NOTES ON A FILTERABLE SPIROCHETE FROM FRESH WATER. SPIROCHETA BIFLEXA (NEW SPECIES).*

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This spirochete was discovered in a filtrate from stagnant water taken from the shores of a fresh water pond in the vicinity of Boston. The filter used was a Berkefeld "V" filter. Bacillus prodigiosis was added as a control and the filtration accomplished by suction. The date of the filtration was July 13, 1913. On August 19 the filtrate which had remained at room temperature was slightly turbid. Examination with the dark field illuminating apparatus showed a very small actively motile spirochete in pure culture. Inoculation experiments done on a variety of media yielded no growth, either of the spirochete or of bacteria. Another tube of this same filtrate was placed in the cold room immediately after filtration and kept at a temperature just above freezing point until October 4, when the tube was placed at 30° C. On examination, November 4 and November 5 with the dark field illuminating apparatus, no spirochetes were found, but on November 7, although the liquid was practically clear, many small spirochetes identical to those found on August 19 were seen. The spirochetes increased in number during several days.

Although we have not been able to cultivate this spirochete, we were able to keep the spirochetes of each tube alive for a period of several weeks and to make a sufficient number of preparations and dark field photomicrographs for purposes of description. Stimulated by our success in cultivating another spirochete obtained from a similar source, many different media were tried consisting of different strengths of hay infusions, broths, synthetic media, sterile water from various sources, all with and without the addition of small quantities of hydrogen sulphide. As these results were unsuccessful the details will not be given. It is sufficient to say that the

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possible importance of slight differences in salt concentration was kept in mind. The failure to secure growth in water from the same pond, rendered sterile by filtration through Chamberland B filters, is perplexing.

This spirochete is characterized by the extreme closeness of the turns, its small size, and curved or flexed extremities (Figs. 1 to 5). The average length is from five to seven microns. The amplitude, or width of the spirals measured from crest to crest is from .2 to .25 micron. The spirals were counted in a small number of individuals and were as follows:

Length of	spirochete,	7	microns;	number of	turns,	22.
"	"	"	8	"	"	"	"	32.
"	"	"	6	"	"	"	"	26.
"	"	"	8	"	"	"	"	30.
"	"	"	7½	"	"	"	"	24.

These counts are based upon photomicrographs taken with the dark field illuminating apparatus at one thousand diameters.

The preparations for photography were made as follows: A drop of the filtrate containing spirochetes in a platinum loop was exposed to the fumes of osmic acid for thirty seconds in order to kill and fix the organisms. This was then rapidly mixed on a warm microscope slide with melted three per cent water agar jelly and a cover-slip put on and pressed down so as to form a very thin layer of jelly containing spirochetes between the slide and cover. It was necessary to resort to this method in order to hold the spirochetes stationary and to obtain them with their length in one plane — which was essential for photography.

The two ends of the spirochetes are thinner than the body and taper to points. They are more or less sharply curved and sometimes have the form of a crook. When alive the spirochete spins with extreme rapidity on its long axis in such a manner that the curved ends give the appearance of solid bulbs or flask-like bodies (Figs. 6 to 10). In addition to this rapid rotation about the long axis there is fairly rapid

translatory motion in either direction of the long axis. The living spirochete is straight and presents the appearance of having a rigid long axis. Occasional slightly flexed forms which are motionless have been seen to straighten out and to begin to spin rapidly. In stained preparations the characteristics of the living organism are lost. The bodies become bent or flexed and the spirals less regular and less closely wound (Fig. 15). Giemsa's stain following osmic acid and alcohol fixation gives the best results. Occasionally very long forms were found showing less perfect spirals (Fig. 14). A few spirochetes with terminal swellings were found (Figs. 12 and 13). Rarely the bodies of the spirochete contain one or more deeply stained chromatophylic granules (Fig. 11).

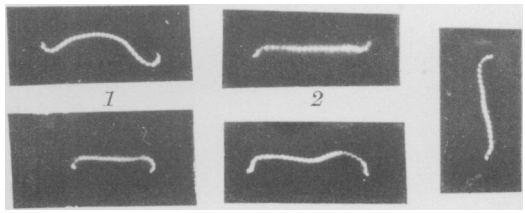
As far as we have been able to determine this is the most delicate of the free living spirochetes. While its filterable nature is not conclusively proved by the single experiment which resulted in its discovery our experiences with another spirochete (Spirocheta elusa), reported in this number, justify the assumption that had a suitable medium been discovered similar results would have been obtained with this one. The curved ends, giving unique appearances to the spirochete in motion, are peculiar to this organism, and for this reason have been utilized in the construction of a name. The name *Spirocheta biflexa* is proposed.

