STUDIES ON THE SPIRILLEAE: METHODS OF ISOLATION AND IDENTIFICATION[1]

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In the recent literature of bacteriology scant attention has been paid to the genus *Spirillum* Ehrenberg. Many of the textbooks treat this as an obscure group. Unfortunately, the most recent edition of Bergey's Manual (1939) is inadequate in its coverage of the genus, in spite of recent corrections. No more than a citation has been given to the one recent general work on the subject, a thesis by Giesberger (1936).

Two difficulties have obscured our knowledge of the spirilla. Their isolation is difficult by the usual bacteriological techniques. And their screw-like morphology, which allows four descriptive measurements, has led to the naming of many species without supporting cultural characteristics. Some forty species are recorded in the literature; probably less than ten of these could be recognized with surety from their original descriptions.

This paper presents a description of techniques of isolation which are comparatively simple and effective, at least for some species, and a characterization of four spirilla and two vibrios thus obtained from stagnant waters of Montana and Minnesota.

METHODS OF ISOLATION AND CULTURE

Since the time of Kutscher (1895) the preparation of an infusion has been the first step in the isolation of spirilla. This is easily

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done with liquid samples such as stagnant water, sewage, and liquid dung considered in the present study. In practice, samples are gathered in wide-mouth jars and brought to the laboratory. A small amount of organic matter such as peptone, salts of organic acids, lettuce leaves, lean meat, or fish is added. The last has proved particularly suitable. After one or two days incubation, motile spiral forms may be microscopically observed in a hanging drop preparation from the extensive flora which develops. If a number of samples are studied, perhaps one or two will show at some time a comparatively high proportion of one or more spiral forms. In the early stages of this study repeated attempts were made to plate out onto beef-peptone agar from such infusions which looked to be teeming with spirilla. Various other methods and media were used without success until the procedure outlined below was tried.

An infusion containing large numbers of spirilla is sterilized by filtration through a Berkefeld filter, portioned out aseptically into a number of sterile petri dishes, and hardened by the addition of sterile 2 per cent agar solution. A rough separation of motile from non-motile forms is made from a hanging drop of another infusion containing large numbers of spirilla by the following method. A glass tube is drawn out to a long capillary of fairly uniform bore. Sterile water or infusion liquid is drawn up in this tube to a length of about 2 or 5 cm. Two marks are made on a piece of paper to indicate the length of the water column. From the hanging drop selected about 2 to 5 cm. more are drawn up into the capillary. This is allowed to lie quietly in a horizontal position for about half an hour. The end of the capillary, up to a point somewhat above the original infusion-sterile liquid junction, is cut off with a knife. The remaining liquid is blown out into a test tube of the previously sterilized infusion liquid. This procedure effects a concentration of spirilla, since they are generally the most actively motile of the bacteria present. While not always necessary, it tends to cut down the number of non-motile forms in some infusions.

The method of plating has been adapted from the single spore technique described by Kauffman (1908). Three or four serial

1:10 dilutions are made into tubes of the sterilized infusion liquid from a tube inoculated with a loopful of infusion, or with the liquid from the capillary containing the "concentrated" spirilla. Capillary pipettes are drawn out from pieces of glass tubing in such a way that water blown out of them emerges in the form of very small droplets. Liquid drawn up from each of the dilutions into these sterilized pipettes is then blown out on to the agar surface of one of the petri plates, so as to give a fairly uniform distribution of droplets. When the appropriate dilution is used, the average droplet contains one or less than one organism. This organism is well isolated on the agar surface and yet immersed in a liquid medium for the first several hours. Such an arrangement is particularly favorable for the spirilla, which generally seem to prefer liquid media.

