

THE SEROLOGICAL CLASSIFICATION OF FUSIFORM BACILLI

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Fusiform bacilli have been known and studied for many years, but relatively little has been done toward classifying these organisms. Several investigators have mentioned the probability that there are several different types. By means of fermentation studies, Krumwiede and Pratt (1913) found two types of fusiform bacilli, one of which fermented sucrose, while the other type did not. Knorr (1922), basing his classification upon morphological characteristics, described three types of fusiform bacilli. No one, however, seems to have studied the serological aspect of this group of bacteria, being deterred possibly by the many obvious difficulties. Broth cultures of fusiform bacilli are impractical for serological work, necessitating the use of surface culture methods for cultivating the organisms. In the past, such methods have been seldom used in the isolation of fusiform bacilli, investigators preferring the shake culture method for this purpose. Knorr was unable to obtain surface cultures of certain of his types of bacilli, even after prolonged cultivation in deep agar tubes to assure acclimatization to artificial mediums. Ellermann (1904), Larson and Barron (1913), Weaver and Tunnicliff (1905), Tunnicliff (1906; 1911; 1923), Mellon, (1919), Krumwiede and Pratt (1913), Brams, Pilot and Davis (1923), and others have isolated and grown these organisms in surface cultures; not, however, in the quantities needed for a serological study. Before such a study could be undertaken, therefore, methods had to be developed both for the rapid cultivation of large amounts of these organisms, and for their rapid isolation from very impure

material; in other words, methods which would enable one to secure surface cultures of known purity, with no possibility of contamination. That such methods are needed may be seen by referring to the literature, in which investigators speak of their "pure" cultures of fusiform bacilli growing aerobically after a few generations; of granules which drop out of fusiform bacilli and give rise to spirilla, and of the transformation of their "pure" fusiform bacilli into motile spirilla, after varying periods of incubation. These observations are sufficient to justify one in the belief that the cultures described by these investigators were impure. As will be shown later, such departures from the typical organism indicate the admixture of other types.

Fusiform bacilli grow in symbiosis with other organisms to a greater extent than has been realized. Some of these contaminating organisms may be so small as to be mistaken for debris, especially when liquid cultures of the organisms are studied. Even in surface cultures, colonies of the contaminating organisms may be too small to be seen with the naked eye. Therefore, since only the surface culture method of isolation, microscopically controlled, offers a means of securing these organisms in a state of unquestioned purity, this method has been used exclusively in the present study. Of course the isolation of a single cell is the most desirable procedure, but it is doubtful if a growth could be secured from an individual cell. The method has not been used in this investigation.

Plaut in 1894 described fusiform-like bacilli found in cases of ulceromembranous angina. Vincent in 1896 described fusiform bacilli and spirilla present in hospital gangrene infections. Bernheim in 1897 reported 30 cases of ulcerative stomatitis and angina in which he found fusiform bacilli in association with spirilla. Vincent in 1898 reported 14 cases of ulceromembranous angina in which these same organisms were present. This infection has since been known as "Vincent's angina."

Veillon and Zuber in 1898 first isolated fusiform bacilli in pure culture. Abel in 1898 succeeded in obtaining the organism in impure culture and kept it alive in this condition for several generations, but was unable to purify his cultures. Lewcowicz

(1903) secured a pure culture of the organism from an infected mouth by isolating it in deep ascitic agar tubes. Pure surface cultures were obtained by Ellermann (1904), who used sodium hydroxide and pyrogallic acid to secure anaerobic conditions.

Fusiform bacilli have frequently been found in lesions about the mouth and throat, usually in association with cocci and spirilla. Weaver and Tunnicliff (1905) and Tunnicliff (1906; 1911; 1923), Krumwiede and Pratt (1913), and others have repeatedly found them in ulceromembranous angina. Matzenauer (1902) Rona (1905), Seiffert (1901), Perthes (1899), Tunnicliff (1911), Krumwiede and Pratt (1913) and others have found them in noma. The two latter investigators have isolated them from carious teeth and pyorrhea. Keilty (1922), while examining the gums of 200 patients, found fusiform bacilli and spirochetes in almost every case. McKinstry (1917) found similar organisms in the mouths of 95 out of 230 healthy recruits during the war. Tunnicliff has observed them in the normal mouth, in diphtheria of the tonsil, and in gingivitis. Brams and Pilot (1923), Tunnicliff (1923) and others have observed them in the normal tonsil. In 1923 the latter investigator isolated a strain of *B. fusiformis* from a normal tonsil, in which she found motile spiral-like organisms. She believed these organisms to be true spirilla, formed in either of two ways: first, by the development of the spirillum from a granule which had fallen out of a fusiform bacillus; secondly, by the rearrangement of the protoplasm within the fusiform bacillus into a spiral-like form, which was then liberated by the breaking down of the cell wall. These observations have not been verified.

Fusiform bacilli in association with various spirochetes and spirilla may cause abscesses in different parts of the body. Lichtwitz and Sabrazes (1899) have observed them in abscesses about the mouth; Schmorl (1907) in a liver abscess; Silberschmidt (1901), Pilot and Davis (1924), Dick and Emge (1914) in brain abscesses. The latter investigators found a brain abscess caused by a pure culture of fusiform bacilli, no cocci or spirilla being present. Pollard (1905) found fusiform bacilli in leg abscesses. Silberschmidt has reported finding them in empyema of the antrum of Highmore.

Pilot and Davis (1924) found fusiform bacilli and spirochetes in pulmonary tuberculosis, lung cavities and bronchiectasis. Rona (1905) found them in two cases of pulmonary gangrene. Greeley (1918), Campbell and Dyas (1917), and others have observed them in bronchitis; Miller (1906) in alveolar abscesses, and McNeill (1924) in an infection of the parenchyma of the lungs, which clinically was indistinguishable from pulmonary tuberculosis.

Müller and Scherber (1904) found these organisms in 50 cases of erosive and 6 cases of gangrenous balanitis, naming the infection "the fourth venereal disease." This disease was previously described by Bataille and Berdal in 1891 under the name of "balano-posthite érosivee circinée." Scherber (1910) later reported 81 cases of this disease. Corbus and Harris (1909), Corbus (1913), Owen and Martin (1916), Brams, Pilot and Davis (1923), Campbell and Dyas (1917), and others have confirmed his findings. Brams, Pilot, and Davis (1913) and Pilot and Kanter (1923; 1924) as well as others have shown that the normal genitalia of the lower classes harbor fusiform bacilli and spirochetes in a large percentage of the cases, both male and female. McConnell (1916) found these organisms in an infection of the cervix.

Generalized infections due to *B. fusiformis* are rare. Larson and Barron (1913) isolated a fusiform-like organism from the blood stream of a patient dying of gangrene. Their description of the organism leads one however to doubt its identity with fusiform bacilli. The same may be said of the "fuso-spirillary" organism isolated by Mellon (1919) from a patient in whom the organism caused a generalized infection, the seat of infection probably being in the appendix.

From the available statistics, it may be seen that fusiform bacilli most frequently attack the mucous membranes. Under special conditions, however, they may attack almost any organ of the body, producing, in some cases, a rapidly advancing necrosis of the tissues, which unless quickly checked, may terminate fatally. It is unfortunate that so few routine anaerobic cultures are made from pathological material, as our knowledge concerning

the distribution of these organisms might otherwise be greatly increased.

ISOLATION AND DESIGNATION OF CULTURES

The data presented in the present paper are based upon a study of 18 pure cultures of fusiform bacilli. In studying this number of cultures, a designation of each strain which would classify it as to its source and morphology was found desirable. Roughly speaking, there are four types of fusiform bacilli which may be separated upon the basis of morphology alone: a narrow, filamentous type; a short, slender type; a broad, stubby type, and one which grows characteristically in long chains, forming wavy filaments which might be mistaken for spirilla. Accordingly, the following system of nomenclature was devised. Cultures from normal areas or from lesions were designated by the letters N or L, respectively. Following these letters were placed letters designating the characteristic morphology of the organism; F for the filamentous type, S for the slender type, B for the broad type, and W for the wavy, spiral type of organism. Similar cultures isolated from different sources were separated by placing Arabic numerals after the type letters.

