

# GRAM-NEGATIVE BACILLI OF THE GENUS BACTEROIDES<sup>1</sup>

JOHN C. HENTHORNE

*Fellow in Pathology, The Mayo Foundation*

LUTHER THOMPSON

*Section on Clinical Pathology*

AND

DONALD C. BEAVER<sup>2</sup>

*Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minnesota*

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This study was undertaken as an extension of two previous reports on the problem of non-sporulating, anaerobic bacilli; namely, that of Thompson and Beaver, (1931, 1932) and that of Beaver, Henthorne, and Macy (1934). The object of this research was the investigation of the morphologic, biologic, and agglutinative properties of several Gram-negative species of non-sporulating anaerobic bacilli; and a comparison of these species with each other and with the other anaerobes encountered, to demonstrate, if possible, the generic relationships of any or all strains.

## HISTORY

Veillon and Zuber (1897) are generally credited with the most important early work on the pathogens of this genus. They reported 25 cases of fetid and gangrenous suppuration, and described various anaerobes as the etiologic agents. One of these anaerobes recovered from osteo-arthritis of an infant was probably

<sup>1</sup> Abstract of thesis submitted by Dr. Henthorne to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Pathology. Read in part before the Society of American Bacteriologists, Chicago, Illinois, December, 1934.

<sup>2</sup> Now residing in Detroit, Michigan.

*Bacteroides funduliformis* which later was described and named by Hallé. In a more detailed report, which appeared later, these authors (Veillon and Zuber, 1898) said that their research was inspired by the paradoxical observation previously made by Widal and Nobecourt, that pus from gangrenous tissues contained many bacteria, as demonstrated by direct smear, but on culture, either very few organisms grew, or the cultures (aerobic) remained sterile. Veillon and Zuber (1897, 1898) then undertook a systematic research of gangrenous processes with fetid suppurations, namely, pulmonary gangrene, otitis, mastoiditis, abscess of the brain, gangrenous angina, dental caries, appendicitis, peritonitis, peri-intestinal phlegmons, bartholinitis, and pelvic suppurations. They found anaerobes in all of these lesions. Some of these anaerobes were cocci, some were Gram-positive, some were spore-formers, but several Gram-negative members of the genus *Bacteroides* were described, and were found to play an important part in producing the inflammatory processes.

Other authors, notably Rist (1905), Guillemot, Hallé, and Rist (1904) and Wegelius (1909), confirmed the results of Veillon and Zuber by more intensive studies on single organ systems. Aside from systematic research, many reports of cases, in which infections were caused by bacilli of this genus, have appeared in the European literature. Two articles, one by Teissier, Reilly, Rivalier, and Layani (1929), and one by Teissier, Reilly, Rivalier, and Stéfanescu (1931), contributed important clear-cut bacteriologic and clinical data on infections caused by *Bacteroides funduliformis*. These authors reported 4 cases in which septicemia was caused by *Bacteroides funduliformis*, which was diagnosed in pure culture by anaerobic blood culture. They demonstrated agglutinins for *Bacteroides funduliformis* (1 to 800) in the blood of one of their patients. Experiments on complement fixation with their four strains, however, failed to demonstrate close serologic relationship between the strains. These authors also gave an excellent review of the anaerobic literature.

The literature on the genus *Bacteroides* has been less extensive in the United States than in other countries.

In 1901, Norris described a case in which pylephlebitis was

associated with an anaerobic bacillus which, as he recognized, belonged to the group of Veillon and Zuber (*fragilis?*).

Harris (1901-1905) reported a case of pyemia that was caused by a non-spore-forming bacillus which he named *mortiferus*.

Tunncliffe (1913a) reported a motile, Gram-negative anaerobic bacillus (*serpens?*), which she isolated from a case of acute rhinitis. She also reported a case of chronic bronchitis from which she isolated an anaerobic Gram-negative bacillus (1913b).

Oliver and Wherry (1921) described a Gram-negative non-sporulating anaerobic bacillus which utilized hemoglobin for the production of an extracellular melanin or melanin-like substance, which they named *Bacterium melaninogenicum*. They isolated this organism from the pharynx, tonsils, infected surgical wounds, urine, and feces. Burdon (1928) found this same organism in the mouths of normal human beings and animals, as well as in chronic and subacute pathologic processes; that is, pulmonary abscess, and pyorrhea. In cases of puerperal fever, Burdon found *Bacterium melaninogenicum* both in the infected uterus and in the blood stream. It was often accompanied by other bacteria. He considered the presence of *Bacterium melaninogenicum* an index of pollution, and believed that this organism played a prominent part in various pathologic processes, although in pure culture it was not pathogenic for laboratory animals.