All bacteria develop slowly on the infusion agar, which is rather poor in nutrients. Generally, a week's incubation at room temperature is required. Unfortunately, in spite of all of the above precautions, there is usually only a small percentage of colonies which contain spiral forms, and the author has found no general characteristic by which the colonies of all spirilla may be recognized. For two large species, *Spirillum undula* and *Spirillum serpens*, individual cells protruding from the colony edge may be recognized under the 16 mm. objective. For smaller species the colonies are indistinguishable from those of many other forms. However, all species so far obtained have come from small, more or less colorless, translucent or transparent colonies; never from large or spreading or highly colored colonies. Colonies from which spirilla can be demonstrated in a hanging drop are transferred to tubes of the infusion liquid previously sterilized by filtration. A second plating-out has seemed unnecessary, since the original colonies are well isolated. Any mixed culture is quickly observed, for contaminants almost invariably grow much faster than the spirilla. From the culture in the sterilized infusion media, transfers are made to the usual beef-peptone broth and subsequently to agar slants.

Several modifications of the above method have been tried. A more easily prepared, and nearly as satisfactory, medium for the

isolations is a fish broth, liquid or solidified with agar. About 10–20 grams of fresh fish are simmered in a liter of tap water over a slow flame for several hours. This is filtered, the pH adjusted to 7.0, agar added if desired, tubed and autoclaved. During the sterilization the pH usually rises to about 7.2–7.4.

The continued cultivation of spirilla on the usual beef-peptone agar is not difficult, provided several precautions are taken. Although no conclusive tests have been made, it has seemed advantageous to dissolve the peptone and beef extract by 20 minutes autoclaving before the pH adjustment is made. For growth on agar heavy inoculations are necessary and should be made into the condensation water of a fresh slant. The liquid is then drawn up over the slant with the inoculating loop. Observing these precautions, some 60 strains have been carried in stock culture without difficulty by transferring once a month.

During the first part of this investigation about 20 strains of spiral organisms were isolated from surface waters near Bozeman, Montana. These were studied in the usual way in regard to their morphology and cultural characteristics. At about this time there came to the author's attention the thesis of Giesberger (1936) describing five species of spirilla isolated by him in Holland. His method of isolation consisted of repeated streaking out and the use of a micro-manipulator, since only very small colonies were produced under his conditions. Some 60 strains were thus obtained for study. Of those which could be kept in culture he was able to obtain a clear-cut differentiation into four groups on the basis of their utilization of various carbon sources. These groups corresponded exactly with the four different types of morphology found. By comparison with previously described forms these were named *Spirillum serpens*, *S. tenue*, *S. itersonii*, and *S. undula*. A fifth species obtained from horse manure, but which could not be cultured so readily, was described as *S. kutscheri*.

Unfortunately my cultures were accidentally lost before Giesberger's methods of study could also be applied. A second set of cultures isolated in 1936 were also lost before they could be adequately studied.

Early in 1939 a third series of cultures was obtained from various sources near Minneapolis, Minnesota. Over a hundred cultures were isolated and carried along in duplicate. Of these, many have been subsequently discarded due to contamination, apparent change in morphology, or to the fact that they seemed to be borderline cases with cells only slightly curved, or with only a small percentage of cells curved. From this experience it seems likely that there may be numerous borderline microörganisms lying between the spirilla and vibrios on one hand and the *Pseudomonas*-type rods on the other. A consideration of such borderline organisms is beyond the scope of the present study, since even the distinct spirilla are still so incompletely known. A few attempts at isolation from barnyard manure were also made, but with little success. The one culture obtained grew poorly on all the media tried and soon died. Giesberger's suggestion (p. 57) that the dung-spirilla belong to species distinctly different from those found in the usual surface waters may indeed be correct.

STUDY OF THE ISOLATED STRAINS

Of the 107 strains originally isolated and cultured in duplicate, 54 which continued to show a typical spiral morphology were retained for study. Previous work on the strains isolated in Montana had indicated temperature optima in the vicinity of 32°C. and pH optima of about 7.2–7.4. This is entirely in accord with the results of Giesberger. Because of the large number of cultures, they were incubated at room temperature (24–30°C.).

