THE BACTEROIDES OF HUMAN FECES

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That human feces may contain a variety of species of non-spore-bearing anaerobic bacilli has been known for some time. Tissier showed in 1900 that the predominating organism in the stools of breast-fed infants was a Gram-positive bacillus without spores and strictly anaerobic, to which he gave the name of *Bacillus bifidus-communis*. In 1908, Tissier described four other anaerobes, also from infants' stools: *Coccobacillus preacutus, Coccobacillus oviformis, Bacillus ventriosus,* and *Bacillus capilosus.* Then, in 1911 and 1912, Distaso reported nine new species of non-spore-bearing anaerobic bacilli from adult stools: *Bacillus multiformis, Diplobacillus acuminatus, Bacillus variabilis, Bacillus pseudoramosus, Bacillus bullosus, Bacillus thetaiotaomicron, Bacillus variegatus, Bacillus cornutus,* and *Bacillus tenuis-spatuliformis* In 1912, Distaso's pupil Debono described *Bacillus tortuosus.*

Tissier, Distaso, and Debono isolated these organisms from stool cultures in deep glucose agar tubes, after the technic of Veillon and Zuber (1898). While they stated that certain species, such as *Bacillus bifidus* in infants, or *Bacillus variabilis* and *Bacillus thetaiotaomicron* in adults, were very common, they did not obtain any data concerning the actual numbers of these organisms per milligram of stool, nor did they ascertain what percentage of the viable, cultivatable organisms of the feces belonged to this anaerobic group. Because of this lack of quantitative data, the usual opinion among bacteriologists is that, in

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the adult at least, these species are rare and unimportant. Thus Ford (1927) states in his text book that "occasionally obligate anaerobes can be isolated by special technic, Veillon agar, etc." Other standard text books make no mention of these obligate anaerobes of adult stools, nor do the larger reference works, such as the Handbuch of Kolle, Kraus, and Uhlenhuth (1928), or the System of Bacteriology of the Medical Research Council (1929–1931). Very recently, Sanborn (1931) and also Torrey and Montu (1931) have observed that non-spore-bearing anaerobes or microaerophiles are frequently much more numerous in the stools of adults than are the aerobes; Sanborn states that the count obtained by planting high fecal dilutions in cooked meat tubes was frequently 300 times as high as that on aerobic plates.

Bergey's Manual of Determinative Bacteriology (1930) lists, under the genus Bacteroides, Tissier's *Bacillus bifidus* and his *Coccobacillus oviformis;* also the species described by Distaso and Debono. The genus Bacteroides is defined as consisting of "motile or non-motile rods, without endospores. Show good growth with ordinary culture media; without pigment formation. Obligate anaerobes." These species are also described in Ford's Textbook of Bacteriology (1927), and in Castellani and Chalmers' Manual of Tropical Medicine (1919).

In our own investigation, 65 stools were cultured; aerobic and anaerobic colony counts were made, and various anaerobic colonies from each stool were fished for replating and further study. Five of these stools were from infants; 40 were from normal adults; 20 were selected from patients that were apparently normal as far as their intestinal flora was concerned.

TECHNIC

Dilution of the feces. The method used by Torrey (1926) was followed. The fresh stool was emulsified in sterile 0.85 per cent salt solution. After allowing the coarse particles to settle, the emulsion was decanted into a sterile test tube having an inside diameter of 16 to 17 mm.; a heavy black pencil mark was made on the wall of the tube and the suspension diluted until the mark was just visible on looking through the tube toward a strong

light. This was dilution 1; from it, higher dilutions were made in four more tubes, each containing 9 cc. of sterile salt solution and 1 cc. of the previous dilution. Tube 4 therefore represents a 1:1000 dilution of tube 1. Torrey has calculated that a 4 mm. loopful from this fourth dilution represents approximately $\frac{1}{3000}$ mgm. of fresh feces of average water content.

Preparation and inoculation of plates. Our basic medium was beef infusion agar, containing 1.5 per cent of agar, 1 per cent of Parke Davis peptone, and 0.4 per cent of di-sodium phosphate. The pH was 7.6 to 7.8. Before pouring the plates, about 5 per cent of sterile blood and 0.15 per cent of sterile glucose (in the form of a 10 per cent solution) were added. With this small amount of glucose, the plates, being well buffered, do not become acid. This is important, as otherwise the more vigorously growing species soon make the medium unsuitable for those of slower growth. Our experiments indicate that this moderate alkalinity is most favorable for initiating growth of the Bacteroides. We tried several other media, including liver infusion, brain infusion, and coagulated egg, but our best results were obtained with the medium described above.

Plates were inoculated with a 4 mm. loopful of the fecal dilutions, the inoculum being well rubbed over the entire surface of the plate so as to obtain well distributed colonies. Anaerobic plates were made from fecal dilutions 3, 4, and 5; aerobic plates from dilutions 3 and 4.

Anaerobic cultivation. We have used two methods for securing anaerobic conditions for plates.

