THE GRAM-POSITIVE NON-SPORE-BEARING ANAEROBIC BACILLI OF HUMAN FECES

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In a previous paper (Eggerth and Gagnon, 1933) the predominance of non-spore-bearing anaerobic bacilli in human feces was reported, and a number of the Gram-negative species were described. As observations on the Gram-positive species were at that time incomplete, they were omitted.

Bergey's *Manual of Determinative Bacteriology* (1934) defines the genus *Bacteroides* as consisting of "motile or non-motile rods without endospores. Show good growth with ordinary culture media; without pigment formation. Obligate anaerobes." If this definition is accepted, the Gram-positive species described in this paper fall into the genus *Bacteroides*. For reasons that will appear later, the writer believes that the Gram-negative *Bacteroides* and the Gram-positive non-spore-bearing anaerobic bacilli of the intestine are biologically quite distinct and should be placed in different genera at least; possibly in different tribes or even in different orders. For the present, however, the nomenclature of Bergey's Manual will be followed, as the Manual is the only authoritative system of classification we have. The Gram-positive species will be designated as *Bacteroides*, though it is certain that they will ultimately be placed in another genus.

The organisms to be described were obtained from plate cultures of 85 stools: 11 of these were from breast-fed infants; the remainder, from normal adults. All of them were strictly anaerobic when isolated; with the exception of certain strains of *Bacteroides bifidus*, all have remained obligate anaerobes.

TECHNIC

The technic in general was that of the previous investigation (Eggerth and Gagnon, 1933) to which the reader is referred for more complete details. The feces were first diluted by the method of Torrey (1926); then a 4 mm. loopful of fecal dilution number 4 was well rubbed over the surface of a blood agar plate. This represents an inoculum of 1/3000 mgm. of fresh feces of average water content. Such a plate will usually grow about 100 colonies after five days of anaerobic incubation.

Plating media. For plating the fecal emulsions, a beef-heart infusion agar containing 1.5 per cent of agar, 1 per cent of Parke Davis peptone, and 0.4 per cent of $Na_2HPO_4 \cdot 12\ H_2O$, was used. The pH was set at 7.6, and 5 per cent of sterile blood and 0.15 per cent of sterile glucose were added before pouring the plates. This medium served very well for the original plating of the feces; but it was found that colonies streaked out on a second plate of the same medium sometimes failed to grow. Much better second-generation growth was obtained when liver infusion was substituted for the above heart-infusion agar.

Anaerobic cultivation. Modified McIntosh and Fildes (1916) anaerobic jars were used, as described in the previous paper. Plates were incubated for from five to six days before opening the jars.

Isolation of pure cultures. From three to ten colonies from each stool were selected for pure culture study. Each strain was replated 4 times, using the method of surface streaking; single, well isolated colonies were fished. The liver-infusion blood agar proved very useful for replating.

After a number of strains had been collected and fermentation tests made, some doubt arose as to whether this method actually produced pure cultures. This doubt was occasioned by the fact that the results of the sugar fermentation tests were so very diverse; no two strains agreed completely in their fermentation characteristics, which made classification very difficult indeed. It seemed possible that this great diversity of fermentative characteristics might be due to impure cultures. Therefore many of the strains were subjected to single cell isolation, using a

Chambers micromanipulator and following the technic of Kahn (1929) and of Gee and Hunt (1928). Twenty-eight single cell cultures were obtained, and the fermentation tests were repeated. In each case, exactly the same results were obtained with the single cell cultures as with the original cultures that had been replated 4 times. It would seem, then, that cultures replated 4 times in the manner described actually are pure.

It often proved very difficult to obtain growth from single cells of these anaerobes, and there were many failures. Various media were tried for the initial propagation of the single cells; by far the best for this purpose was a beef-heart infusion broth containing 5 per cent of sterile blood and 2 per cent of glucose.

Stock cultures. These were carried on brain medium, as described before (Eggerth and Gagnon, 1933).

Medium for carbohydrate fermentation tests. This medium had the following composition: 1 per cent of Parke Davis peptone; 0.5 per cent of NaCl; 0.2 per cent of agar; and brom-cresol-purple as acid indicator. The medium was sterilized in small flasks in the autoclave. The carbohydrates (enough to make 1 per cent of the medium) were autoclaved separately in distilled water and added to the flasks; finally, 4 per cent of sterile serum was added. The completed medium was then distributed in 100 by 11 mm. tubes.

It is important to inoculate the fermentation tubes very heavily, otherwise growth may not take place. For this purpose, a nichrome wire with a spiral at the end was used. Young cultures in brain medium served as the source of the inocula. Incubation of the fermentation tubes was continued for forty days, the jars being opened every five to eight days.

For milk medium, a powdered milk (Klim) was used.

Gelatin medium was prepared by adding 10 per cent of gelatin and 0.15 per cent of glucose to phosphate heart infusion broth and adjusting to pH 7.8. After sterilization, 4 per cent of sterile serum was added.

For indol tests, heart-infusion broth was fermented with *Salmonella Schottmuelleri* to remove the sugar.

Lead acetate broth was prepared by adding 1 cc. of sterile 1

per cent lead acetate to 200 cc. of sterile broth containing 0.5 per cent of glucose. Duplicate tubes were prepared containing also 4 per cent of sterile serum.

Coagulated egg-albumen broth was prepared by cutting cubes of coagulated egg white into tubes of infusion broth and sterilizing.

Nitrate broth was prepared by adding 0.5 per cent of sodium nitrate to infusion broth.