The following list shows the source of each of the cultures studied:

- LF1. Carcinoma of the tongue
- LB1. Ulcerated tonsil. Vincent's angina
- LB2. Carious tooth
- LW1. Tonsillar granule, from excised tonsil
- LW2. Excised tonsil
- NW1. Tartar from teeth
- LS1. Same source as LW1
- LS3. Excised tonsil
- LS4. Excised tonsil
- LS5. Carious tooth
- LS6. Excised tonsil
- NS1. Normal tooth
- NS2. Same source as NW1
- NS8. Tooth with very small cavity

NS10. Normal gingiva, extremely dirty

NS12. Tartar

NS13. Tartar

NS14. Tartar

The organisms were isolated by means of surface culture methods only. Undiluted pus, tartar or mucus from the infected area was streaked over the surface of blood agar plates, by means of a special apparatus, devised solely for this purpose, to which was given the name "inoculating machine." By its aid, the plates were streaked in a series of concentric circles, and a far better separation of the colonies on a plate was possible than by hand streaking. By the aid of this procedure the isolation of fusiform bacilli has become a simple matter. A description of the apparatus will be given elsewhere.

INCUBATION OF CULTURES

The inoculated plates were incubated exclusively in anaerobic jars. The method used was that described by the author (Varney, 1926), in which anaerobiosis is secured by burning phosphorus within a tightly sealed glass chamber. A metal rack is constructed, so as to hold the petri dishes. This fits into a standard sized museum jar, and on top of the plates is placed a container for holding the phosphorus. A little water is placed in the bottom of the jar, the phosphorus placed within its container, and the jar tightly sealed. The phosphorus soon takes fire, and quickly establishes anaerobic conditions. Suitable guards are provided to overcome the danger of breakage of the jar.

In practice, after the jar was loaded, it was placed in the incubator and incubated for forty-eight hours. At the expiration of this period, the jar was opened, the phosphorus container immediately removed to the hood, or flooded with water, and the plates removed for examination.

The high moisture content within the jar during incubation greatly aids the growth of fusiform bacilli, and a much heavier growth is obtained than with any other of the common anaerobic methods. Due to the high moisture content, some liquid may

find its way into the bottom of the inverted plates during incubation. If present, this moisture should be removed before an examination is attempted, by pressing the opened, inverted plate down upon a piece of dry filter paper. The plates may then be safely examined.

EXAMINATION OF PLATES

The characteristic morphology of colonies of fusiform bacilli cannot be easily seen when they are examined under a monocular microscope. A dissecting microscope, fitted with a special base, was used for the examination of plate colonies. Instead of using the regular stage, much better results are obtained if a stage is constructed, at an angle of 10° from the horizontal. Plates are placed on this stage, and the colonies viewed by reflected, rather than by transmitted light. Seen in this manner, they are very characteristic, and are distinguishable from colonies of other organisms. When a typical colony is found, a portion of it is picked, by the aid of a sharply pointed needle and transferred to a slide, where it is stained and examined. If typical fusiform bacilli are found, free from other organisms, the remainder of the colony is picked and streaked over the surface of a fresh blood agar plate, which is then incubated for forty-eight hours in the anaerobic jar. If upon examining this plate, none but fusiform colonies are found, it is usually safe to assume that the culture is pure. If but a single contaminating colony is found, however, a new colony should be picked, and the process repeated.

Before the possibility of a mixed growth of fusiform bacilli and other organisms can be definitely ruled out, a well streaked plate culture must be incubated for not less than 6 days, then very carefully examined under the binocular for the presence of contaminating colonies. By this means it is often possible to detect extremely small colonies, from 0.025 to 0.0125 mm. in diameter, growing in supposedly pure cultures of fusiform bacilli, whereas a stained smear from the plate, or a naked eye examination, would show no impurity. In this work, it was found that when a culture gave off a very foul odor, such as has been re-

peatedly mentioned in the literature, it was invariably an indication of the impurity of the culture. Usually in these cases, the contamination was due to bacteria which grew in the extremely small colonies already mentioned. Pure cultures of fusiform bacilli do not give off a foul odor. It is important to know that fusiform bacilli may be contaminated with bacilli, which, by reason of their small size or minuteness of colony, are not detectable by an ordinary examination of a supposedly pure culture.

CULTURE MEDIA USED

When grown on dissimilar lots of media, fusiform bacilli show remarkable changes of shape, hence every precaution should be observed to keep lots of media as nearly uniform as possible. Even on similar lots of media, some types of fusiform bacilli show wide variations in size in different generations. Most fusiform bacilli, however, retain their characteristic morphology, even after many generations, when grown on identical lots of media, but lose it immediately when placed on a medium unlike that to which they have become accustomed. This is shown in figures 9 and 10. Figure 9 shows the normal form of the organism, grown on a medium to which it had become accustomed. The same generation of the same culture, when grown on a medium containing less blood, grew in the form shown in figure 10. If the characteristic morphology is to be retained, therefore, even these slight differences in successive lots of media must be obviated.

Blood agar is the best medium on which to grow surface cultures of fusiform bacilli. The agar base of the medium used in this work is composed of proteose pepton 1 per cent, Liebig's beef extract 0.3 per cent, sodium chloride C.P. 0.5 per cent, and washed, dried agar-agar 1.7 per cent in distilled water. Tap water should never be used. The ingredients should be melted with as little heating as possible, and the reaction very carefully checked before the final sterilization. The final reaction should be pH 7.4. The agar is flaked in carefully measured amounts, so that the proper amount of blood may be added later. Just before use, a flask of agar is melted, cooled to 50°C., and exactly

4 per cent citrated blood added. After thorough mixing, plates are poured or the medium tubed. In this laboratory, large batches of the agar base are made at one time, for the sake of uniformity, and stored in the ice box.

If human beings are used as a source of blood, certain precautions must be observed if successful results are to be obtained. Human blood, secured from clinic patients, has been used exclusively in this work. From time to time certain lots of media failed to support a growth of fusiform bacilli, or but one to two colonies developed, even after long incubation, following a heavy inoculation of the medium with a vigorous culture. Before the source of the trouble was found, some dozen cultures were lost as a result of their failure to grow on this medium. It was finally discovered that the trouble lay in using blood from persons undergoing treatment for syphilis. This blood may contain enough arsenic to inhibit the growth of fusiform bacilli, or so weaken them that sub-cultures cannot be obtained. Other bacteria, with the exception of spirilla, will grow readily on such media. For routine cultivation of fusiform bacilli, therefore, care must be taken to exclude from the medium blood containing arsenic, if successful results are to be obtained.

Under certain circumstances the presence of arsenic in the medium may be of advantage, however. It may be that a great deal of the confusion which has arisen over the relationship between fusiform bacilli and spirilla has been due to reports based on the study of impure cultures, mixtures of these two organisms. In ruling out all possibility of a symbiotic growth between these two organisms, advantage may be taken of the fact that spirilla are somewhat less resistant to the action of arsenic compounds than are fusiform bacilli. If the culture is grown for one or two generations on media containing enough arsenic barely to permit the fusiform bacilli to grow, all spirilla will be killed.

These phenomena have been well illustrated in the present work. Before using arsenic free media exclusively for isolation purposes, no spiral organisms were ever encountered in any of the cultures examined, though thousands of slides were made from cultures one to 355 days old. Frequently "shadow forms"

were seen: old, degenerate cells from which most of the protoplasm had escaped, leaving merely a light staining shell, but no spiral forms were ever seen. Nor have they been seen in these same cultures grown for many generations on arsenic free media.

In later cultures, however, which were isolated and grown on arsenic free media, both wavy types of fusiform bacilli and true spirilla have been found and grown in pure culture. From one supposedly pure culture of fusiform bacilli, growing on a plate, a true spiral organism was isolated after ten days incubation. Transferred repeatedly to arsenic media, it failed to grow, though in each case a growth of fusiform bacilli was obtained. Accordingly, in this laboratory it has been made a practice to cultivate all strains of fusiform bacilli on arsenic blood agar for several generations, in order to rule out the possible presence of a spiral organism.