Two of us (Thompson and Beaver (1931, 1932) ) reported 2 cases of sepsis with formation of abscesses in the lungs. The lesions in 1 case were caused by *Bacteroides funduliformis*; those in the other case, by *Bacteroides fragilis*. We have since expressed the opinion that we were dealing with *Bacteroides funduliformis* in both cases, because both of our strains were hemolytic. We believed that the difference in morphology between the two organisms noted at the time of the report was attributable to the pleomorphism of *Bacteroides funduliformis* rather than to an actual difference of species.

Cohen (1932) reported a review of the literature, and original research on the bacteriology of abscess of the lung. He found a remarkable disregard for the anaerobic flora of abscesses of the lung in previous reports, especially those from the United States.

The anaerobes he encountered were *Bacterium melaninogenicum*, *Bacteroides furcosus*, *Bacteroides thetoides (funduliformis)*, and *Bacteroides fragilis*, as well as *Fusiformis*, *Leptothrix*, and others. Cohen used the term, *Bacillus*, rather than the generic name, *Bacteroides*.

Two of us (Beaver and Henthorne) with Macy (1934) reported 2 cases of sepsis and hepatic abscess caused by *Bacteroides funduliformis*.

There have been many contributions to the anaerobic bacteriology of stools. The recent one of Eggerth and Gagnon (1933) indicates that anaerobes constitute the predominating organisms in the stool. These authors carried out extensive research on the Gram-negative species of the genus *Bacteroides*, and isolated and studied 118 strains of eighteen species. These strains produced only feeble agglutinins which were strain-specific rather than species-specific. No serologic reactions which could be classed as group reactions were encountered. It is clear then that strict anaerobes which may be classified in the genus *Bacteroides* have been isolated from fetid and gangrenous suppurations of practically all organ systems of the human being, as well as from normal mucous membranes.

#### PATHOGENICITY

It must be admitted that the *Bacteroides* of feces have not been proved to be pathogenic. The pathogenicity of the species isolated from human lesions, however, including *Bacteroides fragilis* and *Bacteroides funduliformis*, has been demonstrated by various investigators through inoculation of laboratory animals. The mouse has proved relatively refractory to infection especially with *Bacteroides funduliformis*, and the guinea pig has proved more resistant than the rabbit. The latter observation corresponds with our own experience with *Bacteroides funduliformis* in that the four strains previously reported from The Mayo Clinic produced subcutaneous abscesses in guinea pigs, while rabbits often succumbed with systemic infections and abscesses of the liver, lungs, and joints. The two strains described in the second report provided a nucleus about which this research was built and

are listed subsequently as *Bacteriodes funduliformis*, strains 1 and 2.

Unfortunately, we have not had an opportunity to test the pathogenicity of our strains of *Bacteroides fragilis* or *Bacteroides A* for laboratory animals.

#### CLASSIFICATION ✓

It is apparent that because of its pleomorphism, *Bacteroides funduliformis* has been variously named (*thetoides*, *fragilis*) by different investigators who, after further observation and collaboration, have realized that they were dealing with *Bacteroides funduliformis*. It is possible that other anaerobes, for instance those which have been named *Leptothrix* or *Streptothrix*, are closely related to *Bacteroides funduliformis*. *Actinomyces necrophorus*, also, is similar to *Bacteroides funduliformis* in several important biologic characteristics. This organism is well described by Cunningham (1930), Shaw (1933), and others and is listed by Topley and Wilson (1929) in the genus *Fusiformis* along with *Fusiformis dentium* and pathogenic species of non-spore-bearing anaerobic bacilli, including *Bacteroides fragilis* but not *Bacteroides funduliformis*.

*Bacillus mortiferus* described by Harris (1901-5) is also similar in most of its biologic reactions to *Bacteroides funduliformis*. One difference exists, that is, *Bacillus mortiferus* formed gas from carbohydrate-free protein mediums. We have not observed this reaction in cultures of *Bacteroides funduliformis*. The nature of Harris' case and the fact that it is the only one reported due to *Bacillus mortiferus* of which we are aware, suggests, however, that he was dealing with one of a closely related group of organisms resembling *Bacteroides funduliformis*.

In general, important biologic characteristics common to all of these variously named organisms are as follows; marked morphologic pleomorphism with production of filaments, requirement of animal tissue in culture mediums, production of indol, production of hydrogen sulphide, production of gas from carbohydrate mediums, and marked hemolytic action on erythrocytes. Whether differences between *Bacteroides funduliformis* and these

otherwise-named non-spore-bearing Gram-negative anaerobes are of major or minor importance must be determined by future comparisons.

Bergey (1934) has classified in the genus *Bacteroides*, several of the fecal bacilli and the pathogens *Bacteroides fragilis* and *Bacteroides fundibuliformis* (it is noted that the spelling of Bergey, with the second syllable "ib" is different from that of the French authors). Among the fecal *Bacteroides*, Bergey has listed several members, which, according to the original describer, do not fit the generic definition, namely, *Bacteroides angulosus*, and *Bacteroides tenuis*, which are described as spore-bearers by Distaso (1911, 1912), and *Bacteroides laevis*, *Bacteroides liquefaciens*, and *Bacteroides rigidus*, which are described by the same author as facultative rather than obligate anaerobes.

