

## THE IDENTITY OF FUSIFORM BACILLI AND SPIRILLA.\*

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It has been observed for several years that fusiform bacilli, usually associated with long spirilla,<sup>†</sup> are present in many pathological conditions. They were first demonstrated in cases of ulceromembranous angina (Vincent's or Plaut-Vincent's) and later in cases of ulceromembranous stomatitis, hospital gangrene, noma, pyorrhea alveolaris, appendicitis, subpectoral, brain, and thigh abscesses, and some other morbid processes. Both organisms have been observed in the healthy mouth and genitalia. In the majority of cases the bacilli and spirilla have been recognized in smear preparations, made from the seat of the disease, or in stained sections of tissues. Both organisms have been grown in mixed cultures. Fusiform bacilli have been isolated in pure culture by Veillon and Zuber, Ellermann and Weaver. The fusiform bacilli isolated by Dr. Weaver, and described in an article by Dr. Weaver and myself,<sup>‡</sup> appeared to be the same as those described by Ellermann, and compared quite closely with those found in smear preparations made from the seat of the disease. Although spirilla resembling those from the tissues have been grown in mixed cultures with fusiform bacilli and other organisms, all efforts to obtain them in pure culture have up to this time been unsuccessful.

Most authors have believed that the fusiform bacilli and spirilla are entirely distinct organisms and that they act in symbiosis, the spirilla serving to increase the virulence of the bacillus. Some observers have maintained that they are different forms of one organism (Seiffert, Perthes, Sobel, and Herrman). As until recently no pure cultures of the bacilli and none of the spirilla have been obtained, it has been impossible to prove whether they were distinct organisms or not.

\*Received for publication January 2, 1906.

<sup>†</sup>The term "spirilla" and "spirochetæ" have been used indiscriminately in the literature on this subject.

<sup>‡</sup>*Jour. Infect. Dis.*, 1905, 2, p. 446.

While studying some pure cultures of fusiform bacilli, it was observed that after they had grown from 48 hours to five days, spirilla were formed which greatly resembled those seen in smear preparations made from the gums and from the various pathological lesions in which spirilla had been observed. No such spirilla had been seen in the cultures previously described by Dr. Weaver and myself, but on examination of some of these old, dead cultures, spirilla were found similar to those observed in the living cultures. The spirilla could be easily overlooked, as often they are present for only a few days in any considerable number, and are more readily seen when stained by carbol-gentian-violet than by carbol-fuchsin, which was usually employed in the earlier cultures. Ellermann states that no "spirochetæ" were present in his pure cultures of fusiform bacilli.

The three organisms which are here described were isolated from the gums of healthy mouths. In one case there was considerable tartar around the teeth, which were badly decayed. In smear preparations made from the gums the fusiform bacilli and spirilla corresponded to those found in the pathological lesions in which these organisms are associated. Their morphology does not need any description at this time. The material from the gums was smeared over the surface of a series of slants of ascites agar (1:3). After anaerobic incubation at 37° for two or three days the growth of fusiform bacilli appeared in delicate, whitish colonies, 0.5-2 mm. in diameter, resembling colonies of streptococci. By inoculation from these colonies pure cultures were obtained. In one case the first two tubes contained streptococci and fusiform bacilli, and the third, fusiform bacilli alone.

#### CULTURAL PROPERTIES.

The organisms are obligate anaerobes growing at 37°, but not at room temperature. The organisms show no progressive motion. They possess considerable vibratory motion, mostly at the extremities, but sometimes it extends from one end to the other. The organisms were examined by Dr. D. J. Davis with the ultramicroscope, and this motion was seen to be similar to that of dead cilia in cerebrospinal fluid, as observed by him.<sup>1</sup> As in the case of the cilia, the motion probably depends upon physical factors. With the ultramicroscope spirilla from the gums were seen to possess no progressive, but a decided cork-screw and lateral motion. This lateral motion is probably similar to the vibratory motion described above. No flagella have

<sup>1</sup> *Trans. Chicago Path. Soc.*, 1904, 6, p. 225.

been demonstrated. Johnston's staining method, which was employed, readily stained typhoid flagella.<sup>1</sup>

The cultures were grown by Wright's method, by saturating the cotton stopper with a strong solution of pyrogallic acid in a 5 per cent solution of sodium hydroxide, and closing the tube with a tightly fitting cork, sealed with paraffin.