The method of differentiation used by Giesberger was closely followed, except that of the 29 carbon sources which he used six were selected which showed clear-cut differences between his various species. To the inorganic medium containing 0.05 per cent $MgSO_4$, 0.05 per cent K_2HPO_4, and 0.05 per cent $CaCl_2$ there was added a nitrogen source (0.1 per cent) and a carbon source (0.5 or 1.0 per cent). The pH was adjusted to 7.0 to 7.2 against the bubbling hydrogen electrode and the media sterilized by filtration through a Seitz filter. (Autoclaving induces precipitation and attendant pH changes.) Tubes were inoculated

with a loopful of a young broth culture. The small additional organic matter thus introduced in the inoculum was apparently too small to influence the results, since all cultures showed zero growth on at least one medium.

With 1.0 per cent calcium lactate as the carbon source, four nitrogen compounds were tried: NH_4Cl, asparagin, KNO_2, KNO_3. As shown below, NH_4Cl gave at least fair growth with nearly all strains and was therefore used in the series of various carbon sources. Glucose, fructose, glycerol, ethyl alcohol, calcium pyruvate, and calcium lactate were studied as sources of carbon. The last two were used in a concentration of 1.0 per cent and without any $CaCl_2$ in the media.

The tubes were examined after seven days incubation at room temperature. Growth was roughly estimated by the degree of turbidity. Results are presented in the summarized descriptions below. A supplementary microscopic examination was made of all tubes showing even the slightest turbidity. This revealed surprising changes and irregularities, both in size and in shape. For instance, on calcium lactate *S. serpens* showed numerous cells in which one end had enlarged into a distinct ball, sometimes comprising a half of the total cell material. And all three strains of *Vibrio* #2 (see below) on several of the synthetic media showed only round or oval cells, though subsequent inoculation to peptone-beef broth always brought back the original curved form. It is apparent that valid cell measurements in these genera can be made only under standard and reproducible conditions.

Microscopic examination of cells in a hanging drop preparation was made on 12–18 hour broth cultures (30°C.) for all strains. This allowed a division of the strains into six groups. Such a morphological grouping showed good correlation with the data on the utilization of the various sources of carbon. In general, the amount of growth on any one synthetic medium was the same for all strains of a morphological group. Exceptions are noted.

Flagellation was studied by staining the cells from a 12–24 hour agar slant culture by the method of Gray (1926). In the author's hands this method, with minor modifications, has given consistently good results with spirilla for the past five years. Charac-

teristics of growth on various routine culture media have been determined by the usual methods. Summarized morphological and cultural descriptions of six of the organisms isolated are presented below. (Characteristics significantly different from those described by Giesberger are marked with an asterisk.)

Spirillum I, *Spirillum serpens* (Müller) Winter

Strains studied: 18, 20, 64, 66, 67, 70, 82, 83.

Cells motile by means of bipolar bundles of flagella. Cytoplasm often highly granular. Cell thickness: 0.8–1.0 μ; cell length: 10–25 μ; width of spiral: 1.5–2.0 μ; wave length: 7.5–9.0 μ.

Gelatin not liquefied.*

Agar colonies: circular, rough, translucent, the edge irregular; flat, the center characteristically raised and radiately ridged.

Agar slant: beaded, gray, glistening.

Broth: scant, turbid growth.

Litmus milk: unchanged.

Potato: no growth.*

Neither nitrites nor gas produced from nitrates.

Indole not produced.

H_2S formed slowly by most strains.

Aerobic (growth only in upper 3 mm. of tube shake culture).

Optimum temperature: about 32°C.

Utilized as sources of nitrogen: NH_4Cl (except strains 18, 20), asparagin; as sources of carbon: lactate (except strains 18, 20), pyruvate.

Isolated from greenhouse pool, sewage effluent, Mississippi River, lake water, stagnant ditch water.

Spirillum II, *Spirillum itersonii* Giesberger

Strains studied: 11, 12, 13, 15, 16, 17, 78, 80, 81, 84.

Cells motile by means of bipolar bundles of flagella. Cell thickness: .5 μ; cell length: 4–8 μ; width of spiral: 1.2–1.8 μ; wave length: 3.0–3.5 μ.

Gelatin not liquefied.

Agar colonies: circular, smooth, glistening, entire, convex.

Agar slant: filiform, glistening, gray.

Broth: strong, turbid growth.

Litmus milk: becoming slightly alkaline.

Potato: moist brown growth.