From these observations, one is justified in the belief that the possibility of a symbiotic growth between fusiform bacilli and spirilla has not been ruled out in those cultures in which motile spirilla have been reported. While the confusion may have arisen through mistaking the wavy type of fusiform bacillus for spirilla, this seems improbable, since there is little real resemblance between the two organisms. It is possible, however, that smears made from liquid or semi-solid cultures of the wavy type of fusiform bacillus could not be differentiated from true spirilla, though no motility is present in the former. The two organisms may be readily differentiated when grown on surface cultures, however.

Experiments have been planned to ascertain the exact quantity of various arsenic salts needed to inhibit the growth of spirilla, while still permitting the growth of fusiform bacilli. At the present time no recommendation can be given as to the exact quantity needed.

STOCK CULTURES

Stocks of all bacilli isolated have been kept in autoclaved brain medium under vaseline, in a modified Eberson's yeast medium, and on the surface of blood agar slants under pyrogallie acid.

Pure cultures of fusiform bacilli grow poorly in Eberson's medium, but in mixed cultures, preferably with streptococci, they grow readily. Brain cultures remain viable for several months, whereas slant cultures on blood agar should be transferred every ten to twelve days in early generations.

IMMUNIZATION OF ANIMALS

Beginning with the tenth to the fifteenth transfer of the pure cultures, rabbits were immunized by the intravenous injection of living forty-eight-hour cultures of LF1, NS13, NS14, and LS5, all of which showed morphological differences when examined under the microscope.

Two methods were used in preparing the antigens. In the first method, the organisms were grown on blood agar slants, incubated in the anaerobic jar for forty-eight hours, then washed off with sterile saline. In the second and preferable method, the organisms were streaked over the surface of blood agar plates by means of the inoculating machine, incubated for forty-eight hours in the anaerobic jar, then washed off with saline. Too heavy an inoculation decreases rather than increases the amount of growth. A well growing plate culture of fusiform bacilli should furnish from 6 to 10 cc. of antigen. Cultures incubated by Wright's method furnish too small a volume of organisms for injection or agglutination purposes.

A few strains of fusiform bacilli form granular suspensions, due to the tendency of the organisms to remain in clumps, or to agglutinate spontaneously. This does no harm when the suspension is to be used for injection, and the antigen need not be shaken before injection. Due to this clumping, however, it is impossible to count the number of bacteria to be injected accurately, hence the injections should be governed either by the number of cubic centimeters of a standard suspension used, or by the number of plate cultures used. Since the amount of growth on plates varies, it is more accurate to inject a definite volume of the suspension each time. The initial volume used is 1 cc., which is increased by 1 cc. at each subsequent injection.

Intravenous injections only were made, with forty-eight hour

intervals between injections. With one exception, no reactions have been observed. In the exception noted, the respiration rate was markedly increased for a period of thirty minutes. Any number of injections may be made without harm to the animals.

The titre of the serum was tested after the fifth to the ninth injection. If this was over 1:5000, which was considered satisfactory, the animal was then bled. Bleeding was first practiced at an interval of ten days after the last injection, but this has been found to be entirely too long an interval, the titre dropping abruptly within this period. The serum of one animal, injected nine times, had a titre of 1:81,920 at the trial bleeding, which was performed two days after the last injection. At the end of nine days, when the animal was exsanguinated, the titre had dropped to 1:20,480. Starin and Dack (1923), studying the immunological response elicited in rabbits immunized against *Clostridium botulinum*, found that the titre of their serum rose steadily for seven to eight days after the last injection, after which it fell. They recommended bleeding five to seven days after the last injection. Other investigators, working with animals immunized against different anaerobes, have found that the titre begins to drop rapidly three days after the last injection, confirming in this respect the results obtained in the present investigation. Accordingly, animals immunized against fusiform bacilli are now bled three days after the last injection.

Blood was drawn aseptically from the carotid artery, and the serum preserved by the addition of 0.1 cc. of 5 per cent phenol in saline to each cubic centimeter of serum collected. The titre was not affected by the preservative.

PREPARATION OF ANTIGENS USED IN THE AGGLUTINATION TESTS

Antigens used for agglutination purposes were prepared in a manner similar to those used for injection, with the exception that those cultures which formed granular suspensions were thoroughly shaken for five or ten minutes to break up all clumps. Too long a shaking should be avoided. Even after this treatment, it was extremely difficult to keep some of the antigens in even suspension during the tests. With some of the strains used, as with other

anaerobes, the tendency toward spontaneous agglutination was very great. The control tube is absolutely essential in all tests, to detect any trace of spontaneous agglutination.

Cultures from twenty-four to 48 hours old are essential for successful results. Old cultures used for preparing antigens usually agglutinate spontaneously. Antigens or cultures which have remained in contact with the air for some time are worthless for agglutination purposes. The more readily a culture auto-agglutinates, the younger the culture should be which is used to prepare the antigen.

Absorption tests probably would be more suitable for typing fusiform bacilli than agglutination tests, but these have not been attempted.

THE TEST

In order to conserve antigen, a preliminary test of each organism was made, with dilution of 1:1 to 1:20. After incubating at 40°C. for ten minutes, the result could usually be read. Agglutination often occurs in five minutes in these low dilutions. In several cases, agglutination with more than one serum occurred, showing the presence of sub-types. In no case did sub-types agglutinate in dilutions greater than 1:20. In dilutions greater than this the reaction is very specific.

Having found the probable type to which an organism belonged by means of this rough test, a series of dilutions of the serum were prepared, ranging from 1:20 to 1:20,480, placing 0.5 cc. of each dilution in a tube. A like amount of antigen was then added to each of the tubes, which, after a thorough shaking, were placed in the water bath at 40°C.

Agglutination often occurred in five or ten minutes in the highest dilutions employed. A period of two hours was allowed as the maximum. In an effort to obviate spontaneous agglutination various temperatures of incubation were tried, with negative results. A temperature of 45°C. is apparently less efficient than one of 40°C., while incubation at 56°C. cannot be practiced, since the bacteria immediately agglutinate and rise to the surface of the liquid. The bacteria settle out rapidly at very low tem-

TABLE 1
Types of fusiform bacilli

TYPE I	TYPE II	TYPE III	TYPE IV
LF1 Sub-type 1 LS5 LS3 LS1 NS2 NS10 Sub-type 2 NS1 NS8 NS13	NS14 LS4 LS6	LW1 LW2 NW1	LB1 LB2

TABLE 2
Agglutination reactions with Type I serum

ANTIGEN	1:1	1:8	1:16	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	CONTROL	TEMPERATURE °C.	TIME
LF1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	-	40	15 minutes
LW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	24 hours
LW2.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	24 hours
NW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
LS1.....	+	+	±	±	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS3.....	+	+	+	-	-	-	-	-	-	-	-	-	0	-	40	2 hours
LS4.....	±	-	-	-	-	-	-	-	-	-	0	0	0	-	40	2 hours
LS5.....	+	+	±	±	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS6.....	-	0	0	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS1.....	±	+	+	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS2.....	+	+	+	±	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS8.....	+	+	+	+	±	±	-	-	-	-	-	0	0	-	40	2 hours
NS10.....	+	+	+	±	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS12.....	+	+	+	±	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS13.....	+	±	±	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS14.....	-	0	0	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS5.....	+	+	+	+	+	+	+	+	+	+	0	0	0	+	56	5 minutes
NS1.....	+	-	-	-	-	-	-	-	-	-	0	0	0	-	45	22 hours

peratures, hence results cannot be read if the tubes are placed in the ice box. Hall and Stark (1923) suggest that this settling out

of anaerobes when placed in the ice box may be due to a negative chemotactic response to oxygen. Fusiform bacilli, however, are more sensitive to cold than to oxygen, settling out immediately after chilling, whereas they will remain in contact with oxygen for several hours without settling out, if kept at 40°C.