#### ORIGINAL RESEARCH

*Material.* Gram-negative species of the genus *Bacteroides* were obtained from sources listed in table 1. We encountered three strains of *Bacteroides fragilis* and one strain which we designated *Bacteroides fragilis* 4. Of this group, strain 2 was obtained from blood culture before death as well as from gangrenous lesions cultured at necropsy. Six strains of *Bacteroides funduliformis* were obtained. Of these, strain 4 was isolated from the blood culture taken before death. A strain of another species, probably undescribed heretofore, has been called *Bacteroides* A.

In addition to these species, a strain of *Fusififormis dentium*, one of *Bacteroides bifidus*, an unidentified Gram-positive anaerobic bacillus which morphologically resembled the diphtheroid bacilli, and a Gram-negative pleomorphic filamentous organism probably a nonpathogenic Actinomycete have been studied for a comparison with the Gram-negative *Bacteroides*.

Information regarding the lesion cultured, whether or not the organism was recovered directly from the lesion in pure culture, the predisposing factor to bacterial invasion, and the final outcome of the condition is listed in table 1.

*Methods.* The plating method advised by Spray (1930) was used for determination of the hemolytic action of organisms on blood agar and for isolation of colonies for pure culture.

TABLE 1  
Source of strains of anaerobic bacilli

STRAIN	SOURCE	TYPE OF CULTURE	PRIMARY PATHOLOGIC CHANGE	END RESULT
<i>Bacteroides fragilis</i> 1	Pelvic abscess	Mixed	Carcinoma of rectum	Fatal
<i>Bacteroides fragilis</i> 2*	Hepatic abscess and appendix	Mixed	Gangrenous appendicitis	Fatal
<i>Bacteroides fragilis</i> 3	Abscess over sacrum	Mixed	Infected pilonidal cyst	Rapid recovery
<i>Bacteroides fragilis</i> 4	Appendix	Mixed	Gangrenous appendicitis	Fatal
<i>Bacteroides funduliformis</i> 1	Hepatic abscess	Pure	Unknown	Fatal
<i>Bacteroides funduliformis</i> 2	Hepatic abscess	Pure	Carcinoma of rectum	Fatal
<i>Bacteroides funduliformis</i> 3	Hepatic abscess	Pure	Carcinoma of rectum	Fatal
<i>Bacteroides funduliformis</i> 4	Hepatic abscess	Pure	Carcinoma of rectum	Fatal
<i>Bacteroides funduliformis</i> 5	Fecal (?) fistula	Pure	Carcinoma of sigmoid flexure	Recovered
<i>Bacteroides funduliformis</i> 6	Pulmonary abscess	Mixed		Fatal
<i>Bacteroides</i> A*	Appendix	Mixed		
<i>Bacteroides bifidus</i> *	Hepatic abscess and appendix	Mixed		
Anaerobic diphtheroid*	Hepatic abscess and appendix	Mixed		
<i>Fusiformis dentium</i>	Putrid pleurisy	Pure	Foreign body and pulmonary abscess	Fatal
Actinomyces (?)	Urine†	Mixed		

\* Four anaerobes from same case.

† No symptoms referable to bladder.

Strains were maintained with cultures in tubes (20 by 1.5 cm.) of glucose brain broth. All strains grew readily in one or two days. Cultures were also easily preserved in similar tubes of soft agar, to which a piece of fresh brain tissue had been added.

Fermentation was determined in tubes which contained 15 cc. of brain broth; to each tube was added 0.75 cc. of 10 per cent carbohydrate solution which had been sterilized by filtration through a Seitz filter. The marble chips, which usually are added to brain broth as a buffer, were omitted in this procedure. The tubes were inoculated, sealed with semisolid petrolatum, and incubated for two weeks. Production of gas was ascertained by observation of the tubes at frequent intervals, and by the elevation of the petrolatum by gas. The pH value of the culture was estimated at the end of the incubation period by addition of a few drops of bromthymol blue or bromcresol green, and comparison with a standard color chart.

Production of hydrogen sulphide was determined by the action of the gas which was produced by cultures, on filter paper impregnated with lead acetate.

Litmus milk reactions were carried out in the long narrow type of tubes used for brain broth.

Potato cultures were incubated in an anaerobic jar, from which air had been displaced by nitrogen. Pyrogallic acid and a solution of sodium hydroxide were added to remove remaining traces of oxygen.