*Slants of ascites-agar.*—At the end of 24 to 48 hours a delicate, whitish, irregular growth appears, usually with delicate colonies along the edge from  $\frac{1}{2}$  to 2 mm. in diameter. A flocculent deposit collects at the bottom of the fluid of condensation.

*Loeffler's blood-serum.*—In 24 to 48 hours a slightly moist, irregular growth appears, with colonies along the border. A flocculent deposit collects at the bottom of the fluid of condensation.

*Rabbit's blood-agar (1:4).*—The growth is similar to the preceding but of a brownish color.

*Agar slant.*—In 24 to 48 hours a very delicate, whitish growth forms with small colonies at the border.

*Glycerin-agar slant.*—There is no growth.

*Glucose-agar stab.*—A delicate, whitish growth with small lateral prolongations develops along the needle track in 24 to 48 hours. A little gas is usually formed.

*Stab in glucose-agar and ascites-fluid (3:1).*—The growth is similar to the preceding, but somewhat more abundant. A little gas is formed.

*Litmus milk.*—In 48 hours there is a moderate growth, the medium being decolorized. There is no coagulation. After oxygen is admitted the medium assumes its original color.

*Potato.*—There is no growth.

*Dextrin-free broth.*—No growth occurs.

*Plain nutrient broth.*—There is no growth. Growth does not occur in broth containing 2 per cent glucose, but does occur if it contains between 0.25 and 1 per cent. The growth appears in 24 to 48 hours. It settles somewhat to the bottom, but the fluid is turbid throughout.

*Ascites broth (1:3).*—A slight, flocculent growth appears in 24 to 48 hours. It settles in a thick mass to the bottom, leaving a clear fluid above. On shaking, the growth may be separated into visible granules.

*NaCl agar (2 per cent).*—No growth.

*NaCl broth (2 per cent).*—No growth.

*NaCl ascites broth (2 per cent).*—No growth.

In all of the successful cultures a somewhat offensive odor is given off.

The fusiform bacilli described by Dr. Weaver and myself grew on glycerin agar, and in dextrin-free broth. They did not grow in milk. Although there are these differences between those cultures and the ones here described, the variations are too slight to conclude that the organisms are not the same. The number and age of the bacilli inoculated are important and may account for the variations in results. However, it doubtless will be found that there are several varieties of fusiform bacilli, since those already isolated differ somewhat from one another.

<sup>1</sup> *Loc. cit.*, 1905, 2, p. 343.

The organisms have retained their vitality 55 days, the length of time which has elapsed since their isolation. It seems to be true that later generations of these organisms have considerably less vitality than the organisms first isolated. The factors which especially influence the formation of spirilla have not yet been ascertained. Further cultivation of the organisms will probably show the best conditions for their development. Occasionally a culture is found in which short bacilli only are present. Sometimes no growth occurs after the first 24 or 48 hours, which may account for the absence of filaments and spirilla. Filaments and spirilla are formed as late as the 10th generation, the generation now under observation.

#### MORPHOLOGY AND STAINING PROPERTIES.

The organisms present the same morphological appearance in whatever media grown. They are extremely polymorphous, appearing as quite different organisms at different periods of their development. They are, usually during the first 24 hours of their growth, delicate, pointed rods from 3 to 10  $\mu$  in length. As a rule they show deeply staining bodies or bands, most often two in number, and not situated at the ends. The bacilli are usually straight, but sometimes bent. The bacilli often strikingly resemble barred forms of diphtheria bacilli. They are often slightly larger in the center, but not always. In these young cultures a few smaller, 1.5 to 4  $\mu$  in length, and plumper bacilli are also sometimes found. They have very thick unstained bodies, with deeply stained rounded ends. The swollen bodies resemble spores, but do not stain as such. Both forms appear in pairs, end to end, at obtuse angles, and in rows.