A rapid decrease in the titre has been observed in all four sera, confirming observations made a year previously, when four

TABLE 3
Agglutination reactions with Type I, Sub-type 1 serum

ANTIGEN	1:1	1:8	1:16	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	CONTROL	TEMPERATURE	TIME
															°C.	
LF1.....	+	+	+	+	±	±	-	-	-	-	-	0	0	-	40	2 hours
LW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
LW2.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
NW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
LS1.....	+	+	+	+	+	+	+	+	+	±	±	0	0	-	40	5 minutes
LS3.....	+	+	+	+	+	+	+	+	+	±	-	0	0	-	40	5 minutes
LS4.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS5.....	+	+	+	+	+	+	+	+	+	+	+	+	0	-	40	5 minutes
LS6.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS1.....	±	±	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS2.....	+	+	+	+	+	+	+	+	+	+	-	0	0	-	40	15 minutes
NS8.....	+	±	±	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS10.....	+	+	+	+	+	+	+	+	+	+	+	0	0	-	40	15 minutes
NS12.....	+	+	+	+	+	+	+	+	+	±	±	0	0	-	40	5 minutes
NS13.....	+	+	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS14.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours

immune sera were prepared against fusiform bacilli. It is necessary, therefore, to use the serum within a few months after bleeding. While the titre drops rapidly, no prezone range has been observed.

Each strain of fusiform bacillus was tested against the four anti-sera. By means of these tests, three main types of fusiform bacilli have been found, together with two sub-groups. A fourth type has been added to the list, on the basis of its morphology only. This, the broad type already referred to, has a

characteristic morphology, differing considerably from that of the first three types. Due to its extremely meagre growth on surface cultures, no antigen could be prepared, hence this organism is included as a separate type only upon the basis of its morphology and character of growth on surface cultures.

No immune serum has been prepared against the wavy type of fusiform bacillus. It differs radically from the broad type,

TABLE 4
Agglutination reactions with Type I, Sub-type 2 serum

ANTIGEN	1:1	1:8	1:16	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10,240	CONTROL	TEMPERATURE	TIME
															°C.	
LF1.....	+	+	+	+	±	-	-	-	-	-	-	0	0	-	40	2 hours
LW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
LW2.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
NW1.....	-	-	-	0	0	0	0	0	0	0	0	0	0	-	40	2 hours
LS1.....	+	+	±	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS3.....	+	±	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS4.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS5.....	±	±	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS6.....	±	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS1.....	+	+	+	+	+	+	+	+	+	±	±	-	-	-	40	2 hours
NS2.....	+	+	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS8.....	+	+	+	+	+	+	+	+	+	+	+	-	-	-	40	2 hours
NS10.....	±	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS12.....	+	±	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS13.....	+	+	+	+	+	+	+	+	+	+	+	0	0	-	40	5 minutes
NS14.....	-	0	0	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS5.....	+	+	+	+	+	+	+	+	+	+	+	0	0	+	56	5 minutes
NS1.....	+	+	+	+	+	+	+	+	-	-	-	0	0	-	45	2 hours

and since it fails to agglutinate with the sera of either types I or II, it is classed as a distinct type.

Results of the agglutination tests are given in table 1, which shows the type to which each of the cultures belongs. Sub-types are those which agglutinate in dilutions of 1:1 to 1:20, but which fail to agglutinate in dilutions greater than this. The other organisms listed all agglutinate with their type serum in dilutions of 1:2560 or higher, but not at all with sera of other types.

Type I organisms have been isolated most frequently from the cases studied, but these results are based on the study of too small a number of strains to enable one to draw any conclusions as to the relative frequency of occurrence of the various types. Type IV is very frequently seen in tartar, and it is also found in Vincent's angina, but it has been isolated in but two cases during this study. Further study will doubtless show a much greater

TABLE 5
Agglutination reactions with Type II serum

ANTIGEN	1:1	1:8	1:16	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	CONTROL	TEMPERATURE °C.	TIME
LF1.....	-	-	-	-	-	-	-	-	-	-	-	-	0	-	40	2 hours
LW1.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
LW2.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
NW1.....	-	-	-	0	0	0	0	0	0	0	0	0	0	-	40	2 hours
LS1.....	-	-	-	-	-	-	-	0	0	0	0	0	0	-	40	2 hours
LS3.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS4.....	±	+	0	+	+	+	+	+	+	-	-	0	0	-	40	15 minutes
LS5.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
LS6.....	+	+	+	+	+	+	+	+	+	±	±	0	0	-	40	5 minutes
NS1.....	±	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS2.....	-	-	-	-	-	-	-	-	0	0	0	0	0	-	40	2 hours
NS8.....	-	-	-	-	-	-	-	-	-	-	0	0	0	-	40	2 hours
NS10.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS12.....	-	-	-	-	-	-	-	-	-	0	0	0	0	-	40	2 hours
NS13.....	-	-	-	-	-	-	-	-	-	-	-	0	0	-	40	2 hours
NS14.....	+	+	+	+	+	+	+	+	+	+	+	+	+	-	40	2 hours
LS5.....	+	+	+	+	+	+	+	+	+	+	+	0	0	+	56	5 minutes
NS1.....	0	0	0	±	-	-	-	-	0	0	0	0	0	-	45	2 hours

percentage of the last three types than is here recorded, and may also show the existence of new types.

No relationship has been found between the type to which an organism belongs and the lesion from which it was secured. It is probable that no single type is constantly present in any one pathological condition.

DESCRIPTION OF TYPE CULTURES

Type I. Type organism LF1

In early generations bacilli of this type may be quite short, measuring 7 to 17 μ in length, by 0.4 to 0.6 μ in width, but in older generations they become filamentous and maintain this shape quite constantly.

The organisms are typically long, slender, and sharply pointed, often growing in long filaments which may attain a length of 225 μ and a width of 0.5 to 0.65 μ . Shorter forms are seen in all cultures. The average length is from 35 to 65 μ . The shorter forms, which gradually disappear in successive generations, are from 4 to 19 μ in length, and from 0.35 to 0.7 μ in width.

Involution forms of various bizarre shapes are frequently found in cultures where the medium is unsuited to the growth of the organisms. Extremely filamentous organisms may be observed which are often over 300 μ in length. These may be extremely tangled and twisted, so as to resemble a piece of tangled thread. As shown in figure 4, a slender organism may broaden out to a width of 3 to 5 μ . Very large granules are sometimes found in these enlarged organisms. At other times, these forms may stain evenly and intensely. Grown on a medium which supports a vigorous growth of the organisms, these involution forms do not develop, and the culture dies out without their appearance.

No granules can be found in young, rapidly growing cultures when the organisms are stained with ordinary aniline dyes. Many granules may be seen, however, in cultures three to ten days old, in which degeneration forms have begun to appear. The protoplasm forms into granules, leaving a bare cell wall which shows as a hollow tube filled with the granules, which vary from one to fourteen. The first or second generation of a culture may contain many of these forms during the first twenty-four to forty-eight hours, but in later generations they do not appear until the culture is several days old. So constant is their appearance in old cultures as to suggest that their presence is a sign of decadence and approaching death and dissolution of the culture. Old stock cultures, stored several months without

transplanting, will also show many of these forms when freshly transplanted.

Single celled filaments outnumber all others, but at times filaments composed of from two to fourteen short, individual organisms of varying length, joined end to end, are seen. The juncture between the cells composing such filaments is often very indistinct, giving the appearance of a long, individual filament.

Observed under the dark field, the filaments appear to bend very stiffly. Single-celled filaments usually bend evenly, similar to a thin piece of steel, while multiple cell filaments may bend sharply, usually at the juncture of two cells.

The organisms stain readily with the strong aniline dyes, such as carbol-fuchsin or gentian violet. The former stain, diluted 1:10, is one of the best for the demonstration of granules. Stained with Atkin's modification of Gram's stain, and decolorized with acetone, the organisms are distinctly Gram-negative, no vestige of the original stain remaining.

Grown in liquid media, the bacilli clump together in huge masses of intertwined, filamentous organisms, even in fresh cultures, making such preparations unsuitable for agglutination purposes. Grown on solid media and suspended in saline, the organisms form an even, homogeneous suspension from which they settle out only after long periods of time. When so prepared, little difficulty due to spontaneous agglutination is experienced with Type I cultures.

Observed under the dark-field, no motility has been observed in any of the many cultures examined. No attempt has been made to stain flagella.

A very slight, characteristic odor, similar to that found in other types of fusiform bacilli, prevails in all cultures. This is by no means unpleasant. In impure cultures, however, a very foul, offensive odor quickly develops. As previously shown, this contamination is seldom detected by ordinary methods.