Slight production of nitrites from nitrates on solid media, none in broth; no gas.

Indole not produced.

H_2S produced.

Aerobic (growth only in upper 3 mm. of tube shake culture).

Optimum temperature: about 32°C.

Utilized as sources of nitrogen: NH_4Cl, asparagin; as sources of carbon: lactate, pyruvate, glucose, fructose, glycerol, ethyl alcohol.

Isolated from greenhouse pool, Mississippi River.

Spirillum III

Strains studied: 14, 37, 38, 39, 40, 41, 42, 45, 46, 47.

Cells motile by means of bipolar bundles of up to six flagella, somewhat attenuated at one or both ends. Cytoplasm highly granular. Cell thickness: 0.7–0.8 μ; cell length: 2.5–5.0 μ; width of spiral: 1.2–1.5 μ; wavelength: 2–3 μ.

Gelatin not liquefied.

Agar colonies: circular, smooth, entire, convex, translucent.

Agar slant: filiform, glistening, gray.

Broth: strong clouding, turbid sediment.

Litmus milk: becoming slightly alkaline.

Potato: brown growth.

Nitrites and gas produced from nitrates.

Indole not produced.

H_2S produced.

Aerobic (growth only in upper 3 mm. of tube shake culture).

Optimum temperature: about 37°C.

Utilized as sources of nitrogen: NH_4Cl, asparagin; as sources of carbon: lactate, pyruvate, glucose, fructose, ethyl alcohol.

Isolated from: greenhouse pool, stagnant ditch water.

Spirillum IV, *Spirillum undula* (Müller) Ehrenberg

Strains studied: 3, 5, 6, 8, 10.

Cells widely-coiled, motile by means of bipolar bundles of flagella. Cytoplasm often highly granular. Cell thickness: 0.9–1.2 μ; cell length: 8–16 μ; width of spiral: 3.0–3.5 μ; wavelength: 6–8 μ.

Gelatin not liquefied.

Agar colonies: circular, smooth, entire, convex, translucent.

Agar slant: beaded-filiform, glistening, gray.

Broth: slight granular growth.

Litmus milk: growth none or very slight; medium unchanged.

Potato: no growth.*

Neither nitrites nor gas produced from nitrates.

Indole not produced.

H_2S not produced.

Aerobic (growth only in upper 15 mm. of tube shake culture).

Optimum temperature: about 32°C.

Utilized as sources of nitrogen: NH_4Cl, asparagin; as sources of carbon: lactate, pyruvate (* not ethyl alcohol as listed by Giesberger).

Isolated from greenhouse pool, stagnant ditch water.

Vibrio #1

Strains studied: 27, 56, 57, 61.

Cells of seldom more than a single arc, motile by means of one or (rarely) two polar flagella. Cell width: 0.8 μ; cell length: 2.5–4.0 μ.

Gelatin not liquefied.

Agar colonies: circular, smooth, entire, convex, translucent.

Agar slant: filiform, gray growth; a characteristic dark brown pigment diffuses slowly down into the agar.

Broth: clouding scant; medium darkened in time.

Litmus milk: becoming slightly acid.

Potato: no growth.

Neither nitrites nor gas produced from nitrates.

Indole not formed.

H_2S not produced.

Aerobic (growth only in upper 3 mm. of tube shake culture).

Utilized as sources of nitrogen: NH_4Cl (except strain 27); as sources of carbon: lactate (except strain 27), pyruvate, fructose.

Isolated from greenhouse pool, liquid entrapped in a pitcher plant.

Vibrio #2

Strains studied: 29, 36, 48.

Cells seldom of more than a single arc, motile by means of one or (rarely) two polar flagella. Cell width: 1.1–1.3 μ; cell length: 3–5 μ.

Gelatin not liquefied.

Agar colonies: circular, smooth, entire, flat, translucent.

Agar slant: filiform, glistening, gray.

Broth: scant clouding; often a surface membrane.

Litmus milk: becoming slightly alkaline.

Potato: brown growth.

Nitrites and gas produced from nitrates.

Indole not produced.

H_2S produced.