One hundred and thirty strains of Gram-positive non-spore-bearing anaerobic bacilli were studied. These were grouped in 11 species, as follows:

1. *Bacteroides lentus* (isolated 23 times; one single cell culture was obtained).

The morphology of different strains of this organism is very variable. In 15 of the 23 strains, the predominating form is a short oval bacillus, 0.5 to 1.5μ broad and 1 to 3μ long; with these, small coccoid forms or long cigar shaped bacilli up to 6μ in length may occur. The bacilli are usually found in chains of 2 to 20 elements; often the bacilli in a chain are set at angles to each other, giving a "rail fence" appearance. In the other 8 strains, the predominating forms are rather slender rods, 0.3 to 0.5μ thick and 1.5 to 3μ long; these may be single, or, more commonly, in long chains. Many of the strains show both the thick oval bacillus and the more slender one, and sometimes both forms occur in the same chain.

A type of cell that is often found is what Tissier (1908) describes as a "peloton de jardinier" (gardener's ball of twine). The middle of the bacillus is swollen, often to 2 or 3 times the diameter of the cell. This cell cannot be described as fusiform or spindle shaped; for the ends are usually not pointed; in fact, they are often slightly thickened. Such a cell resembles a stick with a ball of twine wound around its middle, hence Tissier's simile. These cells usually occur single.

Most of the strains do not show metachromatic granules. In a few strains, these granules are large and numerous, almost filling the entire cell.

This organism is non-motile.

On blood agar and on liver-infusion blood agar the colonies of this species are small, being 0.25 to 0.75 mm. in diameter. They are slightly raised, smooth, and gray. In 3 strains, the colonies show irregular knobs and projections. There is no action on the red cells. As a rule, colonies do not appear on glucose agar, though there is some growth where the inoculation is heavy.

With the possible exception of glucosamine, none of the carbohydrates are fermented by this organism. A slight acidity (pH 6.2 to 6.6) develops in the glucosamine tubes. It seems possible that this is due to the deaminization of the glucosamine rather than to a true fermentation with acid production; this is suggested by the fact that the presence of the glucosamine does not stimulate the growth of this organism, as a fermentable sugar might be expected to do.

Twelve of the 23 strains blacken lead acetate, the others do not.

Only one strain reduces nitrates to nitrites. A single cell culture was obtained of this strain, so it seems quite certain that it is a pure culture.

Indol is not formed, gelatin is not liquefied. There is no action on milk or coagulated egg albumen. Large doses injected subcutaneously into mice and rabbits are without pathogenicity.

2. *Bacteroides ventriosus* (Tissier 1908) (isolated 3 times).

It seems probable that the organisms described by Tissier as *Coccabacillus oviformis* and *Bacillus ventriosus* are identical; the chief differences in Tissier's description are morphological ones, and in dealing with this group of organisms, minor morphological differences cannot be taken too seriously. The writer has chosen the name *ventriosus* rather than *oviformis* because Tissier's description of the former best fits the 3 strains here described.

On blood agar, in glucose serum broth, and in brain medium, this organism appears as a small thin rod about 0.3μ thick and 2μ long. It forms chains of from 3 to 20 members; the chains are often angulated, like a rail fence. The members of the chain may vary greatly in size and appearance; some may be minute cocci; others, normal bacilli; some may be rods with large median swell-

ings, the "peloton de jardinier" of Tissier. On glucose agar the bacilli are more often single, 0.7 to 1.2μ thick and 2 to 4μ long. The organism is non-motile.

On blood agar plates, the colonies are 1 mm. in diameter, smooth, raised, and gray; there is no action on the red cells. Surface cultures on glucose agar show only slight growth where the inoculum is heavy, without visible colonies.

Two of the three strains ferment glucose, levulose, galactose, and mannose. Glucosamine medium is slowly and slightly acidified. The third strain ferments these sugars, and in addition slowly ferments maltose (in thirty-two days). Gas is not formed. No other carbohydrates are fermented.

There is no action on gelatin, milk, coagulated egg white, lead acetate, or sodium nitrate. Indol is not formed. This species is non-pathogenic for white mice and rabbits.

Tissier notes the fact that this organism dies out very readily in cultures. This was also the writer's experience, as all three strains were soon lost through death of stock cultures.

3. *Bacteroides aerofaciens* (isolated 18 times; 5 single cell cultures were obtained).

This organism appears as a bacillus varying in length from 0.8 to 5μ, with a usual length of 2 to 3μ; the thickness varies from 0.4 to 2μ. The ends are rounded or pointed. It forms chains of 2 to 10 elements. The individuals in one chain often vary enormously in size and shape: long bacilli and cocci of various sizes succeed each other in the chain; Tissier's "peloton de jardinier" is common; also geniculate bacilli and bacilli with various irregular projections. Some of the rods are curved, and when several curved forms occur in a chain the chain may be remarkably twisted or knotted.

In 4 of the 18 strains, a few branching forms were found. These were never abundant; they were most often seen in glucose agar cultures, without blood. The branches took the form of short terminal bifurcations. It is probable that the geniculate, nail head, or otherwise irregular forms often seen in this species represent abortive or incomplete branching.

Metachromatic granules are numerous on glucose agar and on potato medium, less common on blood or liver agar.

This organism is non-motile.

On blood agar plates the colonies of this species are 1 to 1.5 mm. in diameter, gray-white, and hemispherical. There is no action on the red cells. On blood liver agar the colonies are 1.5 to 3 mm. in diameter, slightly raised, gray-white, with a more opaque center. On glucose agar the colonies are 1 to 2 mm. in diameter, gray-white; with some strains they are very tough and difficult to emulsify.

