a degree, confusion has arisen in the nomenclature of the bacteria. Almost every investigator, without being concerned with the work of his predecessors, has often designated easily recognized forms quite arbitrarily, assigning to them new names, and the rule of priority, which is the basis of nomenclature of organisms everywhere, is generally disregarded.

In fact, the obstacles encountered in giving these organisms the correct designation and nomenclature are extraordinary. Only Ehrenberg and Dujardin have endeavored to classify entire series of bacteria, to arrange them in relationship (to each other) and to assign them to genera and species, their works, therefore, must serve as the starting point. . .

All of these difficulties make themselves felt when we seek to assign bacteria to their natural genera. The genera of the bacteria have not the same significance as those of higher plants and animals as they are based only on the characteristics of vegetative cell formation and not on reproductive characters. . . . (1872, p.128).

Despite his respect for the classification of Ehrenberg (1838) and Dujardin (1841), Cohn (1872) criticized the diagnostic characters used by these authors to distinguish some of the species. He found it difficult to separate Ehrenberg's Bacterium termo from Vibrio lineola; his Vibrio subtilis from Spirillum tenue; and Vibrio prolifer from Spirillum undula. Concerning these difficulties, Cohn (1872) states:

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The supposedly good diagnostic characteristics of Bacterium, Vibrio, and Spirillum established by Ehrenberg do not appear so distinctive in their application in reality. To be sure we find rigid, small, rod-like forms which can be placed without hesitation in Bacterium, rigid spirals, which can be placed in Spirillum; then we encounter eel-like forms, darting here and there, which both Ehrenberg and Dujardin designated as Vibriones.

My convictions are that the undulating and spiral forms can be applied collectively to the Vibriones and that the morphologically stable Spirillum is not capable of stretching and bending and therefore has no really sinuous motion; it follows that there is no Vibrio corresponding to Ehrenberg's definition of the genus. It is evident that these genera must be defined anew.

Cohn (1872) divided the bacteria into four groups (Tribes), each containing one or more genera. The sinuous and spiral organisms were placed in two Tribes; Vibrio (char. emend.) was placed in the Tribe Desmobacteria and Spirillum Ehrenberg in the Tribe Spirobacteria. Genus 4, Vibrio, contained two species, Vibrio rugula and V. serpens; genus 5, Spirillum Ehrenberg, contained three species, S. tenue, S. undula, and S. volutans. Cohn retained the names of Ehrenberg's (1838) species and reintroduced Müller's (1786) species, Vibrio serpens, since he considered that Ehrenberg was not justified in his rejection of this species. Cohn (1872) described the genus Vibrio (char. emend.) as "characterized by the stable form of the undulating windings of the filaments, which by their rotation produce the appearance of being sinuous and form, therefore, a transition to the spiral bacteria or Spirillum." Cohn also observed that the cells of V. serpens appear as straight rods during motion as earlier noted by Müller (1786).

Cohn separated the genus Spirillum from the genus Vibrio on the basis of "the thicker and tighter coils with a regular form, i.e., stable curves in the filaments" and by the presence of a flagellum in S. volutans. The discovery of a flagellum in S. volutans and not in the other species of Spirillum caused Cohn (1872) to consider transferring this organism to the genus Ophidomonas since Ehrenberg (1838) had described a "snout" on these organisms to be the cause

of motility. S. volutans, however, was colorless and had been so described by Ehrenberg whereas the species of Ophidomonas were described as being colored. Cohn was reluctant to separate S. volutans from the other two species of Spirillum and in discussing a large spiral organism which appeared red in mass, although single cells appeared colorless, Cohn (1875, p. 171) says:

But a second genus controverts the diagnosis of our species in the bacterial genus Spirillum; ever since we discovered flagella in S. volutans, there exists between Spirillum and Ophidomonas no distinction at all, if it can be assumed that an organ of locomotion will later be found in the smaller spirilla. We have, therefore, only one choice, either to discard Ophidomonas as a distinct genus and rename the species Spirillum sanguinea - to some extent the mammoth of the bacteria - or conversely to place the naturally equipped flagellated spiral organisms (S. volutans, S. jenensis, and S. sanguinea) under Ophidomonas and retain the name Spirillum exclusively for the smaller species (S. tenue, S. undula), so long as no flagellum is discovered for the smaller ones. If such happens, the name Spirillum would be adandoned.

