THE DIFFERENTIATION OF BACILLUS FALLAX (WEINBERG AND SÉGUIN) FROM BACILLUS CARNIS (KLEIN)

NICHOLAS D. DUFFETT

Department of Bacteriology and Public Health, University of Colorado School of Medicine and Hospitals, Denver, Colorado

Received for publication November 28, 1934

In preceding articles, Duffett (1933) and Hall and Duffett (1935) identified von Hibler's "*Bacillus VI*" (1908) with *Bacillus carnis* (Klein, 1903, 1904) and indicated the differentiation of this organism from *Bacillus fallax* (Weinberg and Séguin, 1915).

B. fallax, first described under the designation "*Bacille A*" in 1915 by Weinberg and Séguin from a fatal case of gaseous gangrene where it was associated with "*V. septique*" and "*B. oedematiens*," was isolated altogether four times from their 125 cases (1916). Since that time, isolation of *B. fallax* has been recorded by Weinberg and Séguin (1917), Henry (1917), Henry and Lacey (1920) from wounds in cases of gaseous gangrene, by Kendall, Day and Walker (1922) from intestinal contents, by Duthie (1924) and Weinberg, Renard and Davesne (1926) from cases of appendicitis, by Mutch and Mutch (1927) from chronic arthritis, and, according to Weinberg and Ginsbourg (1927), by Weinberg and Aynard from a case of blackleg in sheep. In addition, Stokes (1916) obtained anaerobic bacilli, from blood cultures in cases of septicemia following wound infections, which Robertson (1929) identified as *B. fallax*.

It is to be noted that the name "*Bacillus fallax*" as used by Ornstein in 1920 for a Gram-negative, aerobic, intestinal bacterium is invalid on the ground of priority. The writer has examined a culture of Ornstein's "*Bacillus fallax*" and found it to be closely related to, if not identical with, *Bacterium typhosum*.

In 1917 Weinberg and Séguin concluded from the description

of "*Bacillus VI*," as given by von Hibler (1908), that it was impossible definitely to distinguish "*Bacillus VI*" from *B. fallax*, or to identify them. It was the purpose of this brief investigation of *B. fallax* to show that it is a separate species from *B. carnis*.

Since the various reported strains of *B. fallax* were not available, Hall's *B. fallax* No. 254 (American Type Culture Collection No. 3519) was studied as a type strain, as it was one of Weinberg's four original strains.

The following descriptions of morphology and cultural properties of *B. fallax* agree with those given by Weinberg and Séguin (1915, 1917), Henry (1917), Bulloch et al. (1917), Kahn (1922), Kendall, Day and Walker (1922), Duthie (1924), Mutch and Mutch (1927), and Robertson (1929), except where specific exceptions are noted.

MORPHOLOGY

In young cultures the bacilli were Gram-positive, but tended to become Gram-negative in older cultures. The rods were straight or slightly curved and had pointed ends. The bacilli measured 0.6 micron in width by 1.7 to 5.2 microns in length. The average dimensions were 0.6 micron in width by 2.8 microns in length. It may be noted that Duthie found the organisms to be only 0.1 to 0.4 micron in width. Sluggish motility was observed in coverslip preparations of 24-hour liquid cultures. Figure 1, plate 1, shows two bacilli with peritrichous flagella stained by Bailey's (1930) method in a mount from a 22-hour culture on blood agar slant under alkaline pyrogallol. Morphologically, *B. fallax* was very similar to *B. carnis*, except that *B. carnis* had rounded ends.

Spore formation occurred only in mediums not containing an excess of fermentable carbohydrates. Mature refractile spores were seldom seen even in old cultures, but solidly staining orgonts were observed regularly in small numbers on blood agar slants under alkaline pyrogallol, when stained with the ordinary aniline dyes after 48 hours incubation at 37°C. They were usually subterminal, but frequently central and occasionally terminal, and always much larger in diameter than the rods.

Figure 2, plate 1, shows vegetative rods and orgonts of *B. fallax*, from such a culture, solidly stained by Gram's method.

Spores of *B. carnis* were produced in greater numbers under identical conditions. They were usually refractile, were nearer the ends of the rods, and swelled the rods less. Cultures of both species were boiled for 2 minutes at 94°C. without destruction of the spores. Weinberg and Séguin found spores in only one of their four strains of *B. fallax*, but recorded no observations as to their resistance to heat.