1. The double plate method, first described by Herrold (1920). We used *B. mesentericus* to consume the oxygen. Two covers (or two bottoms) of Petri dishes were chosen which had smooth perfect edges and were of exactly the same size. The glucose blood agar was poured into one and inoculated with the fecal dilution; a very stiff (3 per cent) nutrient agar was poured into the other and inoculated with *B. mesentericus*. Both plates were thoroughly dried in the incubator for 15 to 30 minutes; they were then placed face to face and sealed with two layers of adhesive tape. The double plate was incubated with the *B. mesentericus*

side up. To prevent leakage of air, the two plates must fit perfectly and be free from nicks. This method is widely used to produce a "partial oxygen tension;" it does more than that, however, as very excellent anaerobic conditions are produced (as well as a high concentration of CO_2, which is undoubtedly favorable in certain cases). It possesses the advantage of allowing inspection of individual plates. In our work, we incubated the cultures for 4 to 5 days. We used the double plates extensively in the early part of our investigation, but eventually abandoned them in favor of the second method. However, the most fastidious Bacteroides can be grown by this method; for instance, *Bacteroides bifidus*, which is considered difficult to isolate, can be grown directly from the stool.

2. The other method used by us was that of McIntosh and Fildes (1916), which depends upon the removal of oxygen by the union of hydrogen with oxygen under the influence of a catalyst, palladinized asbestos. For making the apparatus, we have followed the directions given by Zinsser (1930, pp. 151–153). A tube containing methylene-blue in alkaline glucose broth serves as an indicator for the absence of oxygen. This method is almost ideal, being clean, simple, inexpensive, and efficient. We have devised a slight modification which simplifies it still more by making the heating unnecessary. This is based upon the observation that the palladinized asbestos, when in the form of loose shreds, is a much more active catalyzer than when it is packed tightly on a spindle. We have placed about 0.3 gram of the loose palladinized asbestos in a small beaker and covered the top of the beaker with a fine meshed copper gauze, which is bent over and sealed around the edge with adhesive tape, so that there are no large leaks. The beaker is placed in the jar with the cultures and the hydrogen slowly run in. (We allow the hydrogen to bubble through a column of water, so that we can tell the rate of flow.) No heating is necessary; if the room is darkened, the projecting spicules of asbestos can be seen to glow when the hydrogen first enters. The usual precautions against introducing the hydrogen too rapidly must of course be observed. If the catalyser becomes sluggish, heating in the Bunsen burner flame restores its activity.

We now use this method for all of our anaerobic work. Cultures are incubated for 4 to 5 days without opening the jars.

Isolation of pure cultures. From 3 to 6 colonies from each stool were selected for pure culture study. Each strain was re-plated 3 times, single well isolated colonies being fished each time.

Stock cultures. Stock cultures of all of the Gram-negative and some of the Gram-positive Bacteroides were maintained on brain media. This was prepared by passing raw pigs' or sheep's brains through a meat chopper, and stirring up with enough broth to make a paste. The medium was then cooked in the Arnold sterilizer for 30 minutes, adjusted to pH 7.8, and 0.1 per cent of glucose added. It was then tubed and steamed in the Arnold sterilizer for 3 successive days. On this medium, most of the Bacteroides isolated grow out in twenty-four hours and remain viable in the refrigerator for 3 to 6 months. A few of the Gram-positive strains die off rather rapidly in this cooked brain; these are best maintained on blood agar slants.

Special media for subcultures. A 1 per cent peptone water containing 0.5 per cent sodium chloride and adjusted to pH 7.8 was used for fermentation tests. Brom cresol purple was used as an acid indicator. The sugars and other carbohydrates were made up in concentrated solution in distilled water, autoclaved, and then added to the broth tubes.

Milk was brought to pH 7.0 before sterilizing. This usually required 5 cc. of normal sodium hydroxide per liter of milk. If the milk is made more alkaline than this, it becomes yellow on sterilizing. If it is more acid, some of the Bacteroides fail to initiate growth.

Gelatin medium was prepared by adding 10 per cent of gelatin and 0.15 per cent of glucose to phosphate infusion broth and adjusting to pH 7.8.

Peptone water for the indol tests consisted of 1 per cent of Parke Davis peptone and 0.2 per cent of di-sodium phosphate, set at pH 7.8. When growth on this medium was poor, duplicate cultures were made on beef infusion broth.

Lead acetate broth was prepared by adding 1 cc. of sterile 1 per cent lead acetate solution to 200 cc. of sterile beef infusion broth, then distributing in tubes.

Coagulated serum slants were made by adding 1 part of 0.5 per cent glucose broth to 3 parts of serum and coagulating in the inspissator, then sterilizing in the Arnold sterilizer.

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

Cultures on the above media were not discarded as negative until they had incubated for 40 days or longer.

Indol tests. Twelve-day cultures were used for the indol tests. We used two tests on each culture: the Ehrlich test as given by Kligler (1914) and the Zoller (1920) test. The two tests confirmed each other in every case of our series.

AEROBIC AND ANAEROBIC COLONY COUNTS OF STOOL CULTURES

Both aerobic and anaerobic stool culture plates were incubated for 4 to 5 days; cultivation for a longer time did not increase the colony count. The anaerobic plates were given an additional day of aerobic cultivation, to allow any obligate aerobes to grow. The differences between the two sets of plates were usually very striking, as shown in table 1.