The difficulty of preparing antigens, especially with *Bacteroides funduliformis*, has been noted by other authors. Growth on slants in anaerobic jars is particularly difficult to emulsify into uniform suspensions because of the tendency of these organisms to grow in long filaments on solid mediums. For this reason, and because growth under these conditions is uncertain, and often feeble, brain broth cultures were used for the preparation of all antigens. Cultures two to four days old were sufficiently well grown for preparation of a satisfactory suspension. The organisms were killed with 1:500 formalin in physiologic saline solution, and were suspended by shaking with glass beads for from five to twenty minutes. The suspensions were finally filtered through cotton and centrifuged at low speed for a few minutes to



remove clumps and particles of brain. The supernatant fluid usually contained a sufficiently dense and homogeneous suspension for the agglutination reactions.

Immune serum was obtained from rabbits which had received injections, at intervals of three to four days, of an antigen which had a density approximately that of a typhoid standard of 500,000,000 per cubic centimeter. Doses were given as follows: 0.1, 0.2, 0.4, 0.8, 1.0, 1.0, and 1.0 cc. The animals were bled one week after the final dose had been administered.

The antigen for absorption of agglutinins was obtained by centrifuging 20 cc. of a suspension of antigen which had a density approximately twice that used for immunization. This bacterial mass was resuspended in 3 cc. of a 10 per cent solution of immune serum in physiologic saline solution. This suspension was incubated at 37°C. for twenty-four hours and placed in the ice box for a similar period.

*Morphology.* In general, all strains were stained poorly with safranin, but well with dilute carbolfuchsin. Motility was not observed in any strains. Hanging drop preparations proved that the apparent branching, which sometimes was seen in smears, was false. True branching was not observed except in the Actinomyceae. In order to obtain organisms for smears or subcultures in broth, it was usually necessary to withdraw material from the bottom of the tube with a capillary pipet.

Two species that fit the original description of *Bacteroides fragilis* by Veillon and Zuber have been isolated in the course of this study. These three strains of one species have been designated *Bacteroides fragilis* 1, 2, and 3. The dissimilar strain, which represents a subspecies, has been designated *Bacteroides fragilis* 4. Morphologically, the two species cannot be distinguished from each other.

The morphology of *Bacteroides fragilis* in fresh cultures and in pus is not especially characteristic. In this form, the bacillus measures 0.5 by 2.0 to 2.5 micra. Older cultures tend to develop larger forms (0.6 to 0.8 by 2.0 to 9.0 micra) but even in this state the organism cannot be distinguished from aerobic Gram-negative bacilli, by its morphology.

*Bacteroides funduliformis*, on the other hand, is characteristic

morphologically because of its extreme pleomorphism. In the necrotic centers of lesions, growth occurs in such dense clumps of small bacillary forms that it is difficult to pick out the individual bacilli. Bipolar staining is a prominent feature and gives the bacilli a diplococcus-like appearance. Filaments 1.0 by 10.0 micra have been observed in microscopic sections of the granulating periphery of abscesses. On solid mediums, long unbranched filaments develop after six or seven days of cultivation. Fila-

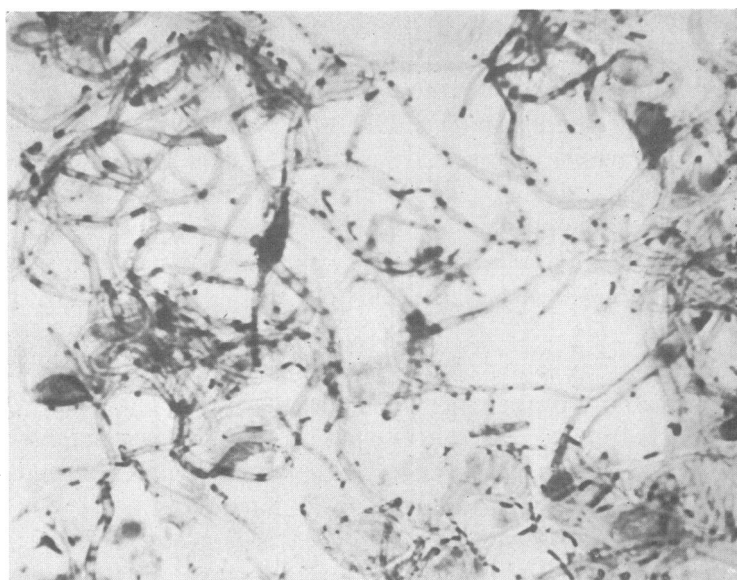


FIG. 1. *BACTEROIDES FUNDULIFORMIS* FROM TEN-DAY CULTURE ON BLOOD AGAR ( $\times 950$ ). STAINED WITH DILUTE CARBOLFUCHSIN

ments of this type are shown in figure 1 (strain 2). These filaments are often 80.0 to 100.0 micra long and are composed of segments 2.0 to 20.0 micra in length. The segments are marked off by granules which are often double. This appearance suggests that the filaments are chains of swollen elongated bacilli and that the granules are the heavily staining beads so frequently seen at the poles of the bacilli. The filaments are 0.6 to 2.5 micra wide and although no "funduliforms" are seen in smears from

solid mediums, there are fusiform swellings 4.0 to 5.0 micra wide. In brain broth the earliest forms are coccobacillary, similar to those seen in pus, or rarely, short curved "comma" shaped rods 0.3 to 0.5 by 2.0 to 4.0 micra. Within two days the characteristic "funduliform," "ball," "leukocyte," or "thetoid" forms appear, together with straight bipolar staining rods which vary in size from coccobacillary up to filaments 20.0 to 40.0 micra long. The ball-shaped organisms (1.5 to 3.0 micra in diameter) seem to be