In glucose broth a slight turbidity develops; there is also a sediment which may be small in amount and flocculent, or it may be a large loose stringy mass. With most strains the final pH in glucose broth (after fifteen days of incubation) is 4.8.

All of the 18 strains ferment dextrin, galactose, glucosamine, glucose, lactose, levulose, maltose, mannose, sucrose, and trehalose. With aesculin, amygdalin, glycogen, methyl glucoside, salicin, and starch, the results are variable (all but 2 strains ferment starch). None of the strains ferment adonitol, arabinose, dulcitol, erythritol, glycerol, inositol, inulin, mannitol, melezitose, raffinose, rhamnose, sorbitol, or xylose.

Abundant gas is formed from the fermentable sugars. Milk is acidified, but not coagulated; coagulation takes place on heating the milk cultures. There is no action on gelatin, lead acetate, or coagulated egg white. Nitrates are not reduced and indol is not formed.

None of the strains of this species are pathogenic for white mice. Several of them, however, produce subcutaneous abscesses when injected into rabbits; the abscesses heal promptly if incised, more slowly otherwise. The organisms are numerous in the pus, appearing as single slender bacilli, or shorter diplo-bacilli. Tissier's "peloton de jardinier" forms may appear in the pus.

4. *Bacteroides biformis* (isolated 7 times; 1 single cell culture was obtained).

In brain medium and in glucose broth, this organism appears chiefly as a short oval cocco-bacillus 0.7 to 1μ thick and 1 to 1.5μ

long; it occurs single, in pairs, or in chains of varying length. Some strains can easily be taken for streptococci. In glucose broth and on blood agar, fusiform bacilli from 2 to 8μ in length may also be found; these occur in short chains. These long rods are often curved. Some strains show still other variations, especially on blood agar: Tissier's "peloton de jardinier;" also hooked, geniculate, and variously irregular forms. In 2 strains a few definitely branching bacilli were found; the branches were short and were either terminal or lateral. On potato, where growth is very poor, the cells appear as large, very irregular masses that have the appearance of several bacilli fused together. Metachromatic granules may be large and and numerous in the bacillary forms, or they may be absent. The organism is non-motile.

The colonies of this species are characteristic. On liver-infusion blood agar they are flat, grayish, with a wavy outline. They are 3 to 6 mm. in diameter. They may be smooth or they may be studded with small knobs. On blood agar plates the colonies are similar but smaller (1.5 to 3 mm. in diameter). On glucose agar, 5 strains fail to grow in colonies, though there is slight growth where the inoculum is heavy. Two strains form a very few flat colonies on glucose agar, 2 to 3 mm. in diameter.

A slight turbidity develops in glucose broth, with a small flocculent sediment. The pH reaches 5.2 to 4.8.

All 7 strains ferment galactose, glucosamine, glucose, levulose, mannitol, mannose, and trehalose. The results are variable with aesculin, amygdalin, cellobiose, dextrin, glycogen, lactose, maltose, raffinose, sucrose, salicin, and starch. None of the strains ferment adonitol, arabinose, dulcitol, erythritol, glycerol, inositol, inulin, melezitose, rhamnose, sorbitol, or xylose.

Milk is acidified and coagulated by those strains that ferment lactose. There is no action on gelatin, coagulated egg white, lead acetate broth, or nitrate broth. Indol is not formed.

Gas is formed from the fermentable carbohydrates, especially from mannitol; with this alcohol, gas formation is more vigorous than it is from glucose.

This species is non-pathogenic for white mice. When injected

subcutaneously into rabbits, it produces abscesses like those formed by *Bacteroides aerofaciens*. The organisms are numerous in the pus, appearing chiefly as coccoid forms in pairs and chains.

This organism is closely related to the previous species, from which it differs in the following respects:

a. In morphology, it often appears like a streptococcus, especially on brain medium and liver-infusion blood agar.

b. The colonies are much larger and flatter.

c. Mannitol is regularly fermented, which is never the case with *Bacteroides aerofaciens*. Raffinose is fermented by 3 of the 7 strains, whereas it is not fermented by *Bacteroides aerofaciens*.

d. All strains of *Bacteroides aerofaciens* ferment dextrin, lactose, maltose, and sucrose. Only 2 out of 7 strains of *Bacteroides biformis* ferment dextrin, 5 strains ferment lactose, 4 strains ferment maltose, and 4 strains ferment sucrose.

5. *Bacteroides tortuosus* (Debono, 1912) (isolated 3 times; one single cell culture was obtained).

The 3 strains here classified as *Bacteroides tortuosus* differ somewhat from one another. Only one of them fits Debono's description very closely. This is the strain from which a single cell culture was obtained (strain 1).

When strain 1 is grown on brain medium, only single slender bacilli, 0.3 to 0.5μ wide and 2 to 4μ long, appear. On glucose agar, blood agar, and liver-infusion blood agar, they are 0.3 to 0.6μ wide and 2 to 6μ long; here they may occur single, or in pairs or short chains. The chains may occasionally be very tortuous, due to the fact that several successive bacilli in the chain are strongly curved. Smears made from the sugar fermentation tubes when they have become acid often show other forms: extraordinarily tortuous chains; rods with huge median swellings (the "peloton de jardinier"); and bacilli with distinct terminal or short lateral branches. Metachromatic granules may be very numerous in some cells. There is no motility.