A portion of Cohn's Plate III (1872), in which drawings of the spiral organisms are shown, is reproduced as Fig. 3. Although Cohn worked only with impure cultures, his observations on the morphology of the bacteria were so acute that his descriptions and illustrations were used by the bacterial taxonomists who followed him in the identification of the species described by him. Cohn's drawings are a decided improvement over those of Müller (1786) and Ehrenberg (1838) in that the morphology of the organisms shown in the drawings is distinctive. In Fig. 3, the drawing of Bacillus ulna could be used for a species of the genus Bacillus today; his spiral organisms are also well drawn.

After the development of the pure culture technique, many systems of classification of the bacteria were published. Insofar as the spiral organisms are concerned, however, many of these systems are unimportant and need not be considered.

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Migula's Taxonomy

Migula published a summary of his first system of classification in 1894 and expanded it in 1895 in Engler and Prantl's *Die natürlichen Pflanzenfamilien, Teil I, Schizophyta*. His later work, *System der Bakterien* (1897, 1900) developed and amplified his ideas on classification. Many of the diagnostic characters used today in the separation of the genera of the bacteria were first proposed by Migula.

Migula, in 1895, clearly distinguishes the several genera of spiral organisms, as shown in the following key:

Migula's Key to the Genera of Spiral Bacteria

- A. Cells rigid, non-flexible, spiral.
 - a. Cells without organs of locomotion. 1. Spirosoma
 - b. Cells with organs of locomotion (flagella).
 - 1. Cells with one, very seldom 2-3 polar flagella. 2. Microspira
 - 2. Cells with polar flagella in tufts. 3. Spirillum
- B. Flexible cells. 4. Spirochaeta

Migula adopted the name Microspira, first proposed by Schröter (1886) for the cholera organism, to replace Vibrio. His (1897, p. 23) reasons for nonrecognition of the Vibrio are stated as follows:

. . . The genus Vibrio had on the whole little good fortune; with every investigator its characteristics changed and the species originally included in the genus were transferred in part to the bacilli and in part to the spirilla.

Migula differentiated the comma or slightly curved organisms from those showing a corkscrew spiral also on the basis of the kind and number of flagella. He placed his spiral organisms either in the genus Microspira (1 - 3 polar flagella) or in the genus Spirillum (tufts of polar flagella). Migula (1900) admitted some difficulty in determining the number of flagella in some species of Spirillum since the

flagella were often agglutinated into bundles resembling a single flagellum. He (1895) noted further that the single flagellum of the species of Microspira usually showed a helical curvature whereas the compound flagellum of Spirillum showed flagella which were seldom curved or at most displayed only a half-curve. Migula's ideas on flagellation are best shown by his illustrations in Die Pflanzenfamilien. A plate of drawings showing flagellation in some species of the bacteria is reproduced as Fig. 4. Differences in flagellation between species of Microspira and Spirillum are shown in the drawings H through M.

Migula (1900, p. 875) confesses to some difficulty in differentiating between species of the genus Pseudomonas and those of the genus Microspira, there being an almost continuous gradient which he described as follows:

The limits given to Microspira are perhaps artificial ones; it is not always possible to judge whether we are dealing with the single flagellated forms of a Microspira or a Pseudomonas. Slightly curved forms, particularly in stained preparations, can be observed in old Pseudomonas cultures.

Migula (1900) relied on the length of the flagellum (illustrated in drawings C and D, Fig. 4, for species of Pseudomonas) to differentiate between the species of the two genera since he states that species of Microspira never showed a flagellum much longer than the length of the cell, whereas the species of Pseudomonas usually had a flagellum which greatly exceeded the cell length. (This character is not shown in his drawing of Pseudomonas pyocanea (Fig. 4)). He also states that all species of the genus Pseudomonas are fluorescent, whereas species of the genus Microspira are not.