No sub-culture could be obtained from cultures exposed to the air in thin layers for twenty four hours, even after 34 generations of anaerobic surface cultures. No growth has been secured on repeated trial. Contrary to the results secured by

certain investigators, it is probable that the organism remains an obligate anaerobe, and that it is impossible to secure aerobic growths of pure cultures, even after prolonged cultivation.

In figure 1, a photograph of a freshly isolated, twenty-four-hour culture of this organism, granule formation is evident. As the organism becomes acclimated to artificial conditions of growth, granule formation in young cultures disappears. A nine-day cul-



FIG. 1. *B. FUSIFORMIS*, TYPE I. SECOND GENERATION, TWENTY-FOUR-HOUR CULTURE. $\times 1045$ DIAMETERS

ture of the same organism, in its twenty-seventh generation, is shown in figures 2 and 3. So-called shadow forms, in which can be seen numerous granules, are prominent in this photograph. The large clumps of filamentous organisms shown are commonly found in this type.

Bacilli of Types I and II cannot be differentiated by means of their surface colonies, but these can be readily distinguished from those of contaminating organisms. Colonies on blood agar plates



FIG. 2. *B. FUSIFORMIS*, TYPE I. NINE-DAY CULTURE, TWENTY-NINTH GENERATION, SHOWING TYPICAL FILAMENTOUS CLUMPS, GRANULES AND "SHADOW" FORMS. $\times 375$ DIAMETERS



FIG. 3. *B. FUSIFORMIS*, TYPE I. SAME AS FIGURE 2, EXCEPT FOR THE MAGNIFICATION OF 1045 DIAMETERS

incubated in the phosphorus jar attain an average diameter of 0.8 mm. They are circular in outline, with a sharply defined, entire edge, rarely slightly indented. They are pulvinate in cross section. No fringe is ever present. Incubated anaerobically by Wright's method, they tend to spread out over the surface of the medium, forming thin, umbonate colonies 3 to 4 mm. in diameter, often with an indented margin, which are less characteristic than colonies grown in the phosphorus jar.

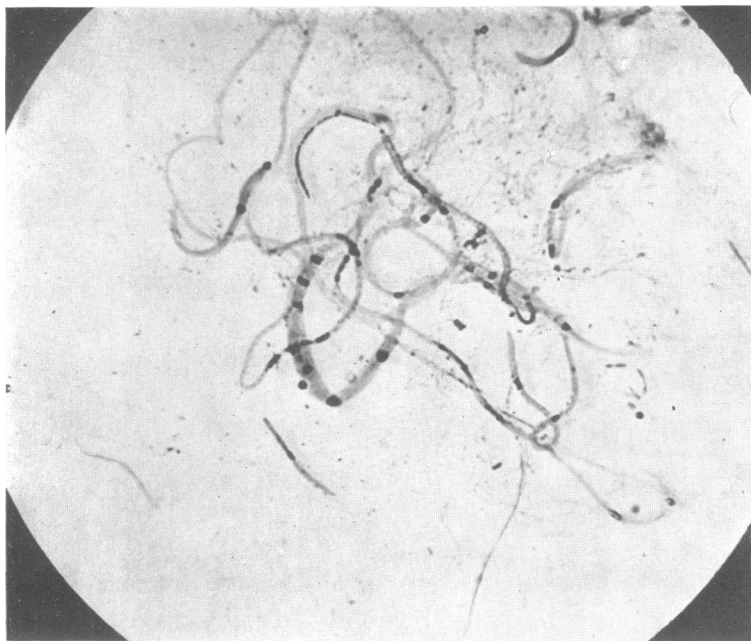


FIG. 4. *B. FUSIFORMIS*, TYPE I. INVOLUTION FORMS. $\times 1045$ DIAMETERS

Extremely fine granular markings, which cannot be seen by strong light, appear on the surface of the colonies. Observed either by transmitted or reflected light, the interior of the colony appears to contain numerous white flecks within a water clear medium. This interior mottling is quite characteristic of Types I and II organisms, although it may be found in other types. Once recognized, the peculiar appearance produced by this mot-

ting aids greatly in isolating the organisms. One accustomed to their appearance can often pick the colonies on a plate by means of a naked eye examination alone, although colonies of certain streptococci may confuse one when they are examined in this way.

The organisms have a creamy white appearance in large masses, but no yellow color, as reported by a few investigators, has been observed. Thin layers of bacilli which have been exposed to the air for some time occasionally have a light violet tint.



FIG. 5. *B. FUSIFORMIS*, TYPE I, SUB-TYPE 1. NORMAL FORM. THREE-DAY CULTURE, TWENTY-FIFTH GENERATION. $\times 1045$ DIAMETERS

Type I. Sub-type 1. Type organism LS5

These organisms grow characteristically as long, straight, sharply pointed bacilli, with fewer curved forms than are found in LF1 cultures. Extreme differences in the length and breadth of the organisms, such as are found in the true filamentous type,

are not encountered in this sub-type. The characteristic form of the organism is shown in figure 5.

Individual bacilli vary in length from 9.4 to 19.3μ , and in width from 0.3 to 0.75μ , the average dimensions being 13.4μ by 0.5μ . Filamentous organisms 90μ long may be found on certain lots of media. Broad involution forms, such as are found in the culture previously described, are very rarely seen in this type. Nests of bacteria, which resemble masses of needle pointed crystals, frequently occur. While other types grow in this form, the longest individual organisms of any of these forms are found in sub-type 1 cultures. Chains of more than two bacilli are uncommon, although tandem forms are frequent. Out of hundreds of slides examined, but one chain of as many as five organisms was seen. This measured 10.5μ by 0.55μ , which is shorter than the average individual bacillus.

The bacilli stain rather weakly with gentian violet, but readily with 1:10 carbol-fuchsin. They are Gram-negative, and are readily decolorized.

In early generations, from two to eight granules form in each of the cells. As the organisms become more accustomed to artificial media granule formation in young cultures ceases to a large extent, occurring mainly in cultures from three to five days old. In such cultures, two, four or six granules form in each organism, with occasionally an odd number.

No motility has been observed in any of the cultures. A very slight odor, similar to that produced by other types of fusiform bacilli, is present in all cultures. No foul odor develops in pure cultures.

Surface colonies are indistinguishable from those of Type I cultures. The organisms are differentiated morphologically from those of the preceding type by reason of their length and tendency to grow in crystal-like masses. Serological tests should be used to identify the organisms positively, however, the morphology changing readily enough to make this an unsafe criterion of differentiation.

Homogeneous antigens are readily prepared from this type of organism, but these rapidly become granular. The organisms

agglutinate with Type I serum in dilutions below 1:20; against their homologous serum in dilutions of 1:10,240 or above.

Type I. Sub-type 2. Type organism NS13

The organisms belonging to sub-type 2 are the shortest of any found in Type I cultures. They are very sharply pointed, and occur characteristically in nests of organisms, similar to

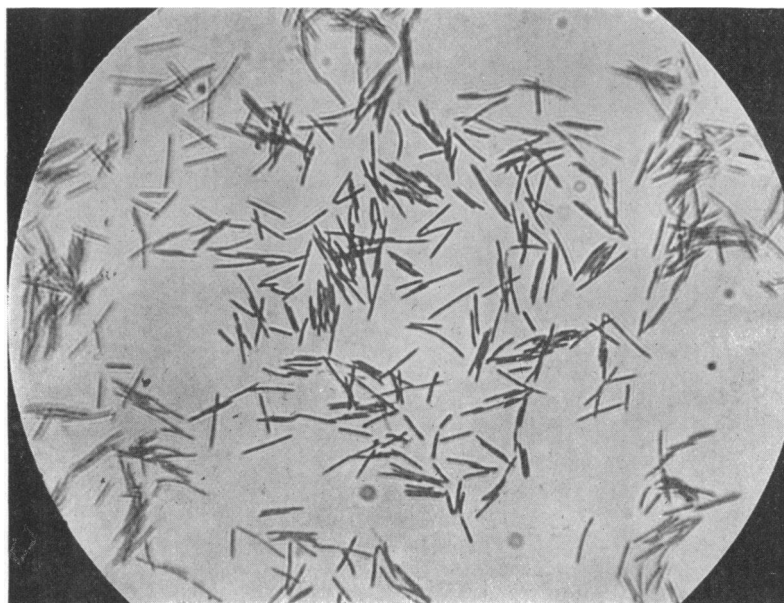


FIG. 6. *B. FUSIFORMIS*, TYPE I, SUB-TYPE 2. SECOND GENERATION, THREE-DAY CULTURE. $\times 1045$ DIAMETERS

those of sub-type 1 cultures. Tandem forms, which may be slightly curved, are frequently seen. V type tandem forms are common. Single organisms are usually very straight.