Aerobic (growth only in upper 3 mm. of tube shake culture).

Utilized as sources of nitrogen: NH_4Cl; as sources of carbon: lactate, ethyl alcohol (except also slight growth in fructose by strain 36 and in glycerol by strain 29).

Isolated from greenhouse pool.

The six forms described above have been designated *Spirillum* or *Vibrio* on the basis of the two coincident characteristics by which these two genera are commonly distinguished [cf. Bergey (1939), Migula (1900), Lehmann and Neumann (1927)]. The genus *Spirillum* is characterized by rigid, truly spiral cells, motile by means of polar bundles of flagella. The genus *Vibrio* (or *Microspira* according to Migula) is distinguished by the occurrence of rigid, curved cells of only a single arc (comma-form) and with only one to three polar flagella at one or both ends.

These characteristics are admittedly poor for the separation of the two genera. Older cultures of a *Vibrio* often show a high percentage of cells in definite spirals, while very young cultures of a *Spirillum* contain a large proportion of cells of only a single turn. On the other hand the difficulties of staining flagella are well known. If inadequately stained, the compact flagella-bundle of a distinct *Spirillum* such as *S. undula* may easily be mistaken for a single flagellum. The difficulty of relying on flagellation is illustrated by the case of *Spirillum virginianum*. This was originally described by Dimitroff (1926) as possessing a single polar flagellum. However, cultures of the same strain obtained from the American Type Culture Collection by Giesberger (1936) and by the author have shown polar bundles of flagella.

The author therefore takes the view that until some clear-cut case to the contrary is discovered, the separation of the genera *Vibrio* and *Spirillum* must be based upon flagellation and spiral length as coincident characteristics, however inadequate these may be.

Most of the 57 strains isolated can be placed in six groups, of which four are definitely spirilla and two are vibrios. Several of the strains were lost or became contaminated and were discarded.

And several others showed distinct differences in morphology and cultural characteristics from all of the six listed above. These strains will not be considered in the present paper since no two of them were sufficiently alike to give duplicates for study.

The literature on the genus *Spirillum* has been carefully studied in order to verify Giesberger's nomenclature and to aid in the identification of the species described above. All of the literature thus reviewed is listed in the bibliography, for the most part without specific citation in the text. A common characteristic of the older descriptions is their inadequacy. Whether or not a newly isolated culture should be identified with one of the species described earlier is at best a delicate problem. In this regard the author recognizes the validity of the species described by Giesberger and follows the principle laid down by him (p. 56):

"schien es mir dennoch emphehlenswert, eine neue Systematik der Gattung *Spirillum* in möglichst engem Anschluss an die Ergebnisse früherer Untersucher durchzuführen und wo dies nur möglich schien, die alten allgemein angewandten Artnamen beizubehalten."

The genus *Vibrio* has been considered only incidentally, since methods of isolation and study were directed principally toward the genus *Spirillum*. Of the two vibrios isolated, *Vibrio* #1 corresponds to *Vibrio nigricans* Weibel (Cf. Migula (1900) p. 1013), while *Vibrio* #2 seems to be a previously undescribed form.

Of the four species of *Spirillum* described above, three agree quite well with the descriptions of *S. serpens*,[3] *S. itersonii*,[3] and *S. undula* given by Giesberger. These names have therefore been applied to the corresponding species descriptions. A fourth species, *Spirillum* III,[3] is clearly distinct from any of those described by Giesberger. This organism is clearly a *Spirillum* rather than a *Vibrio*, since in broth cultures observed during the logarithmic phase of growth (18 hours at 30°C.) most of the cells are of more than a single arc, and since flagella stains show many cells with six or eight flagella in each bundle. It differs from *S.*

[3] Cultures of these organisms have been placed with the American Type Culture Collection, Georgetown University, Washington, D. C.

tenue in its ability to utilize ethyl alcohol and in the wave length of its spiral. It could never be considered a tenuous organism, and in fact was originally tentatively described in our records as "short and fat." Its inability to liquefy gelatin and its short wave length clearly set it apart from *S. virginianum.*