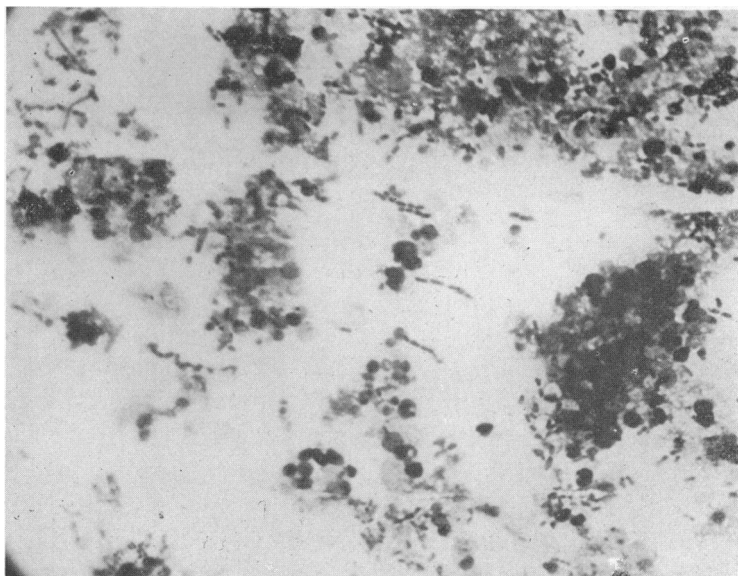


FIG. 2. *BACTEROIDES FUNDULIFORMIS* FROM TWO-DAY CULTURE IN GLUCOSE BRAIN-BROTH ( $\times 1000$ ). STAINED WITH DILUTE CARBOLFUCHSIN

formed by bacilli, swollen so that the bipolar dark staining beads appear as crescentic caps opposite each other on the ball. Figure 2 is made from a smear of a two day glucose-brain broth culture of *Bacteroides funduliformis* (strain 4).

*Bacteroides A* is slightly shorter and more plump (1.0 by 2.0 micra) than *Bacteroides fragilis*. Bipolar staining in day-old broth cultures is prominent. A characteristic feature of this bacillus is its tendency to develop tremendously swollen forms

even in forty-eight hour cultures. These swollen bacilli are less definite in outline than the "funduliforms" of *Bacteroides funduliformis*, and look like bacteria which are undergoing lysis. Practically all of the bacillary forms show this change at about the same time, but the cultures do not seem particularly delicate, since the bacillus has lived in brain broth for several weeks at room temperature.

The strain of *Bacteroides bifidus* corresponds with the description of Topley and Wilson (1929) of *Lactobacillus bifidus*. Gram-positive granules are present in the bacilli only in very fresh cultures.

The strain of *Fusiformis dentium* was Gram-positive only in the pus from which it was obtained.

The long filaments of the Actinomycete develop in cultures seven or eight days old. Fresher cultures contain only bacillary forms.

The anaerobic diphtheroid loses its diphtheroid appearance when it becomes Gram-negative after three to four days of incubation. Its morphology is not characteristic.

*Biologic characteristics.* The main biologic characteristics of the group of organisms studied are summarized in table 2. A few points, however, must be further discussed.

In general, all strains have been able to survive in glucose-brain broth for several weeks at room temperature. None of the organisms survive long, however, if exposed to air. They are able to grow in an atmosphere of nitrogen. Although all strains seem to grow better in brain broth which contains fermentable carbohydrate, growth is not improved on solid mediums by the addition of higher concentrations of glucose.

*Bacteroides fragilis* grows on the surface of plain blood agar as very fine, transparent, moist, grayish white, fimbriated or spreading colonies, without production of hemolysis. Isolated surface colonies do not exceed 2 mm. in diameter. On this medium, growth is seldom perceptible before five days of incubation. Figure 3 is a picture of the surface colonies of *Bacteroides fragilis* on plain blood agar. This picture required special photographic technic because the colonies were so fine and transparent.