On blood agar, the colonies are 3 to 4 mm. in diameter, grayish white, flat, with irregular fimbriated borders. There is no action on the blood cells. On liver infusion blood agar, the colonies are

4 to 5 mm. in diameter, yellowish white, flat, with smooth edges. On glucose agar, the colonies are 2 to 3 mm. in diameter, otherwise as on blood agar.

In glucose broth, a slight turbidity appears, with a flocculent sediment. The pH reaches 4.8.

When strain 2 is grown in brain medium, long chains are formed. The tortuosities which give the species its name are very rare. On liver-infusion blood agar, terminal branchings are frequent. This strain is not very strongly Gram-positive; the Gram stain frequently shows the organism as a Gram-negative bacillus with numerous Gram-positive granules in it.

Strain 3 appears on all media as a slender bacillus, 0.3 to 0.5μ broad, 2 to 3μ long; it is usually single, but may occur in pairs or short chains. Tortuosities and branched forms were never observed.

The colonies of strains 2 and 3 are similar to those of strain 1, except that they are surrounded by a wide zone of hemolysis.

All 3 strains ferment aesculin, amygdalin, cellobiose, galactose, glucosamine, glucose, levulose, maltose, mannitol, mannose, salicin, sucrose, and trehalose. Strains 1 and 2 likewise ferment dextrin, glycogen, lactose, raffinose, and starch; strain 3 does not. None of them ferment adonitol, arabinose, dulcitol, erythritol, glycerol, inositol, inulin, melezitose, rhamnose, sorbitol, or xylose. Abundant gas is formed from the fermented carbohydrates.

Strains 1 and 2 acidify and coagulate milk. Indol is not formed; there is no action on gelatin, nitrate broth, lead acetate broth, or coagulated egg white. This species is non-pathogenic for white mice or rabbits.

6. *Bacteroides catenaformis* (isolated 6 times).

On all the media used, this organism appears as a slender rod, 0.3 to 0.5μ thick, 2 to 3.5μ long, arranged in long chains of 20 to 100 or more elements. Often the bacilli are so closely approximated in the chain that they appear as a continuous filament, no demarcations being evident. Usually the rods are straight, but occasional places will be found where successive bacilli are so strongly curved that the chain is exceedingly twisted as in *Bacte-*

roides tortuosus. The rods in this tortuous portion are often thickened. Another striking feature is the presence of large globular cells, 2 to 3μ in diameter; these may occur in the chains, or, more often, at the ends of the chains, where they may appear pear shaped or club shaped. Occasionally huge sickle shaped cells, 1.5 to 2μ wide at their thickest portion, may occur in the chain. Terminal Y branching may also be found. Metachromatic granules are numerous. The organism is non-motile.

Colonies on blood agar and on liver-infusion blood agar are 2 to 3 mm. in diameter. The edges are flat, but the central portions rise to high, irregular hillocks. The colonies have wavy, irregular edges and are usually radially striated.

In glucose broth cultures a large stringy mass grows at the bottom of the tube, with a clear supernatant fluid. The acidity is low; 10-day cultures in glucose serum broth have a pH of 6.4 to 5.6.

Indol is not formed. There is no action on milk, gelatin, coagulated egg white, nitrate broth, or lead acetate broth. There is no pathogenicity for white mice or rabbits.

Acid, but no gas, is produced by all 6 strains from amygdalin, cellobiose, dextrin, galactose, glucosamine, glucose, glycogen, lactose, levulose, maltose, mannose, raffinose, salicin, and trehalose. The results are variable with aesculin, inulin, mannitol (2 strains out of 6 ferment mannitol), methyl glucoside, methyl mannoside, sucrose (1 strain fails to ferment sucrose), and starch (3 strains). None of them ferment adonitol, arabinose, dulcitol, erythritol, glycerol, inositol, melezitose, rhamnose, sorbitol, or xylose.

7. *Bacteroides pseudoramosus* (Distaso 1912) (isolated 26 times; 4 single cell cultures were obtained).

On blood agar and on liver-infusion blood agar this organism appears chiefly as a slender bacillus 0.2 to 0.4μ thick and 0.8 to 2μ long. It occurs singly, in pairs, or in short chains. The rods have rounded or pointed ends, and are often curved. In glucose broth and on glucose agar, the bacilli are plumper and shorter; they may be oval or wedge shaped; they are often geniculated,

and occasional terminal branching may be observed. Young cultures in brain medium may show bacilli up to 10μ in length. The organism is non-motile.

When first isolated, branching forms of this organism are hard to find, but after prolonged cultivation they may be found more readily, especially on brain medium and glucose agar. One strain (Schafer 1), which is typical in every other respect, now branches freely in brain medium, so that every field in a stained preparation shows definite branching forms.

On blood agar plates the colonies are very characteristic. They are from 0.5 to 2 mm. in diameter, quite markedly elevated, smooth, opaque, either white or yellowish, and are surrounded by a wide zone of hemolysis. On liver infusion blood agar the colonies are from 1 to 2.5 mm. in diameter. Growth is good on glucose agar.

In glucose broth a slight turbidity develops, with a sediment. The pH reaches 4.8.

All strains form acid and a small amount of gas from adonitol, galactose, glucosamine, glucose, glycerol, levulose, mannose, methyl mannoside, sorbitol, and trehalose. Seven out of 26 strains ferment erythritol, 13 ferment mannitol. With dextrin, glycogen, maltose, and starch, the results are very variable: a few strains produce abundant acid; some produce a slight acidity (pH 6.0 to 6.6); most of them fail to ferment. Sucrose and inositol are fermented by one strain only. In the fermentation tubes, lactose is slowly fermented by 5 strains (in thirty to forty days); in milk, however, lactose is fermented by all strains, as the milk reaches pH 5.0 to 5.4. None of the strains ferment aesculin, amygdalin, arabinose, cellobiose, dulcitol, inulin, melezitose, methyl glucoside, raffinose, rhamnose, salicin, or xylose.