In early generations short bacilli 4 to 12μ in length, by 0.5 to 0.7μ in width, are common. The average dimensions of these organisms are 7μ by 0.6μ . Later, both long and short forms are found, but the organisms never attain the lengths common to those of the filamentous type, varying from 2.8 to 19.5μ in length,

by 0.5 to 0.65μ in width. Occasionally short filaments as long as 45μ are found. The average size of the organisms found in older cultures is the same as in young cultures.

Individual organisms may contain from two to six granules. Shadow forms are seldom seen in this type, but when they do occur, usually contain from four to six granules. As with other



FIG. 7. *B. FUSIFORMIS*, TYPE I, SUB-TYPE 2. SECOND GENERATION, THREE-DAY CULTURE. EFFECT OF A SMALL AMOUNT OF ARSENIC IN MEDIUM.
× 375 DIAMETERS

types of fusiform bacilli grown on surface cultures, very few granules form in young, rapidly growing cultures, although they may be present in great abundance in older cultures.

The organisms stain readily with strong aniline dyes, and are Gram-negative. They are non-motile, and give off a faint odor similar to that of other types of fusiform bacilli.

The characteristic morphology of this type of organism is shown in figure 6, in which the crystal like arrangement of the

organisms is apparent. The organisms shown in figure 7, which were grown on a medium containing a trace of arsenic, are of the same age and generation as those shown in figure 6, which were grown on an arsenic free blood medium. The effect of even a trace of arsenic on the development of fusiform bacilli is thus strikingly shown, illustrating the necessity of using an arsenic free medium.

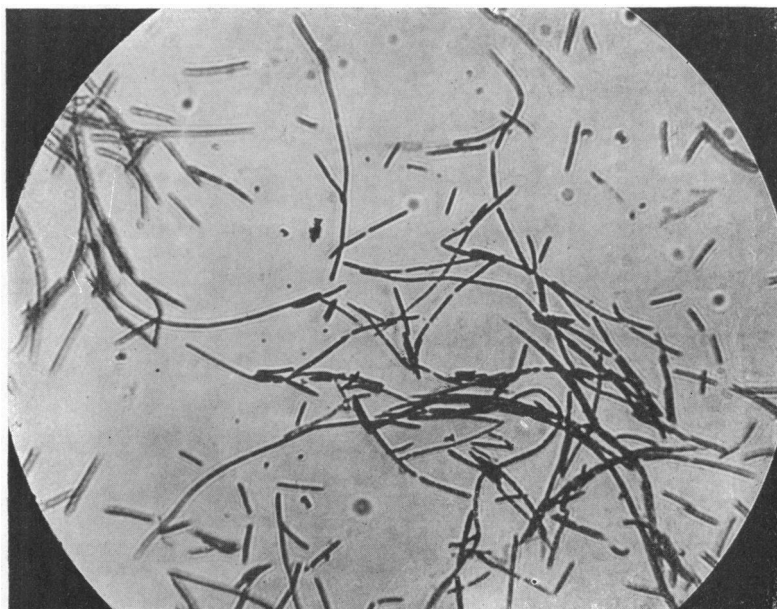


FIG. 8. SAME AS FIGURE 7, EXCEPT FOR MAGNIFICATION OF 1045 DIAMETERS

Type II. Type organism NS14

These bacilli occur either as single organisms or in tandem formation. The ends are sharply pointed, although the juncture of two organisms growing in tandem form is blunt. In young, rapidly growing cultures the individual organisms vary in length from 2.3 to 5.1 μ , and in width from 0.45 to 0.7 μ . Tandem forms are seldom longer than the individual bacilli. The average dimensions of all forms is 3.9 by 0.58 μ .

These organisms degenerate much less rapidly than those of other types so far studied, cultures six days old showing little or no signs of degeneration, excepting a slight increase in the length of the organisms. Cultures older than ten days contain numerous bacilli 10 to 12 μ in length, which often grow in chains of two or more.

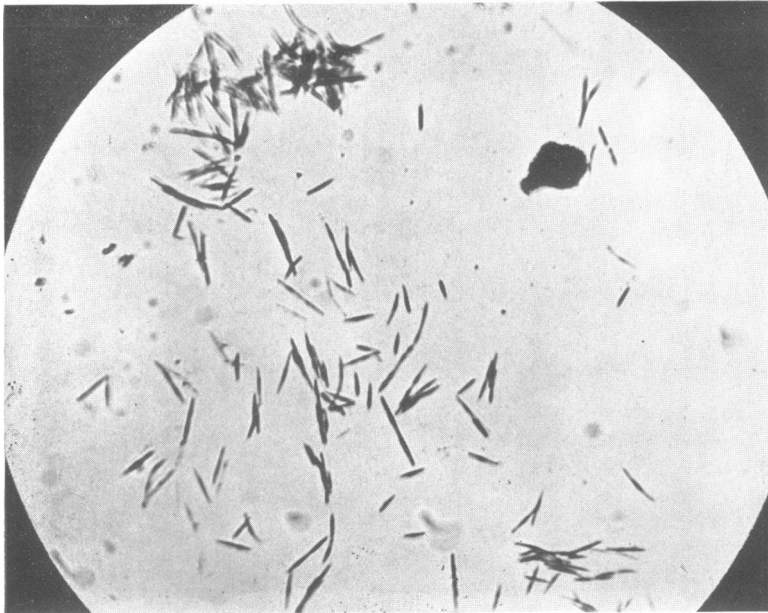


FIG. 9. *B. FUSIFORMIS*, TYPE II. THREE-DAY CULTURE, TWENTY-FIFTH GENERATION. TYPICAL APPEARANCE, SHOWING VERY SHORT FORMS.
× 1045 DIAMETERS

The organisms are straight in young cultures, but in old cultures slightly curved forms are sometimes found, usually in tandem. In chains, the bend usually occurs at the juncture of two cells.

In old cultures short, granular shadow forms are present in large numbers, which, due to the presence of from two to four granules, bear a striking resemblance to diphtheria bacilli. Granules are rarely found in young cultures. Commonly but a single centrally located granule appears, which is usually of a

greater diameter than the cell. Tandem forms may contain either one or two granules.

The characteristic morphology of these organisms is shown in figure 9. Like the sub-types previously described, crystal like nests of bacilli are frequently found. Shorter individual bacilli are found in this type than in any of the other types studied.



FIG. 10. *B. FUSIFORMIS*, TYPE II. TWO-DAY CULTURE, FOURTEENTH GENERATION.
ABNORMAL FORM, GROWN ON 2 PER CENT BLOOD AGAR.
× 375 DIAMETERS

The effect of cultivating the organisms on an agar medium containing less blood than the organism is accustomed to is shown in figure 10. Less change of morphology is produced by this procedure than with Type I cultures. A pure culture of Type II fusiform bacillus was isolated from a tonsillar granule, from which the preparation shown in figure 11 was prepared. The effect of growing the organisms under artificial conditions is well illustrated by comparing this photograph with that of figure 9.

In their natural habitat, fusiform bacilli are less sharply pointed than when grown in pure culture, and are shorter and thicker.

The organisms stain rather poorly with gentian violet, but readily with 1:10 carbol-fuchsin. They are strictly Gram-negative. No motility has been observed in any of the cultures. A slight, characteristic odor prevails in all cultures.



FIG. 11. FUSIFORM BACILLI AND SPIROCHETES FROM A DIRECT SMEAR OF A TONSILLAR GRANULE, FROM WHICH A TYPE II ORGANISM WAS ISOLATED.
× 1045 DIAMETERS

The colony is similar to those previously described, with the same interior mottling. A small brown granular mass is sometimes seen in the center of the colony. On pushing aside the colony with a needle, the mass remains adhering to the media. A slight depression of the medium is produced beneath the colony, the same effect being noted with other types of fusiform bacilli. This can be seen only by washing the colony off the medium with saline.