The anaerobic colony count, as given in table 1, includes the colonies of aerobes that developed on those plates; the true number of obligate anaerobes was computed by subtracting the aerobic colony count from the anaerobic colony count. The obligate anaerobes encountered in this series all have the characteristics of the genus Bacteroides. In these dilutions, spore-bearing anaerobes were never encountered.

Table 1 shows that in the majority of adult human stools, the predominating viable organisms are obligate anaerobes. This confirms the observations of Sanborn (1931) and of Torrey and Montu (1931). MacNeal, Latzer, and Kerr (1909) found that the anaerobic plate count of stools of the adult was, on the average, no higher than that of the aerobic plates; this we believe to be due to the fact that their period of incubation (48 hours) was too short, and their medium (litmus glucose agar) probably not favorable enough. Even under these conditions, they reported a few stools where from 2 to 12 times as many colonies developed on the anaerobic plates as on the aerobic controls.

From these 65 stools (60 of adults, 5 of infants) we isolated 198 strains of anaerobes for further study. Of these, 118 strains were Gram-negative and 80 were Gram-positive. We believe that the division of the Bacteroides into two main groups accord-

TABLE 1

Colony counts from plates inoculated with $\frac{1}{1000}$ mgm. of feces from adults

NUMBER	AEROBIC COLONY COUNT	ANAEROBIC COLONY COUNT	NUMBER	AEROBIC COLONY COUNT	ANAEROBIC COLONY COUNT
1	15	56	31	5	38
2	6	48	32	150	167
3	0	65	33	2	48
4	7	80	34	1	42
5	5	76	35	4	141
6	17	55	36	5	85
7	2	80	37	26	43
8	12	150	38	24	72
9	30	104	39	12	152
10	58	145	40	60	154
11	27	320	41	4	54
12	14	116	42	67	88
13	7	75	43	0	16
14	8	62	44	6	40
15	62	195	45	4	50
16	12	95	46	61	124
17	10	145	47	12	64
18	2	87	48	20	60
19	1	42	49	5	53
20	53	162	50	2	42
21	2	38	51	10	160
22	4	89	52	24	124
23	8	116	53	10	167
24	12	72	54	2	70
25	12	36	55	1	74
26	3	42	56	12	42
27	4	41	57	120	126
28	4	74	58	8	158
29	86	138	59	16	132
30	39	59	60	15	93

Number of stools containing less than 50 per cent of anaerobes..... 5
Number of stools containing between 50 and 70 per cent of anaerobes..... 10
Number of stools containing between 70 and 80 per cent of anaerobes..... 6
Number of stools containing between 80 and 90 per cent of anaerobes..... 8
Number of stools containing 90 per cent or more of anaerobes..... 31

ing to the Gram stain reaction is a fundamental one, for the following reasons:

1. The Gram-positive Bacteroides in our collection either form bifid ends, or they grow in chains or pairs, 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. None of our Gram-positive Bacteroides ferment rhamnose. Many of the Gram-negative Bacteroides do ferment rhamnose.

3. A number of our Gram-positive Bacteroides ferment inositol, glycerol, or mannitol. None of our Gram-negative species ferment inositol; only one ferments glycerol and only one ferments mannitol.

4. Several of our Gram-positive Bacteroides reduce nitrates to nitrites; none of our Gram-negative species do this.

5. With two exceptions, all of our Gram-negative Bacteroides that ferment glucose likewise ferment galactose, levulose, and mannose; and with one exception, those that ferment lactose likewise ferment sucrose and raffinose. In the Gram-positive group, several species ferment glucose that fail to ferment galactose or mannose; and a number ferment lactose that fail to ferment sucrose or raffinose.

6. Most of our Gram-positive Bacteroides give a flocculent growth in infusion broth, with a clear supernatant fluid; most of them grow poorly or not at all in peptone water. The Gram-negative Bacteroides usually grow diffusely in infusion broth, and grow fairly well in peptone water.

For these reasons, we believe that the primary division of the Bacteroides into a Gram-positive and a Gram-negative group is a logical one.

The Gram-positive fecal Bacteroides seem to be made up of 20 or more species. Bergey's Manual (1930) lists 10. The descriptions in the original literature are often so meager that we have found it impossible to identify many of our strains with any of them. We feel it necessary to isolate and study many more

strains of the Gram-positive Bacteroides before we can classify them; hence we leave this group for a later report.

The 118 strains of Gram-negative Bacteroides fall into at least 18 species; 2 of these, *Bacteroides variabilis* and *Bacteroides thetaiotaomicron*, have been described by Distaso (1912); the remaining 16 species are new. We are certain that there are other species that we did not encounter, but our list probably includes all of the commoner fecal Bacteroides and some of the rarer ones.