Migula (1895) placed all the flagellated rigid spiral organisms in one genus, combining Ehrenberg's two genera, Spirillum and Ophidomonas, in the genus Spirillum. He separated the colorless and colored forms by the creation of subgenera, placing the colorless forms (including Spirillum rubrum v. Esmarch) in the new subgenus Euspirillum; the colored forms containing cell inclusions of sulfur were placed in the subgenus Thiospirillum Winogradsky.

Migula (1900) recognized a large number of species of the genus Spirillum; many of these have either been

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transferred by later authors to other genera or have been regarded as inadequately described. In regard to the number of described species, Migula says:

The number of existing species of Spirillum is much greater than those described thus far, as pure cultures are as yet only slightly successful and most of those described are probably collective species. Spirillum leucomelaenum Perty may be said to be not one species but to represent certain developmental stages of various species.

Of the species of Spirillum recognized by Migula (1900), the seventh edition of Bergey's Manual (1957) included S. tenue, S. undula, S. volutans, and S. serpens. Migula considered the culture of S. tenue, isolated by Beijerinck (1893), to be a collective species. Migula transferred Cohn's two species of Vibrio to the genus Spirillum. In regard to this change, in the discussion of the genus Vibrio, Migula (1897) says:

Between Vibrio and Spirillum there is, according to Cohn's diagnosis, no difference, the fact that the undulating curvature is in reality only delicate spirals was suspected by Cohn.

The description of species of Spirillum given by Migula (1900) leaves much to be desired. Although measurements are given for the cells of the species, the dimensions are often rather vague; i.e., in the description of S. rugula, the diameter of the cell is stated to be "twice the thickness of Bacillus subtilis." He also described many species of Spirillum as liquefying gelatin and growing in pour plate cultures; two characters seldom found by recent workers. Two plates of photographs from Migula's System der Bakterien (1897 and 1900), which show the morphology of cultures of Spirillum, are reproduced in Figs. 5 and 6. Although these photographs illustrate the morphology of the organisms observed, apparently they do not illustrate adequately Migula's proposals as to the use of flagella in the differentiation of bacteria as well as do the drawings shown in Fig. 4. Such diagrammatic or composite drawings show general features and are never intended to be taken as illustrating an individual organism. Those who have hunted through preparation after preparation in search of the "typical cell" will attest to the value of such diagrammatic drawings.

PLATE I.

Figure 1

Table VI. O.F. Muller's Animacula infusoria fluviatilia et marina, 1786. (Courtesy, Cornell University Library.)

1. Vibrio lineola
2. Vibrio ruqula
3. Vibrio bacillus
4. Vibrio undula, small mass of cells emerging from a larger mass.
5. Vibrio undula, a mass collected around a filament of one of the Confervae.
6. Vibrio undula, greatly magnified. d. at rest e. swimming
7. Vibrio serpens, magnified.
8. Vibrio serpens, highly magnified. a. intestine
9. Vibrio spirillum

Figure 2

Table V from C.G. Ehrenberg's Die Infusionsthierchen als volkommene Organismen, 1838. (Courtesy, Library of Congress.)

- I. Bacterium trioculare; II. Bacterium enchelys;
- III. Bacterium punctum; IV. Bacterium lineola;
- V. Vibrio tremulans; VI. Vibrio bacillus; X. Spirochaeta plicatilis;
- XI. Spirillum tenue; 25 spiral filaments differing in length of the spiral curves, often exhibiting a vibrating motility; original 300 X.
- XII. Spirillum undula; with two magnifications.
 1. Group of 18 monad-filaments magnified 300 X.
 2. 12 different types of spiral curvature, magnified 800 X.
 3. Indicates the line of motion of single spiral rods.
- XIII. Spirillum volutans;
 1. Organism observed in St. Petersburg. 6 spiral rods, with fewer spiral windings $1/96$ as many.
 2. Magnification of 800 X, with 10 filaments in different degrees of aggregation, from single organisms in fission to coils.
 3. In a plant infusion and is as long as $1/48$ of a line (1 line = $1/12$ inch or 2.2 mm) magnified 800 X.

1

VIBRIONIA CLOSTERINA.

TV.

2

PLATE II.