CULTURAL PROPERTIES

Upon incubation of this organism at 37°C., growth in various anaerobic mediums and devices used in this laboratory (Hall, 1932) appeared in from 24 to 48 hours, as it did with *B. carnis*. Visible growth of both species failed to appear at 20°C. until after three or more days. In contrast to *B. carnis*, *B. fallax* failed to grow aerobically on any medium; *B. fallax* is an obligate anaerobic.

Deep brain medium containing iron wire. Turbidity and gas were produced in 24 hours incubation. There was no blackening or production of tyrosine crystals in 60 days. In these respects there was no difference between *B. fallax* and *B. carnis*. Henry (1917), however, reported the blackening of a meat medium by *B. fallax*.

Glucose broth in constricted tubes with marble seals. Turbidity, acid, and gas were produced by both species on 24 hours incubation. Many of the cells had settled to the bottom of the tube after 48 hours.

Gelatin in constricted tubes. Turbidity and gas were produced by both species in 24 hours, without liquefaction of the medium even in 30 days.

Blood agar under alkaline pyrogallol. Dew-like colonies of both species developed in 24 hours. The colonies of *B. fallax* increased in size to 1 to 1.5 mm. in diameter in 48 hours, and became slightly raised and irregular in shape. On further incubation, the colonies became opaque. The *B. fallax* colonies were found to be unlike those of *B. carnis* in that they were non-

hemolytic and never tended to round up into pearly beads or to become confluent. Figure 3, plate 1, shows colonies of *B. fallax* on a blood agar slant under alkaline pyrogallol after 96 hours incubation. On 1 per cent glucose blood agar the colonies were similar to those on plain blood agar. They remained non-hemolytic.

Milk in constricted tubes. Both species produced gas in 24 hours, but *B. fallax*, in contrast with *B. carnis*, definitely coagulated the casein in about 7 days; neither digested the casein within 30 days.

Deep 1 per cent agar. Minute lenticular colonies appeared with both species in 24 hours. There was some production of gas. *B. fallax* never showed outgrowths such as were seen with *B. carnis*, and the colonies were always much smaller and more compact. I have not seen the "coeurs jaunes" colonies described and illustrated by Weinberg and Séguin (1917). Figures 4 and 5, plate 1, show 8-day-old colonies in deep methylene blue (1:100,-000) agar. Figure 6 shows a similar colony of *B. carnis* for comparison. This particular colony was taken from agar containing no methylene blue. The dye had no effect upon the size and shape of the colonies.

Difco "Bacto-tryptone." No indol was detected with either species in 24-to 96-hour cultures in 1 per cent solutions of "Bacto-tryptone" in distilled water in constricted tubes by the Ehrlich-Böhme and Goré tests as recommended by Conn et al. (1932).

Fermentative reactions. There is general agreement that *B. fallax* ferments glucose, galactose, levulose, mannose, maltose, sucrose, raffinose, and xylose and that it does not ferment dulcitol, sorbitol, glycerol and inositol. Both fermentation and nonfermentation of lactose, arabinose, salicin, mannitol, dextrin, starch, and inulin have been reported. Kahn (1922) found that rhamnose and amygdalin were not fermented.

My attempts to determine the fermentative reactions of *B. fallax* showed that glucose, galactose, levulose, mannose, maltose, lactose, sucrose, raffinose, xylose, rhamnose, arabinose, salicin, amygdalin, mannitol, and dextrin were fermented, and that starch, inulin, dulcitol, sorbitol, glycerol, and inositol were not fermented.

Discordant results in the reports of the various workers are probably due to the difficulty of obtaining growth in the mediums used, and to the possibility that the various strains were not identical with the type strain described by Weinberg and Séguin and studied by the author.

It is to be noted that *B. fallax* fermented raffinose, xylose, rhamnose, mannitol, and arabinose, in addition to the carbohydrates fermented by *B. carnis.*

PATHOGENICITY

According to Weinberg and Séguin (1915, 1917), some recently isolated strains of *B. fallax* were pathogenic for guinea pigs, but this property disappeared upon artificial incubation. They also found that a soluble toxin was produced. Duthie (1924) reported that his strains were weakly pathogenic. Mutch and Mutch (1927) observed their strain to be nonpathogenic for mice and guinea pigs. However, they reported improvement of arthritic patients when *B. fallax* was given as a vaccine.