Key to the species of Gram-negative fecal Bacteroides

A. No gas from peptone.
  B. Acid in arabinose and salicin.
    C. Acid in mannitol.....*Bacteroides gulosus*
    CC. No acid in mannitol.
      D. Acid in rhamnose.
        E. Not capsulated.....*Bacteroides thetaiotaomicron* (Distaso)
        EE. Capsulated.....*Bacteroides variabilis* (Distaso)
      DD. No acid in rhamnose.....*Bacteroides uniformis*
  BB. Acid in arabinose; no acid in salicin.
    C. Gelatin liquefied.....*Bacteroides vulgatus*
    CC. Gelatin not liquefied.....*Bacteroides incommunis*
  BBB. Acid in salicin; no acid in arabinose.
    C. Acid in xylose.....*Bacteroides distasonis*
    CC. No acid in xylose.....*Bacteroides uncatus*
  BBBB. No acid in salicin or arabinose.
    C. Acid in sorbitol.....*Bacteroides tumidus*
    CC. No acid in sorbitol.
      D. Acid in rhamnose.
        E. Acid in lactose.....*Bacteroides ovatus*
        EE. No acid in lactose.....*Bacteroides vescus*
      DD. No acid in rhamnose.
        E. Acid in xylose.....*Bacteroides convexus*
        EE. No acid in xylose.....*Bacteroides exiguus*
AA. Gas from peptone.
  B. Acid in lactose.
    C. Acid in arabinose.....*Bacteroides inaequalis*
    CC. No acid in arabinose.....*Bacteroides insolitus*
  BB. No acid in lactose; acid in glucose.....*Bacteroides varius*
  BBB. No acid in glucose.
    C. Milk coagulated.....*Bacteroides coagulans*
    CC. Milk not coagulated.....*Bacteroides siccus*

DESCRIPTION OF THE SPECIES OF GRAM-NEGATIVE FECAL BACTEROIDES

None of the Gram-negative Bacteroides studied by us attacked cellulose or fermented dulcitol, erythritol, or inositol. None of them reduced nitrates to nitrites. None of them were motile (except for Brownian movement). None of them attacked coagulated serum or coagulated egg albumen (in egg cube broth). None of them were pathogenic for white mice or rabbits when injected subcutaneously in large doses. These negative reactions, common to all, are omitted from the detailed descriptions of the species.

1. Bacteroides gulosus (isolated 7 times)

On blood agar plates, this organism appears as a small oval bacillus 1 to 2 micra long, 0.8 to 1 micron thick. It stains more deeply around the periphery. On glucose broth, the same oval forms occur, as well as various other forms: small cocci; bacilli with marked bi-polar staining; large swollen bacilli, 3 to 4 micra long, 2 to 3 micra thick, staining only at the ends; also longer bacilli up to 6 micra long.

Colonies on blood agar are soft, gray, entire, elevated, 2 mm. in diameter. On glucose agar the colonies are much smaller.

On infusion and glucose broth, the growth is heavy and diffuse. The pH reaches 4.8 on glucose broth.

Fermentation reactions: Acid and a very small amount of gas are produced from aesculin, amygdalin, arabinose, cellobiose, dextrin, glucose, galactose, glycogen, inulin, lactose, levulose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose, and xylose. Sorbitol and mannitol requires 2 to 3 weeks for fermentation. Neither acid nor gas is formed from glycerol.

Gelatin is liquefied in 2 to 3 weeks. Milk is acidified and coagulated in 4 to 20 days. Indol is formed. Lead acetate is blackened.

This species differs from *Bacteroides thetaiotaomicron*, which it most closely resembles, in that it ferments mannitol and sorbitol and liquefies gelatin. It differs from *Bacteroides variabilis*

in that it is non-capsulated and in that it ferments mannitol, sorbitol, melezitose, and trehalose.

2. Bacteroides thetaiotaomicron (Distaso 1912) (isolated 14 times)

On blood agar plates, this organism usually appears as a small single oval bacillus, 1 to 2 micra long, 0.7 to 1 micron thick. It may stain solidly or only at the poles. In some strains, it appears as a slender rod, 2 to 4 micra long and 0.7 to 1 micron thick. In other strains, small coccoid forms predominate. In glucose broth, the bacilli are longer, up to 4 micra long, and often occur in pairs. Some large pale cells, about 3 micra long and 2 micra wide, staining only at the ends, may also be found in glucose broth cultures. No motility was observed; Distaso states that this organism is motile; we believe that he observed Brownian movement, which is very active.

On blood agar, colonies are soft, elevated, entire, and may vary from 1 to 4 mm. in diameter. They may slope straight up to a peak in the center, instead of being rounded. They may be grayish or yellowish. Glucose agar colonies are 0.5 to 1.0 mm. in diameter.

Growth on infusion broth and glucose broth is heavy and diffuse; on glucose broth, the pH reaches 5.0.

Fermentation reactions: Acid and (usually) a small amount of gas are formed from aesculin, amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, mannose, melezitose, raffinose, rhamnose, salicin, starch, sucrose, trehalose, and xylose. Four of our strains failed to produce gas from sugars. Neither acid nor gas is formed from glycerol, mannitol, or sorbitol.

Gelatin is not liquefied, even after 50 days of incubation. Lead acetate is blackened. Milk is acidified and coagulated in 4 to 12 days. Indol is formed. (Bergey's Manual states that indol is not formed; this is not in accord with Distaso's description.)