The descriptions of nearly 40 spirilla recorded in the literature have been examined without finding any with which *Spirillum* III may be surely identified. In a number of respects it is similar to *Spirillum lipoferum* of Beijerinck (1925) and Schröder (1932). This organism was originally isolated from enrichment cultures of *Azotobacter.* It is described as a short, plump, highly granulated *Spirillum,* motile by means of polar flagella-bundles, varying widely in cell size and proportions, and often containing fat globules. Both Beijerinck and Schröder were chiefly interested in the question of nitrogen fixation by this form and neglected to make any complete characterization. They made no mention of cell dimensions. The presence of fat globules is hardly distinctive since it is shown by other spirilla. Giesberger (1936, p. 24) refused to recognize this as an adequately characterized *Spirillum.* Following Beijerinck's directions, he isolated several strains of a similar organism from garden earth and canal water. These cultures contained cells with generally only a single turn and with monotrichic flagellation. He concluded that the organism was not a true *Spirillum.*

Unfortunately Giesberger's experience does not clarify the situation, since there is no way of establishing the identity of the organisms observed by Beijerinck and by Giesberger. Both Beijerinck and Schröder were quite specific in describing the lophotrichic flagellation. And in fact *Vibrio* #2 (see above) could be identified with Beijerinck's description in nearly every respect except that of flagellation.

It is apparent that at present *Spirillum* III can neither be positively identified with, nor differentiated from, the organism described by Beijerinck as *Spirillum lipoferum.* And under these conditions it seems better to leave the organism described in this way than to add what might be merely another name to the already long list of species of *Spirillum.*

SUMMARY

1. A method of isolation has been described which is particularly adapted to a number of species of *Vibrio* and *Spirillum*. The essential feature of this technique is that agar plate colonies are initiated from cells planted in droplets of liquid media scattered over the agar surface. After several transfers on sterilized infusion media most cultures can be carried on the usual agar slants.

2. Four species of *Spirillum* and two of *Vibrio* have been studied in regard to their morphology, routine culture characteristics, and utilization of various carbon and nitrogen sources in synthetic media. After a critical study of the literature, three of the spirilla have been identified with the previously described species, *S. undula*, *S. itersonii*, and *S. serpens*. A fourth, similar in certain respects to *S. lipoferum* of Beijerinck but not definitely the same, has been described but not named.

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REFERENCES

BEIJERINCK, M. W. 1895 Über *Spirillum desulfuricans* als Ursache von Sulfatreduction. Zentr. Bakt. Parasitenk., Abt., II, **1**, 1–9; 49–59; 104–114.

BEIJERINCK, M. W. 1901 Über oligonitrophile Mikroben. Zentr. Bakt. Parasitenk., Abt. II, **7**, 572–574.

BEIJERINCK, M. W. 1925 Über ein *Spirillum*, welches freien Stickstoff binden kann? Zentr. Bakt. Parasitenk., Abt. II, **63**, 353.

*BONHOFF 1896 Untersuchungen über Vibrionen und Spirillen I. Arch. Hyg., **26**, 162–202.

COHN, F. 1875 Beiträge zur Biologie der Pflanzen, **1**, 169–171. Breslau.

* Translations of the references thus designated were made by WPA personnel and have been deposited with the American Documentation Institute. These may be obtained by ordering Document No. 1428 from American Documentation Institute, Offices of Science Service, 2101 Constitution Avenue, Washington, D. C., remitting $2.25 for microfilm form $22.50 for photocopies readable without optical aid.