The type strain studied here has shown no evidence of pathogenicity in contrast to *B. carnis* which has remained pathogenic 36 years after isolation Subcutaneous injection of one cubic centimeter of a 24-hour glucose broth culture of *B. fallax* failed to produce any visible effects in guinea pigs weighing from 250 to 300 grams. Intravenous injection of one cubic centimeter of a similar culture was nonpathogenic for a rabbit weighing 1550 grams No pathogenicity was encountered in the preparation of an agglutinating serum in a rabbit weighing 1750 grams, in contrast to the great difficulty encountered in preparing antiserums with *B. carnis.*

SEROLOGY

Agglutinating serums for *B. fallax* were prepared by Weinberg and Séguin (1915, 1917), Duthie (1924), and Mutch and Mutch (1927). These serums agglutinated the homologous strains, but action on other strains was inconstant.

In order to show serologic differences between *B. fallax* and *B. carnis,* anti-*B. fallax* agglutinating serum was prepared in a white

rabbit weighing 1750 grams by the injection of a total of 19 cc. of 2- to 3-day-old glucose-broth cultures over a period of 38 days. No agglutinins for *B. fallax* were demonstrable in a 1:20 dilution of the serum of the rabbit before the injections The initial dose of 1 cc. was made intravenously. The remainder of the injections, 2 cc. each, were made subcutaneously. The rabbit was bled ten days after the last injection and serum was obtained for agglutination tests.

The serum agglutinated *B. fallax* in all dilutions up to 1:1280. It did not agglutinate any of the four strains of *B. carnis* at 1:20; nor did 1 cc. of the serum protect against 0.05 cc. of a 24-hour glucose broth culture of any strain of *B. carnis* Hall and Duffett (1935) reported cross-agglutination between all of the four strains of *B. carnis*. Serums used in those tests failed to agglutinate *B. fallax* at 1:20 or higher.

SUMMARY

Morphological, cultural, biochemical, pathogenic and serological differences between *B. fallax* and *B. carnis* have been described.

B. fallax and *B. carnis* are separate and distinct species of bacteria.

The author is indebted to Dr. Ivan C. Hall for cultures and suggestions, to Miss Elizabeth O'Toole for the flagellar stain, and to Mr. Glen Mills for the photographs.

REFERENCES

Bailey, H. D. 1930 Science, **52**, 95.
Bulloch, W. et al. 1917 Reports of the Committee upon Anaerobic Bacteria and Infections. Special Report No. 39, 27.
Conn, H. J. et al. 1932 Pure Culture Study of Bacteria, **1**, vi–16.
Duffett, N. D. 1933 Univ. of Colorado Studies, **21**, 22.
Duthie, G. M. 1924 Compt. rend. Soc. de Biol., **91**, 327.
Hall, I. C. 1932 Pure Culture Study of Bacteria, **1**, iii–2.
Hall, I. C., and Duffett, N. D. 1935 Jour. Bact. **29**, 269.
Henry, H. 1917 Jour. Path. and Bact., **21**, 344.
Henry, H., and Lacey, M. 1920 Jour. Path. and Bact., **23**, 281.
v. Hibler, E. 1908 Unters. über die path. Anaëroben, Fischer, Jena.
Kahn, M. C. 1922 Jour. Med. Res., **43**, 155.

KENDALL, A. I., DAY, A. A., AND WALKER, A. W. 1922 Jour. Inf. Dis., **30**, 141.
KLEIN, E. 1903 Centr. f. Bakt., **35**, 459.
KLEIN, E. 1904 Trans. Path. Soc., London, **55**, 74.
MUTCH, J., AND MUTCH, N. 1927 Lancet, **1**, 1021.
ORNSTEIN, M. 1920 Zeit. f. Hyg., **91**, 153.
ROBERTSON, M. 1929 A System of Bacteriology, His Majesty's Stationery Office, London, **3**, 241, 272.
STOKES, A. 1916 Jour. R. A. M. C., **27**, 361.
WEINBERG, M., AND GINSBOURG, B. 1927 Les Microbes Anaérobies, Masson, Paris, 202.
WEINBERG, M., RENARD, C., AND DAVESNE, J. 1926 Compt. rend. Soc. de Biol., **94**, 813.
WEINBERG, M., AND SÉGUIN, P. 1915 Compt. rend. Soc. de Biol., **78**, 277, 686.
WEINBERG, M., AND SÉGUIN, P. 1916 Compt. rend. Soc. de Biol., **79**, 581.
WEINBERG, M., AND SÉGUIN, P. 1917 La Gangrène gazeuse, Masson, Paris, 151.