This species differs from *Bacteroides variabilis*, which it somewhat resembles, in not being capsulated, in not liquefying gelatin, in usually forming gas from fermentable sugars, and in fermenting melezitose and trehalose. It differs from *Bacteroides*

uniformis in morphology, in usually forming gas from fermentable sugars, and in fermenting rhamnose.

3. Bacteroides variabilis (Distaso, 1912), (isolated 8 times)

On blood agar, this organism may be quite round or oval, 1 to 2 micra in length. The periphery stains more heavily than the center. It has a heavy capsule. On glucose agar, one may see very long bacilli, up to 10 or 12 micra in length; these may be swollen, irregular in form, or curved. Stained with safranin or dilute gentian violet, the bacteria (especially when grown on glucose agar) appear reticulated. In glucose broth, oval and coccoid forms predominate; there are also swollen pale ovals, staining at one or both ends or in a band across the middle.

On blood agar, colonies are smooth, glistening, elevated, and very mucoid; they are about 1 mm. in diameter. Glucose agar colonies are pin point in size.

On infusion broth and glucose broth, growth is heavy and diffuse. The glucose broth reaches a pH of 5.0.

Fermentation reactions: Acid but no gas is produced from aesculin, amygdalin, arabinose, cellobiose, dextrin, glucose, galactose, glycogen, inulin, lactose, levulose, maltose, mannose, raffinose, rhamnose, salicin, starch, sucrose, and xylose. No acid or gas are produced from glycerol, mannitol, melezitose, sorbitol, or trehalose.

Gelatin is liquefied in 2 to 3 weeks. Lead acetate is blackened. Milk is acidified; some strains coagulate it in 25 to 35 days; others never coagulate the milk, though it coagulates immediately on boiling. (Distaso states that this species has no action on milk.) Indol is formed.

Bacteroides variabilis differs from the following species, *Bacteroides uniformis*, in that it is capsulated, in that it ferments rhamnose and does not ferment melezitose and trehalose, and in that it regularly liquefies gelatin.

4. Bacteroides uniformis (isolated 8 times)

On blood agar and on glucose agar plates and on glucose broth, this organism appears as a small single bacillus, 0.8 to 1.5 micron

long, with rounded ends. It stains most heavily at the poles and around the periphery; the bi-polar staining is best brought out with safranin. It is very uniform in size and appearance.

On blood agar, colonies are transparent, soft, elevated; they are 0.5 to 0.75 mm. in diameter. On glucose agar, they are pin point in size.

On infusion broth and glucose broth, the growth is diffuse. The glucose broth reaches pH 5.4.

Fermentation reactions: Acid but no gas is formed from aesculin, amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, mannose, melezitose, raffinose, salicin, starch, surcrose, trehalose, and xylose. Melezitose and trehalose usually require 12 to 14 days for fermentation. No acid or gas are formed from glycerol, mannitol, rhamnose, and sorbitol.

Two of our strains liquefied gelatin in 25 to 40 days; the other six failed to do so. Lead acetate is blackened very slowly or not at all. Milk is acidified and coagulated in 8 to 12 days. Indol is formed.

Bacteroides uniformis differs from the following species, *Bacteroides vulgatus*, in that it forms indol and in that it ferments amygdalin, cellobiose, melezitose, salicin, and trehalose, and in that it usually does not liquefy gelatin.

5. *Bacteroides vulgatus* (*isolated 38 times*)

This is the commonest species found in the feces of the adult.

On blood agar, this organism usually appears as an oval bacillus, 0.7 to 2.5 micra long. It usually stains solidly, though some stains will show bi-polar staining. One of our strains (M1) forms filaments up to 10 micra long. The bacilli are usually single, though some strains show a tendency to appear in pairs. This species is very variable in glucose broth: sometimes small oval bacilli are found exclusively; sometimes the bacilli are 4 to 8 micra long; or they may be swollen, vacuolated, or distorted or very irregular in shape. A few strains grow in chains of 3 to 6 elements in glucose broth.

On blood agar, the colonies are soft, translucent, grayish,

elevated; they are 1.5 to 2 mm. in diameter. About one-half of our strains are markedly hemolytic on blood agar plates; agar plates made up with washed red cells, however, do not show any hemolysis. With some strains, the colonies are tough and stringy, and hard to emulsify. Growth on glucose agar is as good as on blood agar.

In infusion broth and in glucose broth, growth is heavy and diffuse. The pH of the glucose broth reaches 5.0.

Fermentation reactions: Acid and a small amount of gas are produced on arabinose, dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, mannose, raffinose, rhamnose, starch, sucrose, and xylose. Seven of our strains ferment aesculin, the others do not. Neither acid nor gas is produced from amygdalin, cellobiose, glycerol, mannitol, melezitose, salicin, sorbitol, or trehalose.

Gelatin is liquefied in 4 to 20 days (one strain, KA, which is typical in every other reaction, does not liquefy gelatin even after 50 days of incubation). Milk is acidified; some strains coagulate the milk in from 5 to 25 days; others fail to coagulate milk, though it coagulates promptly on boiling. Lead acetate is blackened. Indol is not formed.

Bacteroides vulgatus differs from the preceding species in that it does not form indol and does not ferment cellobiose, amygdalin, melezitose, salicin, or trehalose. It differs from *Bacteroides incommunis* in that it does not ferment amygdalin and cellobiose but does ferment glycogen and starch; and in that it liquefies gelatin.