TABLE 2  
*Biologic reactions of anaerobic bacilli\**

	BACTEROIDES FRAGILIS, 1, 2, AND 3	BACTEROIDES FRAGILIS, 4	BACTE- ROIDES FUNDULI- FORMIS, 1, 2, 3, 4, 5, AND 6	BACTE- ROIDES A	BACTE- ROIDES BIFIDUS	FUSI- FORMIS	ACTINO- MYCES
Glucose.....	+++†	++	+‡	++	++	++	++
Maltose.....	++	++	++	++	++	++	++
Mannitol.....	-	-	-	-	-	++	-
Lactose.....	++	++	-	++	++	++	-
Sucrose.....	++	++	-	++	++	++	-
Levulose.....	++	++	+	++	++	++	++
Inulin.....	++	++	-	-	-	+	-
Dextrin.....	++	++	-	++	-	++	++
Inositol.....	-	-	-	-	-	+	-
Xylose.....	++	++	-	-	-	+	++
Raffinose.....	++	++	-	++	++	+	-
Rhamnose.....	-	++	-	-	++	++	-
Arabinose.....	-	++	-	-	-	-	-
Galactose.....	++	++	+	++	++	++	-
Dulcitol.....	-	-	-	-	-	-	-
Glycerol.....	-	-	+	++	-	-	-
Salicin.....	-	-	-	++	++	++	-
Trehalose.....	-	++	-	-	++	+	-
Glycogen.....	++	++	-	-	-	++	-
Sorbitol.....	-	-	-	-	-	-	-
Production of H <sub>2</sub> S...	-	-	+§	-	-	-	-
Production of hemo- lysis.....	-	-	+++	-	-	-	-
Production of gas on CHO.....	+ (S1)	+ (S1)	++	++++	-	-	-
Growth on potato...	-	-	-	-	-	-	-
Production of indol..	∓	∓	+	-	-	-	+
Reduction of nitrates.	-	-	-	-	-	-	-
Reaction on litmus milk.....	Acid, coagula- tion	Acid, coagula- tion	-	Acid	Acid	-	-
Liquefaction of gela- tine.....	-	-	-	-	-	-	-

\* Anaerobic diphtheroid produced indol, rendered all mediums alkaline; pH of mediums before inoculation was 6.7-6.9.

† ++, pH 4.6-5.0.

‡ +, pH 6.0.

§ In this part of table, positive reactions were graded on basis of + to ++++.

Growth on hormone blood agar is more profuse and more rapid, and the colonies are yellow and opaque. Deep colonies are yel-

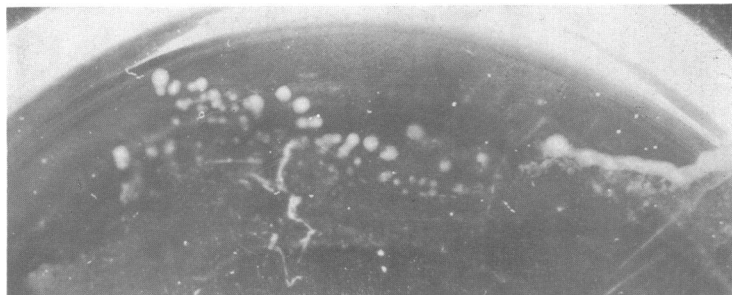


FIG. 3. BACTEROIDES FRAGILIS; SURFACE COLONIES ON BLOOD AGAR

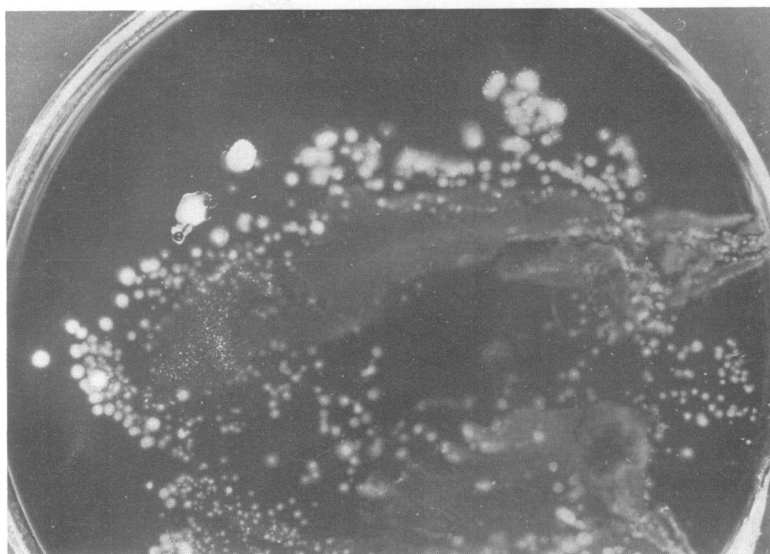


FIG. 4. BACTEROIDES FUNDULIFORMIS; SURFACE COLONIES ON BLOOD AGAR

low, opaque, and lens-shaped. They do not exceed 1 mm. in their longest diameter.