Figure 3

F. Cohn's Table III, Beitrage zur Biol. d. Pflanzen I. (Heft 1), 1872. (Magnification 650 X)

15. Bacillus ulna, single filament and longer rod, with several filaments breaking in division.
16. Vibrio ruqula, single cells or those in division with one showing an apparent swelling in consequence of the quick rotation.
17. Vibrio serpens, long or short rods, dividing into smaller rods; two of the rods twisted about each other.
18. A cluster of V. serpens with the cells matted together.
19. Spirillum tenue, single cells and clusters matted together.
20. Spirillum undula.
21. Spirillum volutans with two of the spiral rods wound around each other.
22. Spirochaeta plicatilis.

PLATE III.

Figure 4

W. Migula's figure 1 from Schizomycetes, Teil 1, in Engler and Prantl's Die natürlichen Pflanzenfamilien, 1895.

- A. Bacillus subtilis and Spirillum undula, cell wall which has been separated from the plasma body with the flagella attached.
- B. Planococcus citreus (Menge) Migula.
- C. Pseudomonas pyocyanea (Gessard) Migula.
- D. Pseudomonas macroselmis Migula.
- E. Pseudomonas syncyanea (Ehrenberg) Migula.
- F. Bacillus typhi Gaffky.
- G. Bacillus vulgaris (Hauser) Migula. Filament and single cell.
- H. Microspira comma (Koch) Schroter.
- J. Spirillum rubrum v. Esmarch, short cells.
- K. Spirillum rubrum v. Esmarch, long cell.
- L. Spirillum undula (Müller) Ehrenberg.
- M. Spirillum undula (Müller) Ehrenberg. The flagella are agglutinated into a bundle.

Fig. 1. A *Bacillus subtilis* Cohn und *Spirillum Undula* Ehrenb., Membran mit daran hängenden Geißeln, vom Plasmakörper abgehoben. — B *Flavococcus citreus* (Münge) Migula. — C *Pseudomonas pyocyanea* (Gessard) Migula. — D *P. macroleimnis* Migula. — E *P. pyocyanea* (Ehrenb.) Migula. — F *Bacillus typhi* Gaffky. — G *B. vulgaris* (Hausser) Migula, Faden und einzelne Zellen. — H *Microspira Comma* (Koch) Schröder. — I *Spirillum rubrum* v. Esmarch, kurze Zellen. — J *S. rubrum* v. Esmarch, lange Zelle. — K *S. undula* (Müller) Ehrenb. — L *S. undula* (Müller) Ehrenb., Geißeln zu einem Strang verklebt. — M *S. undula* (Müller) Ehrenb., Geißeln zu einem Strang verklebt. — Sämtliche Abbildungen nach mit der Löffler'schen Beize behandelten Deckglastrockenpräparaten, 1000/l. (Original.)

PLATE IV

Figure 5

Table III from V. Migula's System der Bakterien, Volume I, 1897.

3. Microspira nigricans (Weibel) Migula. Flagella stain.
4. Spirillum serpens (Müller) Winter. Flagella stain.
5. Spirillum undula (Ehrenberg). Flagella stain.
Flagella agglutinated into unequal bundles.
6. Spirillum undula (Ehrenberg). Flagella stain.
Flagella agglutinated in a single thick bundle.
7. Thiospirillum rufum (Perty) Winogradsky. Flagella stain.
8. Spirillum rubrum v. Esmarch. Flagella stain. By repeating the mordant and stain, very thick flagella are obtained.

(All flagella stains by Löffler's method. Original 1000 X)

Figure 6

Reproduction of Table XVII from W. Migula's System der Bakterien, Volume II, 1900.

1. Microspira albis. Flagella stain after Löffler. 1000 X.
2. Microspira tyrogena. Ramifications. Old broth culture. 1000 X.
3. Spirillum rubrum. Long spirals twisted together. Broth culture. 1000 X.
4. Spirillum rubrum. Flagella stain after Löffler.
5. Spirillum sporiferum. From a decaying bean infusion.
6. Spirillum tenue. Flagella stain after Löffler.
7. Spirillum kutscheri. Flagella stain after Löffler.
8. Spirillum undula. Flagella stain after Löffler. From a straw infusion.

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