6. Bacteroides incommunis

We have 2 strains, which differ slightly from each other.

On blood agar, the bacilli are single, 1 to 2.5 micra long, about 0.5 micra thick. The staining is solid. In glucose broth, they are swollen and irregularly vacuolated, and sizes vary from 1 to 3 micra long and 0.7 to 1.5 micra thick.

On blood and glucose agar, the colonies are about 1 mm. in diameter, elevated, and slightly yellowish. One strain forms soft colonies; the other is stringy when emulsified.

On infusion broth and on glucose broth, the growth is diffuse. The pH on glucose broth reaches 4.8.

Fermentation reactions: Acid and a small amount of gas are formed from amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, inulin, lactose, levulose, maltose, mannose, raffinose, rhamnose, sucrose, and xylose. One of our two strains ferments glycogen and starch, the other does not. Neither strain ferments aesculin, glycerol, mannitol, melezitose, salicin, sorbitol, or trehalose.

Gelatin is not liquefied. Indol is not formed. Lead acetate is blackened. Milk is acidified but not coagulated; it coagulates promptly on boiling.

7. *Bacteroides distasonis* (*isolated 20 times*)

This species ranks next to *Bacteroides vulgatus* in frequency of occurrence.

On blood agar, this organism appears as a single, solid staining bacillus, 1.5 to 2.5 micra in length, 0.5 to 0.8 micra in thickness, with rounded ends. Some strains will show a few bacilli 5 to 8 micra long. The same forms appear on glucose agar and glucose broth.

The colonies on blood agar are soft, grayish, elevated, from 1 to 1.5 mm. in diameter. Two of our 20 strains are markedly hemolytic but not on agar made up with washed red cells. On glucose agar, the growth is poor, the few colonies that develop being pin point in size. Growth in infusion broth is light and diffuse. On glucose broth the growth is heavier and also diffuse. The pH reaches 5.2.

Fermentation reactions: Acid but no gas is formed from amygdalin, cellobiose, dextrin, galactose, glucose, inulin, lactose, levulose, maltose, mannose, melezitose, raffinose, rhamnose, salicin, sucrose, trehalose, and xylose. All but 5 of our 20 strains likewise ferment aesculin and all but 5 (though not the same 5) slowly ferment starch. No acid or gas are formed from arabinose, glycogen, glycerol, mannitol, or sorbitol.

Gelatin is not liquefied by 16 of our strains; the remaining 4 liquefy gelatin in 35 to 50 days. Indol is not formed. Lead

acetate is blackened. Milk is acidified, and all but 4 of our strains coagulate the milk.

Bacteroides distasonis differs from *Bacteroides vulgatus* in that it ferments amygdalin, cellobiose, melezitose, salicin, and trehalose but fails to ferment arabinose and glycogen; and in that it usually fails to liquefy gelatin. It differs from *Bacteroides incommunis* (which it resembles most closely) in that it ferments melezitose, salicin, and trehalose, while it fails to ferment arabinose.

8. Bacteroides uncatus (isolated once)

On blood agar, extreme variation in size and form occurs. Some of the organisms are very minute slender bacilli, 0.5 to 1.0 micron long; on the other hand, filaments 20 to 30 micra long are also found. The ordinary length is 5 to 8 micra. Curved and hooked forms are plentiful. Bacilli may be club shaped or fusiform or show other types of irregularity. On glucose broth, small slender bacilli, 1 to 2 micra long, with pointed ends, are the predominating forms.

Colonies on blood agar are very minute and transparent. Growth is very poor on glucose agar.

On infusion broth and glucose broth, growth is slow and light; the broth is clouded. Glucose broth reaches an acidity of pH 5.6 only after 8 to 20 days of incubation.

Fermentation reactions: After 8 to 30 days of incubation, acid but not gas is formed from dextrin, galactose, glucose, lactose, levulose, maltose, raffinose, rhamnose, salicin, starch, and sucrose. No acid is formed from aesculin, amygdalin, arabinose, cellobiose, glycerol, glycogen, inulin, mannitol, mannose, melezitose, sorbitol, trehalose, or xylose.

Gelatin is liquefied in 16 days. Indol is not formed. Lead acetate is not blackened. Milk is neither acidified nor coagulated.

This organism may be more common than our results indicate, as we have a number of times fished colonies that might well have been *Bacteroides uncatus*, but failed to obtain growth on transplant.

Bacteroides uncatus does not resemble any other species in our

series. We have classified it with *Bacteroides distasonis* because it ferments salicin; possibly *Bacteroides vescus* may be more closely related, as the latter also ferments rhamnose without fermenting xylose.

9. Bacteroides tumidus (isolated 4 times)

On blood agar, small thick oval bacilli predominate; they are solid-staining, occur singly, and are 1 to 1.5 micra long. On glucose broth, many swollen forms with irregular staining may be seen. These vary greatly in size; bacilli having the following dimensions were measured: 1 by 3 micra; 1.5 by 6 micra; 4 by 5 micra; 3 by 6 micra; 2 by 10 micra; 4 to 10 micra. The bodies of these swollen forms are usually very pale, with only the ends staining.