Growth of *Bacteroides fragilis* in glucose-brain broth takes place in twenty-four hours and produces a diffuse clouding of the medium. A characteristic feature of the growth in fermentable

carbohydrate mediums is the slight but definite production of gas. In an anaerobic jar, growth is obtained in peptone-water sugar mediums, but not in plain broth. The biologic reactions of the first three strains are uniformly similar, and those of the fourth strain, which is a subspecies, vary only slightly.

The production of indol by *Bacteroides fragilis* is listed as either negative or positive. For the most part, tests for indol were negative but we have encountered an occasional faintly positive reaction in fourteen-day old cultures.

*Bacteroides funduliformis* grows on plain blood agar with as much difficulty as does *Bacteroides fragilis*, but the colonies are larger (3 mm.) and more opaque. The centers of the colonies are grayish-white and opaque, the borders are fimbriated and may spread to coalesce with other colonies on a sufficiently moist medium. Figure 4 shows the surface colonies. On slants, when the moisture has drained from the surface, colonies are discoid, discrete, and have slightly raised centers and edges.

The colonies when they first appear are fairly moist, but after incubation for ten days they are hard and dry, and can be picked entire from the medium. Deep colonies are lens-shaped or triangular and measure 2 mm. in their greatest diameters. They occasionally produce gas in blood agar which contains glucose, particularly if the medium is not too stiff. The hemolytic action of this species is an important characteristic. The radius of the zone of hemolysis measures 4 to 5 mm.

In brain broth, *Bacteroides funduliformis* grows in clumps at the bottom of the tube. In fresh cultures, a few bacilli are diffused throughout the broth, but they are scarcely sufficient in number to produce appreciable clouding. Often, the only indication of growth is the presence of bubbles of gas caught in the particles of brain at the bottom of the tube.

The biologic reactions of the six strains of *Bacteroides funduliformis* were very uniform, except that strain 6 did not ferment galactose. This fact was remarkable, since Teissier, Reilly, Rivalier, and Stéfanesco (1931) found their four strains variable. A comparison of the fermentation reactions of their four strains and of the six strains used in this study appears in table 3. The

uniformity of fermentation reactions in our six strains was attributable either to efficiency of the brain-broth carbohydrate medium or to chance, in that strains of only one species were isolated. Very slight reductions of the value for the pH occurred in mannitol and lactose in cultures of the first four strains, but since no production of gas was observed, the fermentation reaction was considered negative.

*Bacteroides A* differs from both *Bacteroides fragilis* and *Bacteroides funduliformis* in that it grows on plain agar. The colonies are pale yellow and discrete. Surface colonies are 1 mm. in

TABLE 3  
*Characteristic fermentation reactions of Bacteroides funduliformis\**

	FOUR STRAINS OF TEISSIER, BILLY, RIVALIER, AND STÉFANESCO				SIX STRAINS USED IN PRESENT STUDY					
	1	2	3	4	1	2	3	4	5	6
Glucose.....	+	++	+	+	+	+	+	+	++	++
Levulose.....	+	++	+	+	+	+	+	+	++	++
Lactose.....	-	-	-	-	-	-	-	-	-	-
Sucrose.....	++	+	-	-	-	-	-	-	-	-
Maltose.....	++	++	++	++	++	++	++	++	++	+
Mannitol.....	-	++	++	++	-	-	-	-	-	-
Galactose.....					+	+	+	+	+	-
Glycerol.....					+	+	+	+	+	+

\* Appreciable amounts of gas formed in all positive reactions.

diameter. Deep colonies produce gas in agar which contains glucose. The most characteristic biologic feature of this organism is its ability to metabolize certain carbohydrates very rapidly with gas production that amounts to a "stormy fermentation." Like *Bacteroides fragilis*, this organism grows in peptone-water sugar mediums but not in plain broth, when incubated in the anaerobic jar.

*Agglutination.* The agglutination reactions, which are summarized in table 4, support the biologic reactions in proving that *Bacteroides fragilis* 4 belongs to a serologic group which is distinct from the other three strains, and that the six strains of *Bacteroides funduliformis* belong to one and the same species.



The variations recorded in the agglutination of the several strains of *Bacteroides funduliformis* were attributable more to variations in the quality of the antigen than to any marked difference of the strains. As has been mentioned, satisfactory suspensions of this organism were most difficult to obtain in sufficient quantities to use in titrations with all available serums. The results listed in the table are those obtained with relatively large quantities of antigen. Results of agglutination were just as variable in single strains when antigens were later prepared from