DESCRIPTION OF PLATE VI.

FIGS. 1 to 5 inclusive. — Photomicrographs, dark field illumination. Optical system and camera extension calculated for 1,000 diameters. The method of preparation is described in the text.

FIGS. 6 to 10 inclusive. — Sketches made upon smoked glazed paper to show appearance of the spirochetes in motion.

FIGS. 11 to 15 inclusive. — Photomicrographs of stained preparations; 2,000 diameters. The films were dried in the air, exposed for thirty seconds to the fumes of osmic acid, fixed for ten minutes in absolute alcohol and stained with Giemsa's stain for fourteen to sixteen hours.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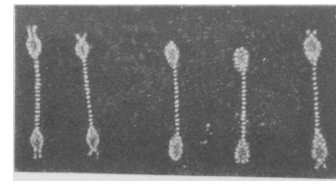
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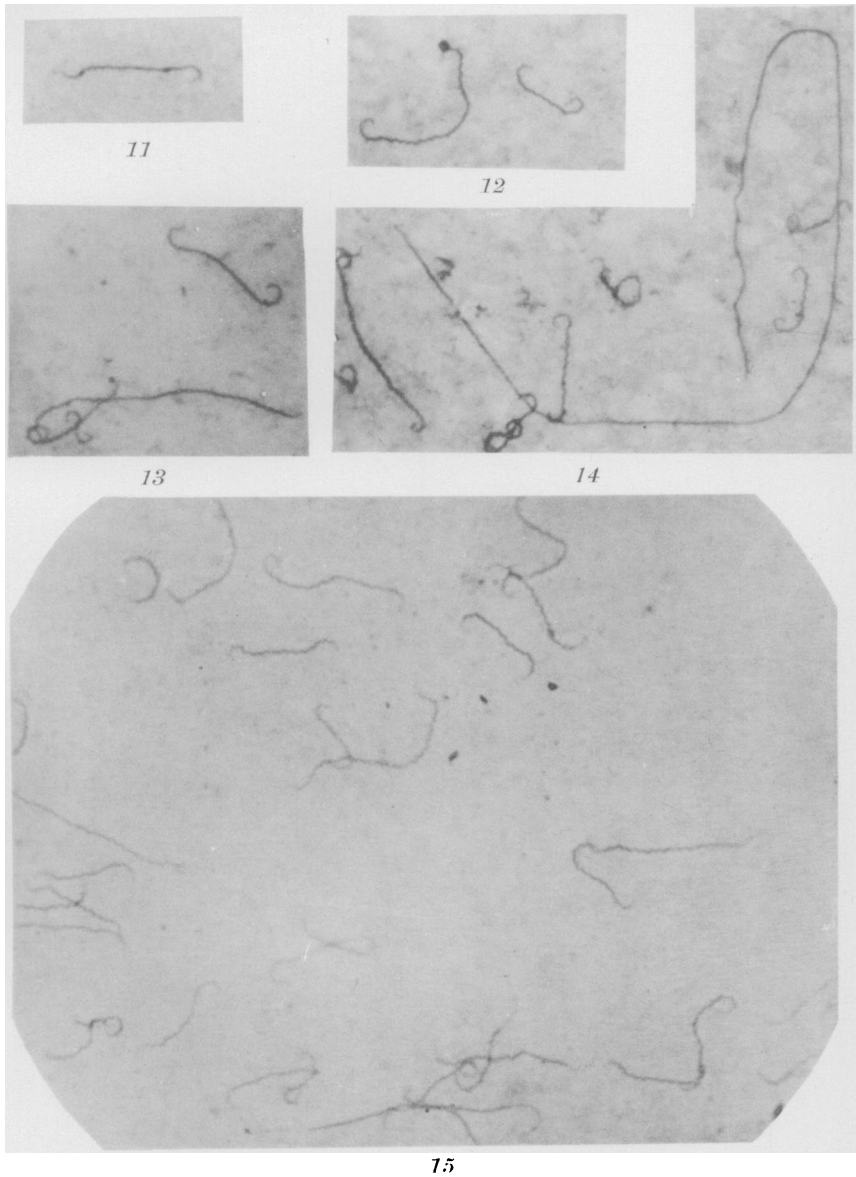
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