Coagulated egg white is not digested. Milk is acidified and coagulated. Gelatin is liquefied. Indol is formed. Nitrates are reduced to nitrites. Lead acetate is feebly and inconstantly blackened.

This organism is not pathogenic for white mice or guinea pigs. When injected subcutaneously into rabbits, abscesses develop. Unless incised and drained, these abscesses persist for weeks;

they eventually heal up completely. The organisms appear in the pus in large numbers as small cocco-bacilli, single or in pairs.

At one time during the progress of this work, the writer believed that this organism was a human pathogen of some importance. It was found repeatedly in various anaerobic cultures, such as those made from abdominal fluid in appendicitis, perinephritic abscess, etc. In a series of anaerobic blood cultures in acute rheumatic fever, it appeared in about 40 per cent of the cultures. However, when the controls were made, it was found that this organism is a very common contaminant. Strange as it may seem, this strict anaerobe is frequently present in the laboratory air. Its presence in cultures must therefore always be looked upon with suspicion. It is even possible that in stool cultures it is a contaminant. The writer believes, however, that *Bacteroides pseudoramosus* has its habitat in human feces, chiefly because no other source could be found. Throat cultures were uniformly negative; so also were cultures of the feces of the horse, dog, cat, rabbit, guinea pig, and white mouse, and of the intestinal contents of the cockroach. Several samples of soil were negative for this organism. Distaso (1912) also found this organism to be very common in human feces.

8. *Bacteroides avidus* (isolated twice; 1 single cell culture was obtained).

This organism appears as a thick bacillus, 0.5 to 1μ broad, 1 to 2.5μ long. The ends are rounded or pointed, and the rod is often slightly curved. Occasional branching forms may be found. Metachromatic granules are few. It is non-motile.

On blood agar and glucose agar plates the colonies are raised, yellowish white, and smooth; they are 2 to 3 mm. in diameter. They are not hemolytic.

Growth in glucose broth is diffuse; the pH reaches 4.8.

Both strains rapidly form acid and a small amount of gas from adonitol, dextrin, erythritol, galactose, glucose, glycerol, inositol, levulose, maltose, mannose, melezitose, starch, sucrose, and trehalose. Both strains slowly acidify glucosamine, inulin, lactose, and raffinose (in twenty to forty days). One strain fer-

ments glycogen, the other does not. Neither strain ferments aesculin, amygdalin, arabinose, cellobiose, dulcitol, mannitol, methyl glucoside, methyl mannoside, rhamnose, salicin, sorbitol, or xylose.

Milk is acidified and coagulated, then partly digested. Cubes of coagulated egg albumen, while not digested away, become completely transparent. Indol is not formed. Nitrates are not reduced. Gelatin is liquefied. Lead acetate is blackened. The organism is non-pathogenic for white mice and rabbits.

9. *Bacteroides limosus* (only one strain isolated; this was obtained as a single cell culture).

This organism is 0.5 to 1.5μ thick and 1 to 5μ long, the usual length being 3 to 4μ. There is much pleomorphism; short ovals, wedge-shaped bacilli, curved, hooked, and bifid forms are numerous. Metachromatic granules are absent. The organism is non-motile.

On blood agar and on glucose agar, the colonies are from 2 to 4 mm. in diameter, raised, and cream colored. They are exceedingly mucoid and adherent.

In glucose broth a diffuse cloudiness with a heavy mucoid sediment appears. The pH reaches 4.8.

Acid and a large amount of gas are formed from adonitol, erythritol, dextrin, glucose, levulose, mannitol, and trehalose. A slight acidity (pH 6.0 to 6.5) without detectable gas slowly develops in aesculin, cellobiose, glucosamine, inulin, glycogen, maltose, mannose, methyl mannoside, raffinose, salicin, starch, and sucrose. No acid is formed in amygdalin, arabinose, dulcitol, galactose, glycerol, inositol, lactose, melezitose, methyl glucoside, rhamnose, sorbitol, or xylose.

Milk is not acidified or coagulated. Lead acetate is not blackened. Gelatin is slowly liquefied. Indol is not formed. There is no action on coagulated egg white or nitrate broth. The organism is non-hemolytic, and non-pathogenic for white mice and rabbits.

10. *Bacteroides cornutus* (Distaso, 1912) (one strain isolated).

Distaso (1912) describes this species as being very common in the feces of human adults. It is strange, then, that only one strain was isolated in this investigation.

Bacteroides cornutus appears chiefly as a slender bacillus, 0.2 to 0.4μ thick and 0.7 to 3μ long. It occurs single or in pairs. In morphology it resembles *Bacteroides bifidus*: bifid, geniculate, and curved forms are common; also the "peloton de jardinier" of Tissier. Metachromatic granules are common. It is non-motile.

A slight turbidity develops in glucose broth, with a granular sediment. The pH reaches 4.8.

On blood agar plates the colonies are from 0.5 to 0.75 mm. in diameter; they are gray, smooth, and hemispherical, and are slightly hemolytic. On liver infusion blood agar, they are from 1.5 to 2 mm. in diameter and are brown in color. Growth was not obtained on glucose agar.