The colony is slightly gelatinous and is hard to remove from the plate. No hemolysis of blood occurs in cultures kept under anaerobic conditions, but a slight hemolysis, due to hydrogen peroxide formation, is observed in cultures exposed to the air for some time.

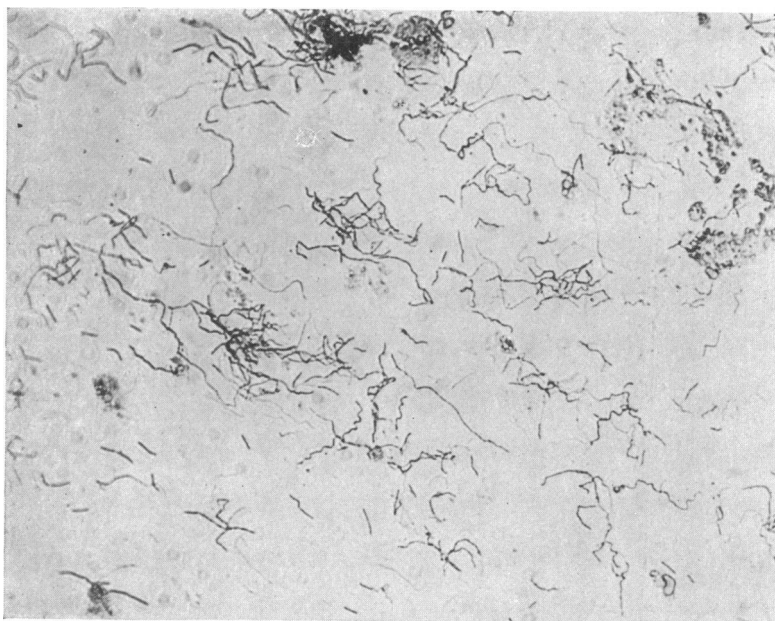


FIG. 12. *B. FUSIFORMIS*, TYPE III. WAVY AND SPIRAL-LIKE FORMS, WHICH RESEMBLE SPIRILLA. SIXTH GENERATION, FORTY-EIGHT-HOUR CULTURE.
× 375 DIAMETERS

Type III. Type organism LW1

Individual organisms of this type are less sharply pointed than are those of Types I and II. In twenty-four to seventy-two-hour cultures they appear characteristically in the form of long, wavy chains, as many as 35 individual organisms forming one chain. Chains 250μ in length are not uncommon. In chains, individual organisms longer than 37μ have not been seen. The majority are far shorter than this, varying from 4.2 to 7.1μ , with an average of 5.5μ . Extreme variations in width do not occur, the average being 0.5μ .

In cultures twenty-four to seventy-two hours old the organisms are extremely wavy, as illustrated in figures 12 and 15. These wavy forms often grow in huge clusters, as shown in figures 13 and 14. Some of the bacilli so closely resemble true spirilla as to be mistaken for them on cursory examination, or when they are examined under magnifications less than 1000 diameters. Under higher magnifications, these spiral forms can be seen to be

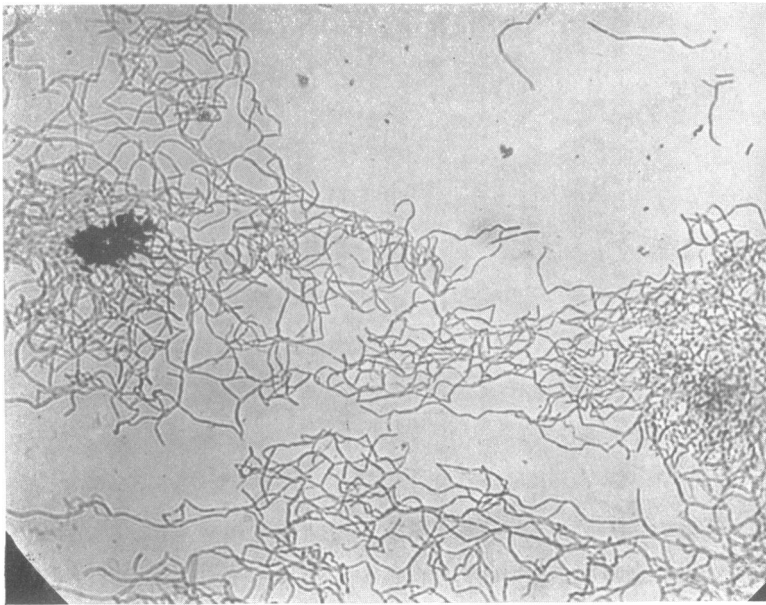


FIG. 13. *B. FUSIFORMIS*, TYPE III. SIXTH GENERATION, THREE-DAY CULTURE SHOWING CLUSTERS IN WHICH THESE ORGANISMS TYPICALLY GROW.
× 375 DIAMETERS

composed of several individual fusiform bacilli, each of which forms a single curve of the spiral like element. Grown in liquid cultures, it is probable that this type of organism could not be differentiated from spirilla except by means of motility tests, so closely does it resemble this organism at certain stages of its growth.

In contrast to other types of fusiform bacilli, it is unusual to find perfectly straight organisms in young cultures. Some bacilli

may be but slightly curved; others, like those which form the spiral like elements, may be bent into the form of a half circle. So characteristic is this wavy or spiral-like form that a culture can be immediately identified by its appearance.

The spiral-like forms begin to disappear in cultures older than seventy-two hours, developing into straight or slightly curved, sharply pointed bacilli. These are slightly wider than young

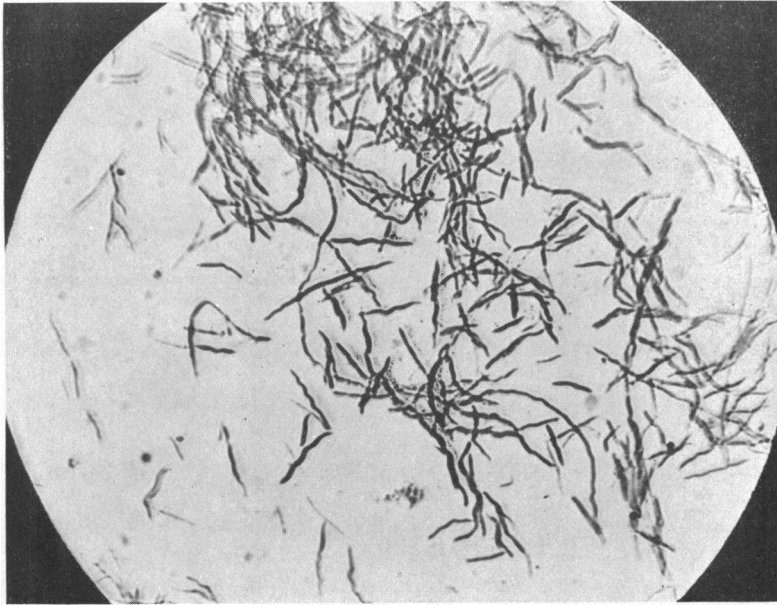


FIG. 14. *B. FUSIFORMIS*, TYPE III. SIXTH GENERATION, TWO-DAY CULTURE, SHOWING TYPICAL SPIRAL-LIKE FORMS. $\times 1045$ DIAMETERS

bacilli, having an average diameter of 0.7μ . Interspersed with these forms, which stain deeply, are numerous shadow forms.

No special stain has been needed to demonstrate these organisms, which is contrary to Tunncliffe's experience with her spiral-like forms. They stain readily with both gentian violet and 1:10 carbol-fuchsin at all periods of their growth. They are Gram-negative.

Two granules are usually found in the individual cells. In

old, degenerate forms, as many as six granules have been observed in a single organism. Two, however, are most commonly found.