PLATE 1

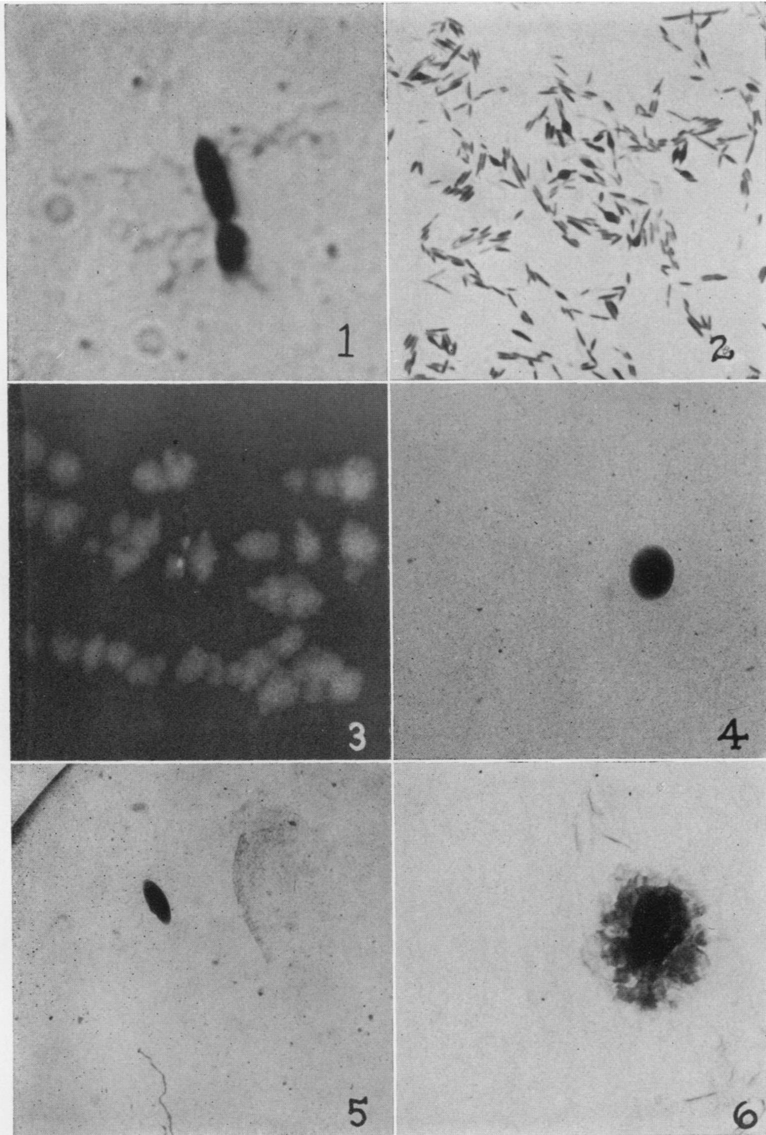
Fig. 1. *B. fallax* with flagella stained by Bailey's method from a 22-hour culture on blood agar slant under alkaline pyrogallol. × 3300.

Fig. 2. Vegetative rods and orgonts of *B. fallax* stained by Gram's method from a 48-hour culture on blood agar slant under alkaline pyrogallol. × 1000.

Fig. 3. Colonies of *B. fallax* on blood agar slant under alkaline pyrogallol after 96 hours incubation. × 6.

Figs. 4 and 5. Colonies from 8-day culture of *B. fallax* in deep methylene blue 1 per cent agar. × 10. Figure 5 shows a colony viewed edgewise.

Fig. 6. Colony from 8-day culture of *B. carnis* in deep 1 per cent agar. × 10.



(Nicholas D. Duffett: Differentiation of B. Fallax and B. Carnis)