Colonies on blood agar and on glucose agar are soft, grayish, and elevated, about 1 mm. in diameter.

Growth is heavy and diffuse on infusion broth and on glucose broth. On the latter, the pH reaches 4.8.

Fermentation reactions: Acid but not gas is formed from dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, mannose, raffinose, sorbitol, starch, and sucrose. Neither acid nor gas is formed from aesculin, amygdalin, arabinose, cellobiose, glycerol, mannitol, melezitose, rhamnose, salicin, trehalose, or xylose.

Gelatin is liquefied in 12 to 20 days. Indol is not formed. Lead acetate is blackened. Milk is acidified but not coagulated.

10. Bacteroides ovatus (isolated once)

On blood agar and on glucose agar and on glucose broth, this organism appears as a single small oval, 1 to 2 micra long, 0.5 to 1 micron thick. The staining is solid.

Colonies on blood agar and glucose agar are soft, grayish, elevated, 1 to 1.5 mm. in diameter.

Growth is diffuse and heavy on infusion broth and glucose broth. On the latter, the pH reaches 4.8.

Fermentation reactions: Acid and a small amount of gas are formed from aesculin, amygdalin, cellobiose, dextrin, galactose,

glucose, glycogen, inulin, lactose, levulose, maltose, mannose, raffinose, rhamnose, starch, sucrose, and xylose. Neither acid nor gas is formed from arabinose, glycerol, mannitol, melezitose, salicin, sorbitol, or trehalose.

Gelatin is liquefied in 4 days. Milk is acidified and coagulated in 4 days. Lead acetate is blackened. Indol is formed.

11. Bacteroides vescus (isolated once)

On blood agar, glucose agar, and glucose broth, this organism appears as a slender pointed bacillus, 1 to 2 micra long, sometimes slightly curved, with bi-polar staining.

Colonies on blood agar are very minute and transparent; usually there is no growth on glucose agar.

Infusion broth and glucose broth are diffusely clouded; the pH on glucose broth reaches 5.6.

Fermentation reactions: Acid but not gas is formed from cellobiose (in 30 days), dextrin, glucose, maltose, mannose, and rhamnose. Neither acid nor gas is formed from aesculin, amygdalin, arabinose, galactose, glycerol, glycogen, inulin, lactose, levulose, mannitol, melezitose, raffinose, salicin, sorbitol, starch, sucrose, trehalose, or xylose.

Gelatin is liquefied in 8 to 25 days. Milk is neither acidified nor coagulated. Lead acetate is not blackened. Indol is not formed.

12. Bacteroides convexus (isolated 5 times)

On blood agar and on glucose agar, this organism appears as a thick oval bacillus, 0.8 to 1.5 micra long. It occurs single or in pairs. On glucose broth it is usually 2 to 3 micra long.

Colonies on blood agar and glucose agar are elevated, grayish, somewhat opaque. They are 1 to 1.5 mm. in diameter.

Infusion broth and glucose broth show heavy diffuse growth; the pH of the latter reaches 4.8.

Fermentation reactions: Acid and a small amount of gas are formed from aesculin, amygdalin, cellobiose, dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, mannose, raffinose, starch, sucrose, and xylose. No acid or gas are formed

from arabinose, glycerol, mannitol, melezitose, rhamnose, salicin, sorbitol, or trehalose.

Gelatin is liquefied in 20 to 30 days. Lead acetate is blackened. Milk is acidified and coagulated in 4 days. Indol is not formed.

13. Bacteroides exiguus (isolated twice)

On blood agar and glucose agar and on glucose broth, this organism appears as a very small slender bacillus, 0.5 to 1.0 micron long, occurring singly and in pairs.

Colonies on blood agar are of two types: the first type is pin point in size; the second type is large, gray, and moist, 1 to 1.5 mm. in diameter. On glucose agar, no growth occurs except where the inoculation is very heavy; here a thick moist film develops.

Infusion broth and glucose broth are diffusely clouded; the glucose broth reaches pH 5.6.

Fermentation reactions: Acid but no gas is formed from galactose, glucose, lactose, levulose, maltose, mannose, sucrose, and trehalose. One of our two strains likewise ferments raffinose. Neither acid nor gas is formed from aesculin, amygdalin, arabinose, cellobiose, dextrin, glycerol, glycogen, inulin, mannitol, melezitose, rhamnose, salicin, sorbitol, starch, or xylose.

Gelatin is liquefied in 16 to 20 days. Milk is acidified and may or may not be coagulated in 35 to 40 days. Indol is not formed. Lead acetate is not blackened.

14. Bacteroides inaequalis (isolated once)

Marked pleomorphism occurs on blood agar. Some forms are coccoid, 0.5 micron in diameter; others are slender filaments, often curved or hooked, 3 to 12 micra long. On glucose agar and on glucose broth, small ovals, 1 to 2 micra long, predominate. These show bi-polar staining and (in glucose broth) may form short chains.

Colonies on blood agar are pin point in size. There is no growth on glucose agar. Infusion broth and glucose broth are diffusely clouded; the glucose broth reaches a pH of 5.2.