This organism forms acid but not gas from dextrin, galactose, glucose, inositol, levulose, maltose, mannose, trehalose, and xylose. Glucosamine is slightly acidified (pH 6.4). Neither acid nor gas is formed from adonitol, aesculin, amygdalin, arabinose, cellobiose, dulcitol, erythritol, glycerol, glycogen, inulin, lactose, mannitol, melezitose, methyl glucoside, methyl mannoside, raffinose, rhamnose, salicin, sorbitol, starch, or sucrose.

Milk, gelatin, lead acetate broth, nitrate broth, and coagulated egg white show no change. Indol is not formed. The organism is not pathogenic for white mice or rabbits.

11. *Bacteroides bifidus* (Tissier, 1900) (isolated 40 times; 14 single cell cultures were obtained).

In young cultures (18 to 48 hours) on a favorable medium such as glucose serum broth, brain, or chopped liver medium, the organisms appear as rods varying from 0.4 to 1μ in thickness and 0.75 to 8μ in length. The usual length is 2 to 4μ. They occur singly, more rarely in pairs or short chains. Terminal or central thickenings and various irregular projections are very frequent. Most strains show evident branching. The branches are from 0.5 to 1.5μ in length; they may be either terminal or lateral. At

this stage, the organism uniformly retains the Gram stain. Metachromatic granules are very numerous; each bacillus usually contains two terminal granules, and often one or more centrally located. They are usually small, but in some strains large bands or masses occupying a third or more of the bacillary body may stain metachromatically. These granules are conspicuous in the living bacillus as seen in the hanging drop, where they have a distinct reddish coloration.

In cultures four to six days old new features may appear, especially if the culture has become acid. Branched forms may become more abundant (in some strains, however, they become more difficult to find). Cells with as many as 6 to 8 lateral branches may be found in some strains; compound branching may occur. Rods with large bulbous swellings, 2 to 4μ in diameter, may be very numerous. Variously curved, hooked, or tailed forms may appear, also giant bacilli 10 to 12μ long. In some cultures the bacilli become very granular, so that they have the appearance of a chain of close-set, irregular cocci. If the culture has become acid, the Gram stain is taken in an irregular way. Some organisms retain the stain entirely, others will be Gram-negative, and still others will be Gram-negative with Gram-positive granules or bands, variously located. In still older cultures, most or all of the organisms may be Gram-negative. Metachromatic granules are less abundant in the older cultures and may disappear entirely.

The colonies on blood agar plates are from 0.5 to 2 mm. in diameter; they are hemispherical, smooth, or faintly stippled; they are gray in color, glistening, and have a smooth circular outline. The medium is unchanged. On liver infusion blood agar, the colonies are from 0.5 to 3 mm. in diameter; they are hemispherical or conical, smooth or faintly stippled, and glistening; the outline is usually circular but may be slightly crenated. The color of the colonies on liver infusion blood agar varies from white to dark brown; the medium around the colonies is turned brown.

Glucose broth cultures show a slight turbidity with a stringy sediment. The pH after ten days of incubation varies from 4.2 to 5.0.

TABLE 1

Showing the variable fermentation reactions of Bacteroides bifidus

STRAINS	ORIGIN	ARABINOSE	XYLOSE	MELEZITOSE	MANNOSE	METHYL MANNOSIDE	AESCULIN	AMYGDALIN	SALICIN	CELLOBIOSE	MANNITOL	SORBITOL	INULIN
						Group 1							
Infant 4	Infant's stool	−	−	−	+	+	−	−	−	−	−	−	−
Infant 7	Infant's stool	−	−	−	+	+	+	−	+	+	−	−	+
Michel	Infant's stool	−	−	−	+	+	−	−	−	−	−	−	+
Martin	Infant's stool	−	+	−	+	+	+	−	+	+	−	−	+
Hoff	Infant's stool	−	−	−	+	+	+	−	+	+	−	−	+
Rodwin 3	Infant's stool	−	−	−	+	+	−	+	+	+	+	+	+
Infant 2	Infant's stool	−	−	−	+	+	−	−	−	−	−	−	+
O'Leary 3	Infant's stool	−	−	−	+	+	+	+	+	+	+	+	+
Grotti 1	Infant's stool	−	−	−	+	+	+	+	+	+	+	+	+
Lucille 1	Infant's stool	−	−	−	+	−	−	−	−	+	−	+	−
Larson	Infant's stool	−	−	−	+	+	−	−	−	−	−	−	+
Eisner	Adult stool	−	−	−	−	−	−	−	−	−	−	−	−
Helg. 3	Adult stool	−	−	−	+	−	+	+	+	−	−	−	−
Z 6	Adult stool	−	−	−	−	−	−	−	−	−	−	−	−
J 2	Adult stool	−	−	−	+	−	+	+	+	−	−	−	+
						Group 2							
Andy 4	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
E 2	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
Dev. 7	Adult stool	+	+	+	+	−	+	+	+	+	−	+	+
Vogel 1	Adult stool	+	+	+	−	−	−	−	−	−	−	−	+
Vogel 2	Adult stool	+	+	+	−	−	−	+	+	+	−	+	+
Duffy 1	Adult stool	+	+	−	+	+	−	−	−	−	−	−	+
Clem. 9	Adult stool	+	+	+	−	−	−	+	−	−	−	+	+
Winton 1	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
Tiffany 3	Adult stool	+	+	+	+	+	−	−	−	−	−	−	+
Price 1	Adult stool	+	+	+	−	−	−	−	−	−	−	−	+
E A 1	Adult stool	+	+	+	+	+	−	−	−	−	−	−	+
Helen 1	Adult stool	+	+	+	−	−	+	−	+	−	−	+	+
Rogers 1	Adult stool	+	+	+	−	−	−	−	−	−	−	−	+
Dana 4	Adult stool	+	+	+	+	−	+	+	+	+	−	+	+
Dana 7	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
Helen M	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
Z 5	Adult stool	+	+	−	−	−	+	+	+	+	−	−	+
Rotter 3	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
Brown 3	Adult stool	+	+	−	−	−	+	+	+	+	+	−	+
E H	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
O 3	Adult stool	+	+	−	−	−	+	+	+	+	−	−	+
Lucille 3	Adult stool	+	+	−	−	−	+	+	+	+	−	+	+
Gu 3	Adult stool	+	+	−	−	−	−	+	+	+	−	+	+
J 5	Adult stool	+	+	+	−	−	+	+	+	+	−	+	+
E N 1	Adult stool	+	+	−	−	−	+	+	+	+	−	−	+