DIMITROFF, V. T. 1926 *Spirillum virginianum* nov. spec. J. Bact., **12**, 19–50.
EHRENBERG, C. G. 1838 Die Infusionstierchen als Volkommene Organismen. Leipzig.
ELLIS, D. 1903 Untersuchungen uber *Sarcina*, *Streptococcus*, und *Spirillum*. Zentr. Bakt. Parasitenk., Abt. I, **33**, 88–96.
EMMERLING, O. 1908 Vergärung von Calciumtartrat. Zentr. Bakt. Parasitenk., Abt. II, **21**, 317–318.
FLÜGGE, C. 1896 Mikroörganismen. vol. 2, pp. 527–599. Leipzig.
FUHRMANN, F. 1910 Die Geiszeln von *Spirillum volutans*. Zentr. Bakt. Parasitenk., Abt. II, **25**, 129–160.
*GIESBERGER, G. 1936 Beiträge zur Kenntnis der Gattung *Spirillum* Ehrenberg. Dissertation. Delft.
GRAY, P. H. H. 1926 A method of staining bacterial flagella. J. Bact., **12**, 273–274.
*HÖLLING, A. 1907 *Spirillum giganteum* und *Spirochaete balbianii*. Zentr. Bakt. Parasitenk., Abt. I, **44**, 665–668.
ITERSON, G. VAN 1903 The decomposition of cellulose by aerobic microörganisms. Proc. Konink. Akad. Wetenschappen Amsterdam, **5**, 685–703.
KAUFFMAN, C. H. 1908 A contribution to the physiology of the *Saprolegniaceae*, with special reference to the variations of the sexual organs. Ann. Botany, **22**, 361–387.
*KITASATO, S. 1889 Über die Reincultur eines *Spirillum* aus faulenden Blute, *Spirillum concentricum* n. sp. Zentr. Bakt. Parasitenk., Abt. I, **3**, 72–75.
*KUTSCHER 1895a *Spirillum undula minus* und *Spirillum undula majus*. Zentr. Bakt. Parasitenk., Abt. I, **18**, 614–616.
KUTSCHER 1895b Die Vibrionen und Spirillenflora der Dungerjauche. Z. Hyg. Infektionskrankh., **20**, 44–59.
LEHMANN, K. B., UND NEUMANN, R. O. 1927 Bakteriologie, **2**, 551–557.
MIGULA, W. 1900 System der Bakterien, **2**, 955–1028. Jena.
MÜLLER, O. F. 1786 Animalcula Infusoria, pp. 43–49. Berlin.
SCHRÖDER, M. 1932 Die Assimilation des Luftstickstoff durch einige Bakterien. Zentr. Bakt. Parasitenk., Abt. II, **85**, 177–211.
*SOROKIN, N. 1890 Noch einmal über *Spirillum endoparagogicum*. Zentr. Bakt. Parasitenk., **7**, 123–124.
*SWELLENGREBEL, N. H. 1908 Erwiderung auf die Arbeit des Hern Dr. Hölling —"Spirillum giganteum und Spirochaete balbianii." Zentr. Bakt. Parasitenk., Abt. I, **46**, 1–3.
SWELLENGREBEL, N. H. 1909 Neuere Untersuchungen über die vergleichende Cytologie der Spirillen und Spirochaeten. Zentr. Bakt. Parasitenk., Abt. I, **49**, 529–550.
VAHLE, C. 1910 Vergleichende Untersuchungen über die Myxobakteriazeen und Bakteriazeen, sowie die Rhodobakteriazeen und Spirillazeen. Zentr. Bakt. Parasitenk., Abt. II, **25**, 178–260.
*VINZENT, R. 1926 Isolement et Culture des spirilles et spirochaetes des eaux. Compt. rend. soc. biol., **95**, 1472–1474.

*Vogt 1899 Beitrag zur Kenntnis der Lebensbedingungen des *Spirillum volutans*. Zentr. Bakt. Parasitenk., Abt. I, **25**, 801–804.

Winter, G. 1884 Die Pilze. In Rabenhorst's Kryptogamen-Flora, **1**, 62–66. Leipzig.

*Zettnow, E. 1891 Über den Bau der Bakterien. Zentr. Bakt. Parasitenk., Abt. I, **10**, 689–694.

Zettnow, E. 1896a Bilder von *Spirillum undula majus* bei freiwilligen Absterben. Zentr. Bakt. Parasitenk., Abt. I, **14**, 177–180.

Zettnow, E. 1896b Nährboden für *Spirillum undula majus*. Zentr. Bakt. Parasitenk., Abt. I, **19**, 393–395.

*Zettnow, E. 1897 Über den Bau der grossen Spirillen. Z. Hyg. Infektionskrankh., **24**, 72–92.

