

## FUSIFORM BACILLI: CULTURAL CHARACTERISTICS.\*

CHARLES KRUMWIEDE, JR., AND JOSEPHINE S. PRATT.

(From the Research Laboratory, Department of Health, New York City.)

In a previous communication we reported a simple method for isolating fusiform bacilli, and methods for cultivation without the use of complex anaerobic apparatus. The strains thus isolated have come from varied sources and have been studied to determine whether they could be differentiated, one from the other, according to their cultural characteristics, and whether, if differentiation is possible, the grouping of the cultures has any relation to their source.

Altho there is an extensive literature on the cultural characteristics of the fusiform bacillus, most of the reports deal with one or at most a few strains and are of little value for comparison and differentiation of strains. For this reason we shall report simply our own results.

### CULTURES STUDIED AND THEIR SOURCES.

1. Noma—recovering after salvarsan.
2. Ulceration of tongue from carious tooth.
3. Vincent's angina.
4. Discharging ear.
5. Discharging ear.
6. Pyorrhea.
7. Spongy bleeding gums.
8. Vincent's angina.
9. Vincent's angina.
10. Pyorrhea.
11. Vincent's angina.
12. Noma.
13. Carious tooth.
14. Vincent's angina.
15. Discharging ear.

*Morphology.*—The typical organism from the lesion is a double-pointed bacillus, containing one or more granules. The morphology is very variable in cultures, varying with the culture medium employed. On or in solid media the morphology is similar, tho the forms are somewhat longer. In the water of condensation of agar

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slants or in fluid media there is a tendency toward the development of filamentous forms, often forming tangled threadlike masses. In some of the filaments the granules may be absent. The typical organism is straight or somewhat bent. The filaments are at times sinuous and where granules are absent simulate a loosely wound spirochete. These are more common in fixed preparations. In fluid media, on or in solid media, when grown under identical conditions our strains showed no morphological characteristics that would be of value in differentiation of one strain from another. The only variation noted was the tendency of Nos. 2 and 3 to produce a greater proportion of short forms.

*Motility*.—Repeated examinations, in hanging-drop preparations and with dark field illumination, of original material and of bacilli from the most varied types of culture media, have failed to show any evidence of motility.

*Gram stain*.—None of the strains retained the dye.

*Colony morphology*.—The characteristic colony, as already described, develops only in the depths of agar.

*General characteristics of growth*.—As we have stated, serum or blood is necessary for satisfactory cultivation of the strains. In our previous article we referred to the use of a semi-solid agar-gelatin mixture which facilitated the transfer of stab cultures. We have found that one-half of 1 per cent agar with the addition of one-third of its volume of serum gives satisfactory results and is much easier to prepare. After isolation, growth may take place in serum-free media for several generations, but for continued vigorous development and surety of transfer a serum medium has been found necessary.

On or in agar, the growth of all the strains was the same. In stab or shake culture a puff-ball appearance is present, as one would expect from the appearance of the colony. On serum or blood agar the growth is less characteristic, consisting of a delicate greyish-white non-adherent layer becoming more opaque with longer incubation and developing a heaped-up appearance.

In broth a flocculent growth occurs at the bottom of the tube, leaving the upper portion clear. The simplest method for cultivation in serum broth is to sterilize the broth with a layer of albolene and add to each tube a small amount of heated serum.

*Indol production.*—All strains produced a large amount of indol.

*Odor.*—All cultures had a similar disagreeable odor.

*Viability.*—After cultivation for several generations the strains remain viable for a long period. Some strains were viable 67 days when preserved on ice. In the incubator three of four strains were viable up to six months.

The viability of freshly isolated strains was not tested. Altho various batches of media are unequally adapted for their cultivation, they can be easily kept alive in semi-solid serum media. Some of our strains are now in the fiftieth to sixtieth generation.

SUGAR FERMENTATIONS.

No.	Dextrose	Galactose	Levulose	Arabinose	Raffinose	Lactose	Saccharose	Dextrin	Maltose	Mannite	Mannose	Dulcite	Rhamnose	Inulin	Glycerin
1.....	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
2.....	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
3.....	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
4.....	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
5.....	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
6.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
7.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
8.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
9.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
10.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
11.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
12.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
13.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
14.....	++	++	++	—	—	—	+++	—	—	—	—	—	—	—	—
15.....	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—

No gas formation was noted in any of the tubes. This fact, as well as the differences in morphology, differentiates the fusiform bacillus from the bacillus necrosis which produces gas from dextrose.

*Method.*—To a sugar-free semi-solid agar sufficient sugar solution was added to bring the content to 1 per cent, and finally the necessary serum. Litmus was used as an indicator, the color being restored by softening the agar and pouring into a petri-dish. Nearly all the results were later checked by titration which gives sharper readings. For sterilization the sugar solutions were usually heated 10 minutes. In the case of maltose, sterilization was accomplished by filtration. In a few series the inoculated tubes showed a slight increase of acidity over the controls. As the non-fermenting controls gave the same change, this was referable to

slight cleavage of the sugar and the formation of fermentable monosaccharides. This deduction was further proved by the fact that the positive controls gave a marked increase of acidity comparable with the acid production of some fusiform strains with saccharose.

*Relation of the fusiform bacillus to the spirochetes from the same source.*—In the smears made from the original material, loosely wound spirochetal forms comparable with those found in fusiform cultures are present. The typical spirochetal forms seen in the original material have never been encountered in pure cultures of the bacillus. The cultural forms simulating spirochetes have been found in fixed preparation. Repeated examinations with dark field illumination have revealed no forms that could in any way be compared with those found in original material. Furthermore the spirochetes in the original material, morphologically at least, may be of two or possibly three varieties. These can be kept alive in mixed culture in coagulated horse serum, the fusiform bacilli dying out on transfer. When pure cultures of fusiform bacilli are planted in this medium no change of morphology takes place. The motility of the spirochete and the lack of motility of the fusiform bacillus form the strongest evidence against their identity. The observations of cultural forms have embraced the growth on the most varied media and at every stage of incubation.

#### SUMMARY.

The fusiform bacilli isolated by us fall in two groups, one group fermenting saccharose, the other not.

There is no relation between these groups and the source of the culture.

The fusiform bacillus and the spirochetes accompanying it are separate and distinct organisms.