Milk is acidified by all strains and coagulated by most of them. There is no action on nitrate broth, lead acetate broth, gelatin, or coagulated egg white. Indol is not formed. This organism is non-pathogenic for white mice or rabbits.

None of the 40 strains ferment adonitol, dulcitol, erythritol, glycerol, or rhamnose. Only 2 strains ferment inositol. All strains form acid but not gas from dextrin, galactose, glucosamine, glucose, glycogen, lactose, levulose, maltose, methyl glucoside, raffinose, starch, sucrose, and trehalose.

With the remaining carbohydrates, the results of fermentation tests are variable. On the basis of acid production from arabinose, xylose, melezitose, and mannose, it is possible to divide the 40 strains into 2 groups: group 1 being negative on arabinose, xylose (except for one strain), and melezitose, but positive on mannose; group 2 being positive on arabinose and xylose and usually melezitose, but usually negative on mannose. This grouping acquires considerable significance from the fact that all of the strains from infants' stools fall into group 1, while most of the strains from adults' stools fall into group 2. This classification is brought out in Table 1. To the 15 strains in group 1, one may properly add the 31 strains studied by Weiss and Rettger (1934); these were all negative on arabinose, xylose, and melezitose (their action on mannose was not stated) and they were all isolated from nurslings' stools.

Strain "Lucille 1," given in Table 1, was isolated from the stool of a breast-fed infant; strain "Lucille 3" was isolated from the stool of the same child, but two years later. It is interesting to note that with the change in age and diet, there was a change in the type of *Bacteroides bifidus* found. In the cases of two stools from adults, both types of *Bacteroides bifidus* were isolated from the same stool (Z 5 and Z 6, and J 2 and J 5).

No consistent morphological differences between the two groups of *Bacteroides bifidus* could be discovered. Attempts were made to ascertain the serological relationship of these two groups, but with unsatisfactory results. Often the antisera agglutinated only the homologous organism; one serum (for Infant 4) agglutinated 6 different strains, 4 in group 1 and 2 in group 2.

There is considerable disagreement in the literature concerning the oxygen requirements of *Bacteroides bifidus*. The writer's experience has been that for primary isolation and for the first few transplants, strict anaerobiosis is necessary. After several transplants, aerobic growth is obtained with about 60 per cent of the strains; the remaining 40 per cent have remained obligate anaerobes, though some have been on artificial media for as long as three years.

That *Bacteroides bifidus* is the predominating organism in the feces of infants has been recognized since the work of Tissier (1900) who estimated (1908) that 85 to 90 per cent of the colonies from nurslings' stools consist of this species. What place this organism occupies in the flora of the adult is not clear from the literature. Tissier (1908) states that a normal child of five years on a mixed diet will show 70 per cent of *Bacteroides bifidus* in its stools; on a high protein diet, 50 per cent; and on a vegetable diet, 80 per cent. If this is true, the stool of the adult should contain approximately the same number. But Distaso (1912) states that *Bacteroides bifidus* is very rare in the stools of the adult, and Torrey (1918) says that this organism "is not likely to occur in large numbers in the stools of human adults except under conditions of intestinal abnormality."

In the writer's experience, *Bacteroides bifidus* (group 2) is a common inhabitant of the stool of normal adults. The actual numbers vary a great deal; some stools contain very few, others may show as high as 90 per cent of the colonies to be of this species. Of 7 individuals whose stools were cultured several times over a space of three years, 4 yielded large numbers of this organism on each occasion.

The chief biological reactions of the above 11 species of Gram-positive non-spore-bearing anaerobic bacilli are summarized in abbreviated form in table 2.

Bergey's *Manual* (1934) classifies the following Gram-positive organisms under the genus Bacteroides:

Bacteroides oviformis (Tissier, 1908). As indicated above, this species is probably identical with *Bacteroides ventriosus* (Tissier,

1908). Bergey's *Manual* states that sucrose is fermented; this is contrary to Tissier's description.

Bacteroides dimorphus, variety *longa* (Distaso, 1912). According to Distaso's description, this organism does not grow in sugar media (broth?); yet in another place he states that it grows in deep tubes of gelatin containing sugar. It acidifies and coagulates milk. Distaso's description is too meagre to enable one to recognize this organism.