Zettnow, E. 1908 Über Swellengrebels Chromatinbänder in *Spirillum volutans*. Zentr. Bakt. Parasitenk., Abt. I, **46**, 193–195.

Zettnow, E. 1916 Breite und Geiszeln von *Spirillum parvum*. Zentr. Bakt. Parasitenk., Abt. I, **78**, 1–3.

PLATE 1

Flagella Stains

Fig. 1. *Spirillum* I (*S. serpens*). 1000×.
Fig. 2. *Spirillum* II (*S. itersonii*). 1000×.
Fig. 3a. *Spirillum* III. 1000×.
Fig. 3b. *Spirillum* III. 1700×.
Fig. 4. *Spirillum* IV (*S. undula*). 1000×.
Fig. 5. *Vibrio* #1. 1000×.
Fig. 6. Undetermined *Spirillum* isolated from stagnant water in Montana. 1000×.

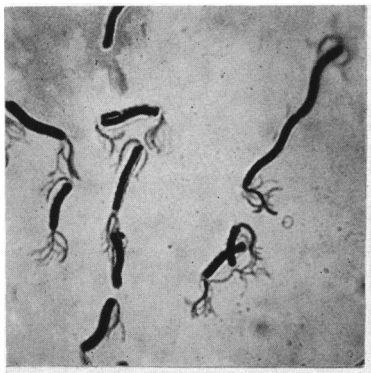

fig. 1

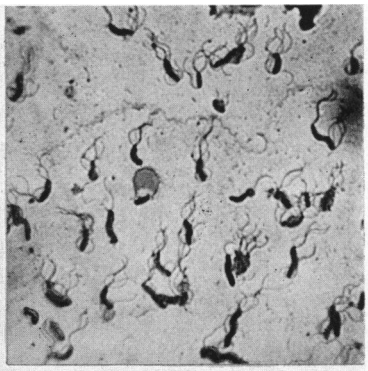

fig. 2

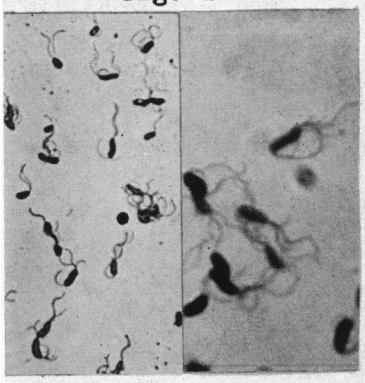

fig. 3a fig. 3b

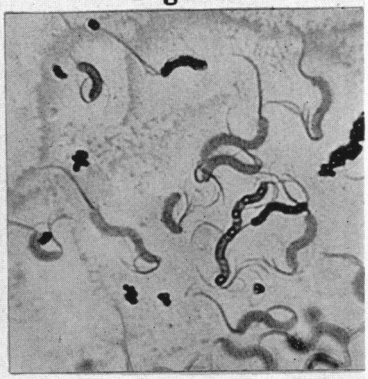

fig. 4

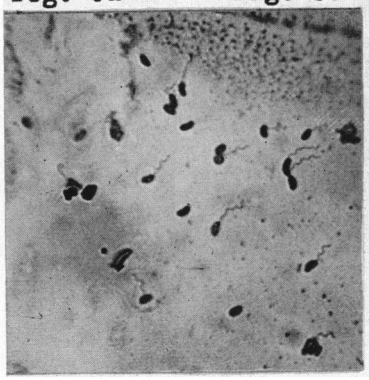

fig. 5

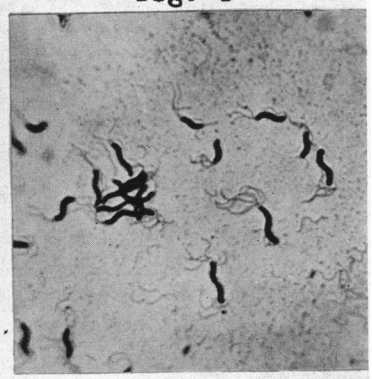

fig. 6

(Jack Myers: Studies on Spirilleae)