In some of the longer bacilli, usually during the first days of their growth, a few spores are seen. There is usually one in a bacillus, but occasionally there are two. They are situated either at one extremity or near the center. They may be seen within, or partly without, or entirely outside, the bacillus. The development from the round spores into the very short, plump bacilli with dark extremities may be observed in a hanging drop. The various stages can also be found in stained preparations. The spores are best seen when stained with carbolfuchsin. They retain the stain when treated as tubercle bacilli and decolorized with 1 per cent sulphuric acid solution in water.

In 24 to 48 hours, or even later, filaments of various lengths are formed. Some of the filaments are of the same diameter throughout and contain, as a rule, deeply staining bodies sometimes round, oftener like bands. Similar ribbon-like forms are frequently seen in smear preparations from the gums. Some of the filaments stain uniformly. As a rule many of the filaments are seen to be made up of strings of bacilli, which are joined at the dark bodies. The bacilli forming the filaments vary in size and shape as the bacilli in the earlier cultures do.

The filaments are sometimes straight, sometimes wavy. Involution forms in a great variety of shapes are frequently observed. In the older cultures the filaments often stain irregularly. Clear spaces resembling vacuoles are occasionally seen. They are simply the unstained bodies of the short, plump bacilli, which in chains are so close together as to appear like vacuoles.

Soon after, or simultaneous with, the appearance of the filaments, most often on the fourth or fifth day, spirals are observed, sometimes in enormous numbers. As a rule they stain uniformly; others show the dark bodies seen in the short bacilli and filaments. Often it is easily seen that the spirilla are made up of chains of short bacilli similar to the straight filaments. The spirals are sometimes in the form of cork-screws, more often the turns are not so sharp, nor so deep. They form from one to twenty curves. The turns are sometimes rounded, sometimes very pointed. The pointed ones are especially marked when the spirilla can be seen to be made up of bacilli and when there is only one bend. Some of the longer spirals extend across the whole field, more often they are shorter, showing four or five turns. These shorter forms are from 5 to 10  $\mu$  in length. They vary considerably in the depth of the curves as do the uncultivated spirilla. The ends are usually pointed. Toward the extremities the curves sometimes become more and more broad. Involution forms are seen in the spirilla as well as in the filaments. In some of the cultures the spirilla alone are found, but usually filaments and short bacilli are also present. By the 10th to the 15th day fewer filaments and spirilla are seen, but as a rule, even in the older cultures (55 days), spirilla can still be found.

Both the bacilli and spirilla stain by methylene blue, gentian-

violet, Giemsa, Romanowsky, carbolfuchsin and carbol-gentian-violet. The last stain was found to be the most satisfactory for staining the spirilla. A solution was used containing 1 c.c. of alcoholic gentian-violet and 10 c.c. of a 5 per cent carbolic acid solution. The smear preparation is allowed to dry in the air. Without pre-

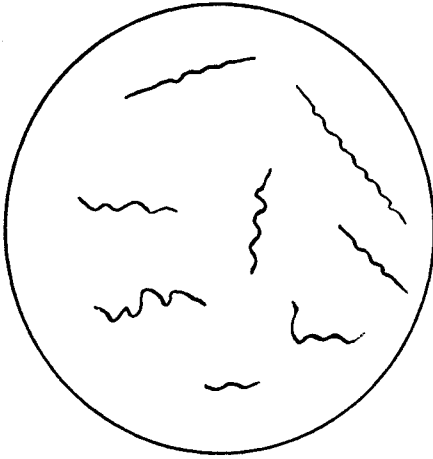


FIG. 5.—Drawing shows spirilla of different shapes seen in four-day-old culture on ascites agar.  $\times$  about 1000.

viously fixing the specimen, the stain is dropped on and is allowed to boil. The preparation is washed in water, dried, and mounted. The deeply staining bodies appear with all the stains. Neither the bacilli nor the spirilla stain by Gram's method. That the spirilla are not artefacts follows from their appearance in hanging-drop preparations, their appearance in stained preparations, whether fixed by heat or not, and when

stained with all the various stains previously mentioned, and from their occurrence with the various culture media in which the bacilli grew.

#### INOCULATION EXPERIMENTS.

Cultures containing short forms and also cultures containing spirilla were injected subcutaneously and into the muscles of guinea-pigs without any result up to this time.