TABLE 2

The chief biological reactions of the Gram-positive non-spore-bearing fecal anaerobic bacilli

SPECIES	GAS FROM GLUCOSE	INDOL	GELATIN	LEAD ACETATE	GLYCEROL	MANNITOL	SORBITOL	ADONITOL	LACTOSE	SUCROSE	TREHALOSE	GLUCOSE	MANNOSE	ARABINOSE	MELEZITOSE
B. lentus	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−
B. ventriosus	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
B. aerofaciens	+	−	−	−	−	−	−	−	+	+	+	+	+	−	−
B. biformis	+	−	−	−	−	+	−	−	±	±	+	+	+	−	−
B. tortuosus	+	−	−	−	−	+	−	−	±	+	+	+	+	−	−
B. catenaformis	−	−	−	−	−	∓	−	−	+	±	+	+	+	−	−
B. pseudoramosus	+	+	+	+	+	±	+	+	∓	−	+	+	+	−	−
B. avidus	+	−	+	+	+	−	−	+	+	+	+	+	+	−	+
B. limosus	+	−	+	−	−	+	−	+	−	∓	+	+	∓	−	−
B. cornutus	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
B. bifidus (1)	−	−	−	−	−	∓	∓	−	+	+	+	+	+	−	−
B. bifidus (2)	−	−	−	−	−	∓	±	−	+	+	+	+	∓	+	±

Bacteroides acuminatus (Distaso, 1912). Distaso states that this is a very common organism in the stools of adults. He describes it as a short rod with rounded ends, occurring singly or in pairs. It forms acid but not gas from glucose, lactose, and sucrose. Milk is acidified and coagulated. Indol is not formed. (Bergey's *Manual* states that indol is formed.)

It seems possible that this organism is the same as that described here as *Bacteroides bifidus*, group 2, and that Distaso missed the branching forms, as other investigators have done (Cruikshank, 1925).

Bacteroides angulosus (Distaso, 1912). Distaso describes this

organism as bearing spores; it has, therefore, no place in this genus.

Bacteroides multiformis (Distaso, 1911) and *Bacteroides tenuis* (Distaso, 1911). Distaso describes these two species as spore bearers related to *Clostridium Welchii*. Le Blaye and Guggenheim (1914) also list them as spore bearers.

DISCUSSION

As stated above, the writer believes that the Gram-positive non-spore-bearing fecal anaerobic bacilli and the Gram-negative *Bacteroides* are biologically quite distinct. Reasons for this opinion were given in a previous paper (Eggerth and Gagnon, 1933); however, as the work on the Gram-positive species was at that time very incomplete, it becomes necessary to revise that list of reasons. They may now be stated as follows:

1. The Gram-positive non-spore-bearing fecal anaerobic bacilli frequently form bifid ends or lateral branches, or appear as rods with swollen central portions (Tissier's "peloton de jardinier"); or they grow in chains; or they have the aspect and arrangement of the diphtheria group. The Gram-negative *Bacteroides* are characteristically single organisms, often oval in shape, and frequently staining more heavily at the ends or around the periphery.

2. The Gram-positive species rarely ferment the pentoses, whereas the Gram-negative *Bacteroides* do this commonly. None of the Gram-positive species were found to ferment rhamnose; only 2 (*Bacteroides cornutus* and *Bacteroides bifidus*, group 2), ferment xylose; and only the latter ferments arabinose. On the other hand, 9 out of 18 species of Gram-negative *Bacteroides* ferment rhamnose; 7 ferment arabinose, and 10 ferment xylose.

3. The Gram-negative *Bacteroides* rarely ferment the higher alcohols, whereas the Gram-positive species do this much more commonly. Of the Gram-negative species, only one ferments glycerol, one ferments mannitol, 2 ferment sorbitol, and none ferment erythritol or inositol. On the other hand, 2 Gram-positive species ferment glycerol, 7 ferment mannitol, 3 ferment sorbitol, 3 ferment adonitol, 3 ferment erythritol, and occasional strains of 4 ferment inositol.

4. Of the disaccharides, lactose, sucrose, maltose, cellobiose, and trehalose, the Gram-positive species ferment trehalose *most* readily, whereas the Gram-negative species ferment this sugar *least* readily. Nine of the 11 Gram-positive species ferment trehalose; furthermore, the fermentation is usually more rapid than that of the other disaccharides. On the other hand, only 5 out of 18 Gram-negative species ferment this sugar, and those that do, often ferment it very slowly.

5. As a group, the Gram-negative *Bacteroides* are more proteolytic than the Gram-positive species. Out of 18 of the former, 12 liquefy gelatin, 9 produce indol, and 15 produce hydrogen sulfide. On the other hand, only 3 out of 11 Gram-positive species liquefy gelatin, only one produces indol, and 3 produce hydrogen sulfide.

6. The Gram-positive species usually give a flocculent growth in glucose broth, with only a slight turbidity; most of them grow poorly or not at all in peptone water without sugar. The Gram-negative *Bacteroides* usually grow diffusely in glucose broth, and grow fairly well in peptone water without sugar.

For these reasons, the Gram-positive species described in this paper should be classified separately from the Gram-negative *Bacteroides*. Some of them might be placed in the genus *Lactobacillus*; Weiss and Rettger (1934) have shown a very close relationship between *Bacteroides bifidus* and *Lactobacillus acidophilus* and there is possibly a close relationship between *Bacteroides aerofaciens* and *Lactobacillus acidophil-aerogenes*. Or the entire group might be placed in a new genus, possibly in the order *Actinomycetales*.

SUMMARY

1. One hundred and thirty strains of Gram-positive non-spore-bearing anaerobic bacilli from human feces have been studied and classified in 11 species.

2. These 11 species seem sufficiently closely related to be included in one genus. As they differ in many respects from the Gram-negative *Bacteroides*, it is suggested that they be classified either in the genus *Lactobacillus* or in a separate genus.

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