No "external granules" have been observed in any of the many cultures examined, confirming in this respect similar observations made of all the other types of fusiform bacilli studied. In old cultures, a solidly staining organism lying end to end with a spiral-like shadow form is occasionally found, but there is no evi-

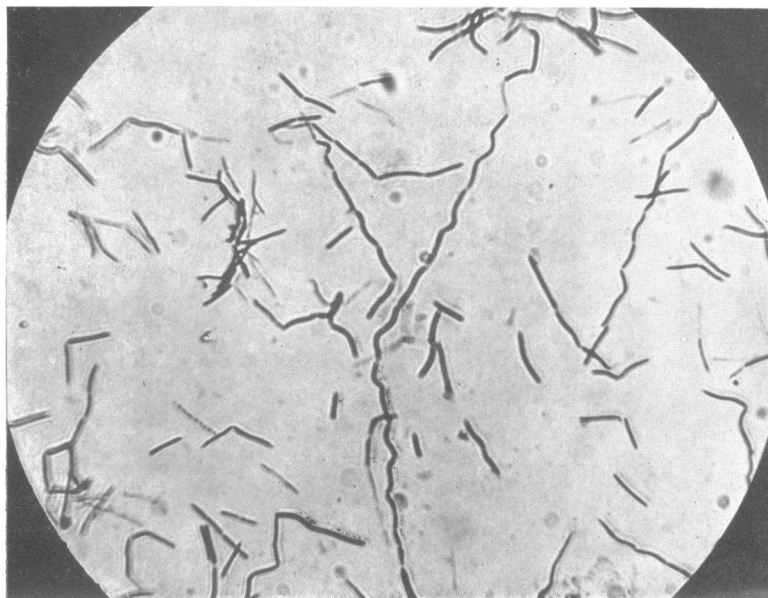


FIG. 15. *B. FUSIFORMIS*, TYPE III. TWO-DAY CULTURE. $\times 1045$ DIAMETERS

dence that this spiral-like, weakly staining shadow form has emerged from the former. No evidence has been secured in the present study in support of the theory that spirilla may develop from granules which have dropped out of fusiform bacilli, or by the rearrangement of the protoplasm within the fusiform bacilli into spiral-like forms, which emerge from the cell as true spirilla through the breaking down of the cell wall. These theories are to be doubted. Efforts of several investigators to substantiate

them has led to a great deal of confusion in the study of fusiform bacilli. Careful surface culture study of this organism should readily clear up these disputed points.

No motility has been observed under the dark-field in any of the cultures, even in those showing a large number of spiral-like forms.

Colonies of Type III fusiform bacilli are slightly larger than those of the preceding types, the average diameter being 1 mm. They are perfectly circular in outline, with a sharply defined, entire edge. No fuzzy outgrowth is present. They are pulvinate in cross section, though slightly more rounded than colonies of Types I and II organisms. The surface is covered with a rather coarse granulation, giving to the colonies a pearly lustre, or an appearance which might be roughly described as resembling the surface of cast iron. Due to these markings, the interior mottling of the colony cannot be seen unless one first gently breaks it up with a needle. There are no central granular masses.

The colony is slightly viscid, and it is difficult to remove it from the surface of the medium. Heaped into irregular masses, the colonies soon flatten out.

In saline, an even, homogeneous suspension is formed, which settles out only after long periods of time. The organism does not agglutinate with any of the four immune sera prepared.

Type IV. Type organism LB1

Organisms of this type are much larger than those of preceding types, and have less sharply pointed ends, some organisms having blunt ends. They are frequently seen in direct smears from tartar, in which they occur as long, broad bacilli with pointed ends, sometimes staining evenly, sometimes unevenly. They are frequently found in tandem form, in which the two organisms are often of dissimilar size. Growing in short chains, the centrally located cells are often blunt ended, appearing sausage shaped. Again the ends may be square cut, the cells resembling chains of anthrax bacilli. In either case, the terminal organisms of the chain are pointed.

The bacilli vary in length from 3.8 to 17.4 μ , with an average of

12 μ . The shorter forms are found only in chains, never individually. Chains have been seen 54 μ in length. The width is greater than that of the other three types, varying from 0.8 to 0.95 μ , with an average of 0.9 μ .

The organisms stain readily with aniline dyes, and are Gram-negative. Very minute, Gram-positive granules, unlike typical fusiform bacillus granules, are sometimes seen in a few of the

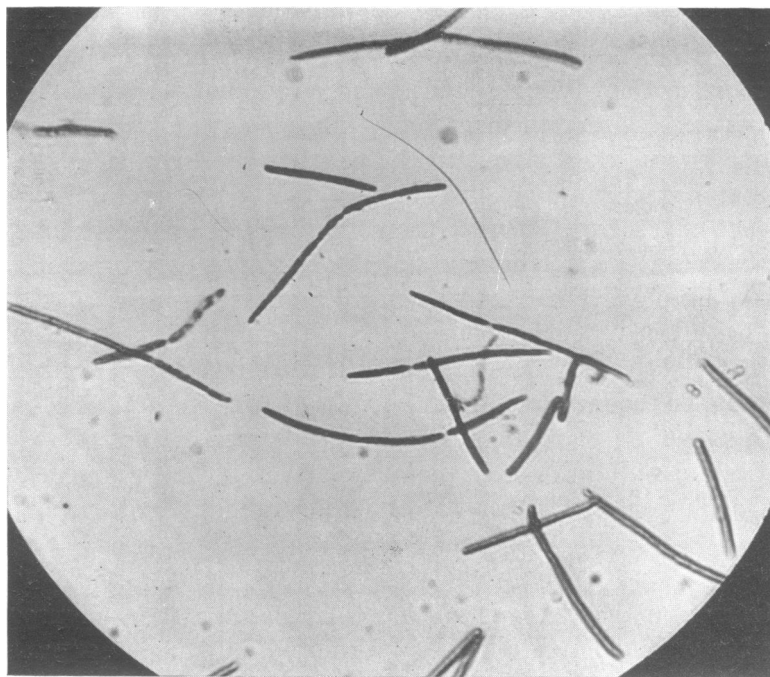


FIG. 16. *B. FUSIFORMIS*, TYPE IV. THREE-DAY CULTURE, THIRD GENERATION.
× 1045 DIAMETERS

cells. These are found scattered throughout the entire cell, two or three often lying abreast. As many as fifteen have been counted in a single cell measuring 4.8 μ in length. This "sprinkling" of small granules causes the organisms containing them to stand out sharply in a Gram-stained smear. Very few organisms contain them, however.

Degeneration sets in rapidly, beginning in forty-eight-hour cultures. The protoplasm shrinks away from the cell wall, forming into large, solidly staining granules regularly spaced. The cell wall between these granules is enlarged, and takes a very weak stain. This is shown in figure 16.

Due to the rapid degeneration of the surface cultures, and the extremely meagre growth obtained, no organisms belonging to this type have been kept alive for more than seven generations. A culture nine days old was the oldest from which a sub-culture could be obtained. Of the four types, this is by far the most difficult to isolate and culture.

The organisms are non-motile. Seen in liquid cultures, a mass of the organisms resemble a bunch of floating logs, so stick-like is their appearance.

An odor, similar to that formed by other types of fusiform bacilli, is given off by this type of organism. This is very faint, due, doubtless, to the small amount of growth present on a plate or slant.

Surface colonies are very thin and spreading, with an irregular margin. Seen with the naked eye, they resemble to some extent colonies of *B. tetani*. Well isolated colonies may attain a diameter of 3 to 4 mm. Closely spaced colonies show a marked diminution in size, few attaining a diameter greater than 1.5 to 2 mm.

The surface of the colony has distinct granular markings, while the edges are slightly curled. On surface culture, this type may be differentiated from those previously described not only by means of its morphology, but by means of its thin, spreading colony and meagre growth, in contrast to the sharply circumscribed colonies and heavy growth of the other types.

SUMMARY

From 18 pure cultures of fusiform bacilli, isolated by a new streak method, four different types have so far been identified by serological and morphological studies. Of these, Types III and IV can often be identified by morphological appearances alone, but the organisms of Types I and II, which vary greatly in their size and shape, can be safely differentiated from each

other only by serological tests. A classification of fusiform bacilli upon cultural and morphological grounds only should not be attempted.

Surface culture methods are adapted to the isolation and cultivation of all types of fusiform bacilli.

A wavy type of fusiform bacillus has been isolated, in which may be found spiral-like forms, so closely resembling true spirilla at certain stages of their growth as to lead to confusion. These spiral forms, which are non-motile and are present only for a short period of time, have no relationship to true spirilla.

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