The cultivated fusiform bacilli and spirilla were compared with the fusiform bacilli and spirilla as found in smear preparations from 35 different sources: three from cases of ulceromembranous angina; two from cases of combined ulceromembranous angina and stomatitis; one from a case of noma; 14 from tartar and gums of healthy mouths. The artificially cultivated and uncultivated organisms closely resemble each other in their morphological and staining properties. All the variations, which are many, in form and size, seen in the uncultivated organisms are to be found in these cultures.

In certain fields the spirilla and fusiform bacilli present pictures strikingly similar to those seen in smears made direct from the mucous surfaces.

The bacilli have been described as non-motile, both in fresh specimens and in cultures. The uncultivated spirilla seem to vary in the degree of their motility, but are sometimes immobile, so that the fact that the cultivated ones show no motility would not speak against the identity of the two spirilla.

As the cultures described, although isolated from healthy mouths, correspond so closely with those previously studied, which were obtained from cases of ulceromembranous angina and stomatitis, and diphtheria, it is probable that they are all identical. This is possible since apparently the same fusiform bacilli and spirilla occur in normal as well as in pathological conditions. There is good evidence that these organisms are the exciting agents of the various morbid processes with which they are associated on account of their presence in the lesions in large numbers and because of successful animal inoculations. From these facts and from the similarity between the cultivated and the uncultivated organisms, it would seem that the spirilla found in these lesions are also simply a stage in the growth of the fusiform bacillus, and that the bacilli and spirilla are not distinct organisms, as has been thought. Further study of these organisms will be necessary to settle this question indisputably. That spirilla or spirochetæ can develop from bacilli is now evident; whether they all do can be shown only by further investigation. If the spirilla found in the tissues are simply a later stage in the development of the fusiform bacillus, the fact may be explained why sometimes bacilli alone, spirilla alone, or a combination of the two occur. As a rule, if lesions are examined early, the bacilli only are found and later the spirilla. It has been observed that the cases in which the bacilli only occur are milder than when the two forms are associated. This may be accounted for by the fact that conditions are unfavorable for further growth of the bacillus so that the spirillary stage is not reached. It is stated that in cases in which deep destruction of tissue occurs, the spirilla are constantly present. This may be explained by the possibility that the length

PLATE 2



FIG. 1.



FIG. 2.

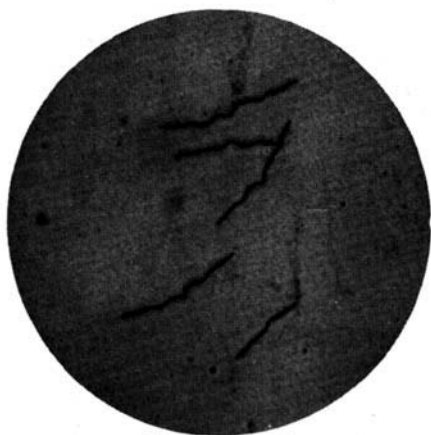


FIG. 3.



FIG. 4.



of time required for the destruction of tissue and for the growth of spirilla may be the same.

It may be true that other bacilli, which up to this time have not been artificially cultivated, also formed spirilla in their growth.

The theory has been advanced by Wright<sup>1</sup> and Mackie<sup>2</sup> that fusiform bacilli and spirilla are forms of trypanosomes. From the study of these pure cultures, no evidence of their protozoan nature could be obtained.

I wish to thank Dr. Hektoen and Dr. Weaver for many suggestions.

<sup>1</sup>*Lancet*, 1904, 2, p. 73.

<sup>2</sup>*Ibid.*, 1905, 2, p. 110.

#### EXPLANATION OF PLATE 2.

(Photomicrographs 1, 2, and 3 are of smears stained with carbol-gentian-violet, and 4 is of a smear stained with carbofuchsin.  $\times 1500$ .)

FIG. 1.—Pure culture grown 48 hours anaerobically on Loeffler's blood-serum.

FIG. 2.—Pure culture grown 48 hours anaerobically in the fluid of condensation of Loeffler's blood-serum.

FIG. 3.—Pure culture grown four days in ascites broth.

FIG. 4.—Smear from gum in normal mouth.