TABLE 4  
Cross agglutinations

ANTIGEN	SERUM					
	<i>Bacteroides fragilis</i> 1	<i>Bacteroides fragilis</i> 4	<i>Bacteroides funduliformis</i>	<i>Bacteroides</i> A	<i>Bacteroides bifidus</i>	<i>Fusiformis</i>
<i>Bacteroides fragilis</i> 1.....	640	160	80	0	0	0
<i>Bacteroides fragilis</i> 2.....	640	0	20	0	0	0
<i>Bacteroides fragilis</i> 3.....	320	80	0	0	0	0
<i>Bacteroides fragilis</i> 4.....	0	640	0	0	0	0
<i>Bacteroides funduliformis</i> 1.....	0	0	320	0	0	0
<i>Bacteroides funduliformis</i> 2.....	0	0	640	40	0	0
<i>Bacteroides funduliformis</i> 3.....	0	0	640	0	0	0
<i>Bacteroides funduliformis</i> 4.....	0	40	160	40	0	0
<i>Bacteroides funduliformis</i> 5.....	0	20	160	40	0	0
<i>Bacteroides funduliformis</i> 6.....	0	40	80	20	0	0
<i>Bacteroides</i> A.....	0	0	0	320	0	0
<i>Bacteroides bifidus</i> .....	0	0	0	0	320	20
<i>Fusiformis</i> .....	0	0	0	0	0	1,280

time to time. An antigen was prepared from every strain which was agglutinated at 1:320 in the course of the investigation.

Growth of the Actinomycete and the diphtheroid-like bacillus was so sparse, even in glucose-brain broth, that satisfactory antigens could not be prepared either for immunization or for agglutination reactions.

*Bacteroides bifidus* and *Fusiformis dentium* agglutinated well in their homologous serums, but cross agglutination did not occur with any other strain.

There was some cross agglutination among the Gram-negative species in lower dilutions. These cross agglutinations were not sufficiently definite, however, and not constant enough to demonstrate group agglutinins clearly. The assumption that group agglutinins were not present in the serums was supported by the experiments on the absorption of agglutinins.

TABLE 5  
*Absorption of Bacteroides funduliformis agglutinins\**

ABSORBING ANTIGEN	TITER
Unabsorbed.....	320
<i>Bacteroides funduliformis</i> 1.....	40
<i>Bacteroides funduliformis</i> 2.....	160
<i>Bacteroides fragilis</i> 1.....	320
<i>Bacteroides fragilis</i> 4.....	320
<i>Bacteroides</i> A.....	320
Control.....	320

\* *Bacteroides funduliformis* 1 serum and *Bacteroides funduliformis* 1 antigen were used.

TABLE 6  
*Absorption of Bacteroides fragilis agglutinins\**

ABSORBING ANTIGEN	TITER
Unabsorbed.....	1,280
<i>Bacteroides fragilis</i> 1.....	40
<i>Bacteroides fragilis</i> 2.....	160
<i>Bacteroides fragilis</i> 4.....	1,280
<i>Bacteroides funduliformis</i> 1.....	1,280
<i>Bacteroides</i> A.....	1,280
Control.....	1,280

\* *Bacteroides fragilis* 1 serum and *Bacteroides fragilis* 1 antigen were used.

Normal human serum was titrated with each antigen in all agglutinations, as a control.

*Absorption of agglutinins.* These experiments may be summarized by reiteration of the fact that agglutinins were not absorbed by antigens prepared from species other than the one against which the animal was immunized. There was, however,

absorption of agglutinins by different strains of the same species. The results of the two most important agglutinin absorption experiments are listed in tables 5 and 6. These two experiments were repeated several times and varied only slightly. When the quantities of absorbing antigens were too small, agglutinins were absorbed only by the antigen homologous with the serum. A second absorption with similar quantities of antigen was necessary under these circumstances in order to demonstrate agglutinin-absorbing properties of various strains of the same species.

The quality of antigens was tested by agglutination before they were used in the absorptions. The control antigen was a suspension of *Escherichia coli*. The immune serum was again controlled by normal human serum.

#### COMMENT AND SUMMARY ✓

A cultural, morphological and serological study has been made of eleven Gram-negative strains of *Bacteroides* isolated from human inflammatory lesions. Of these, four corresponded closely to the published descriptions of *Bacteroides fragilis*, and six resembled *Bacteroides funduliformis*. We were unable to find a previous description of a strain of another species which we have designated *Bacteroides A*.

*Bacteroides fragilis* and *Bacteroides funduliformis* are the only species of this genus which we have encountered to which a definite pathogenic rôle can be attributed.

These two species can be differentiated by their morphology and by biologic and serologic tests.

Of the four strains of *Bacteroides fragilis* studied, three seemed identical serologically while the fourth strain belonged to a different serologic group.

The six strains of *Bacteroides funduliformis* fall into one serologic group. We feel, however, that the difficulties attendant on the production of satisfactory antigens make it wise to interpret the results of agglutination tests with caution.

*Bacteroides funduliformis* has been commonly obtained by us in pure culture from lesions where it is obviously the only pathogen present, and in all but one of the cases in which we have found it, the result has been fatal.

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