Fermentation reactions: This species, as well as the succeeding ones, has the rather unusual characteristic of forming gas from peptone water, in the complete absence of carbohydrates. Only a small amount of gas is formed (about 5 per cent of the closed arm of a Smith fermentation tube). None of this gas is absorbed by alkali.

Acid, but no additional gas, is formed from aesculin, amygdalin, arabinose, galactose, glucose, lactose, levulose, maltose, mannose, raffinose, salicin, sucrose, and xylose. No acid is formed from cellobiose, dextrin, glycerol, glycogen, inulin, mannitol, melezitose, rhamnose, sorbitol, starch, or trehalose.

Gelatin is not liquefied in 45 days. Lead acetate is blackened. Milk is acidified but not coagulated. Indol is formed.

Like the succeeding 4 species that form gas from peptone, this species rapidly decolorizes the sulphonphthalein dyes brom cresol purple and phenol red, in the presence of meat infusion; slowly or not at all in peptone water.

15. Bacteroides insolitus (isolated once)

On blood agar and glucose agar and in glucose broth, the predominating form is a short thick bacillus, 1 to 2 micra long; there are often some slender, curved bacilli, 2 to 3 micra long.

Colonies on blood agar and glucose agar are minute and transparent.

Growth in infusion broth and glucose broth is heavy and diffuse; the pH in glucose broth reaches 5.4.

Fermentation reactions: A small amount of gas is formed from peptone. Acid but no additional gas is formed from galactose, glucose, glycerol, lactose, levulose, maltose, and mannose. Acid is not formed from aesculin, amygdalin, arabinose, cellobiose, dextrin, glycogen, inulin, mannitol, melezitose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose, or xylose.

Gelatin is not liquefied in 45 days. Milk is acidified and coagulated in 30 to 35 days. Lead acetate is blackened. Indol is formed. Brom cresol purple and phenol red are rapidly decolorized in the presence of meat infusion.

16. Bacteroides varius (isolated twice)

On blood agar, slender bacilli, 1 to 2 micra long, predominate. On glucose agar, the bacilli are longer and thicker, being 2 to 3 micra long; the staining is very uneven, barred and vacuolated forms being common. In glucose broth, the bacilli are oval or coccoid.

The colonies on blood agar appear like very flat cones, 2 to 3 mm. in diameter. On glucose agar, the colonies are 1 to 2 mm. in diameter. Infusion broth and glucose broth are diffusely clouded. The pH of the latter reaches pH 5.0.

Fermentation reactions: Gas is formed from peptone. Acid and considerable additional gas is formed from galactose, glucose, levulose, and mannose. No acid and no additional gas are formed from aesculin, amygdalin, arabinose, cellobiose, dextrin, glycerol, glycogen, inulin, lactose, maltose, mannitol, melezitose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose, or xylose.

Gelatin is not liquefied in 45 days. Lead acetate is blackened. Milk is neither acidified nor coagulated. Indol is formed. Brom cresol purple and phenol red are decolorized in the presence of meat infusion.

17. Bacteroides coagulans (isolated once)

On blood agar, this organism is a minute oval bacillus, 0.5 to 1 micron long, with marked bi-polar staining. On glucose agar and on glucose broth, it is longer and more slender, 1 to 2 micra long.

Colonies on blood agar are 0.5 mm. in diameter, soft and transparent. On glucose agar, the colonies are of the same size, but very few colonies develop. The growth on infusion broth and on glucose broth is diffuse.

Fermentation reactions: A small amount of gas is formed from peptone. No carbohydrates are fermented with acid or gas production.

Gelatin is liquefied in 8 to 12 days. Milk is coagulated in 8 days without acid production; the coagulum partly redissolves

after 3 to 4 weeks. Indol is formed. Phenol red and brom cresol purple are decolorized in the presence of meat infusion.

18. Bacteroides siccus (isolated twice)

On blood agar and on glucose agar, this organism appears as a short thick bacillus, about 1 micron long. In glucose broth, it is coccoid and often grows in chains of 4 to 6 elements.

TABLE 2

The chief biological reactions of the Gram-negative Bacteroides

	SPECIES	GAS FROM PEPTONE	GAS FROM GLUCOSE	GLYCEROL	MANNITOL	SORBITOL	ARABINOSE	SALICIN	TREHALOSE	AMYGDALIN	CELLOBIOSE	GLYCOGEN	RHAMNOSE	XYLOSE	LACTOSE	LEVULOSE	GLUCOSE	GELATIN	MILK	INDOL	LEAD ACETATE
1	*B. gulosus*	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	*B. thetaiotaomicron*	−	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
3	*B. variabilis*	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	±	+	+
4	*B. uniformis*	−	−	−	−	−	+	+	+	+	+	+	−	+	+	+	+	∓	+	+	∓
5	*B. vulgatus*	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	+	±	−	+
6	*B. incommunis*	−	+	−	−	−	+	−	−	+	+	∓	+	+	+	+	+	−	−	−	+
7	*B. distasonis*	−	−	−	−	−	−	+	+	+	+	−	+	+	+	+	+	∓	±	−	+
8	*B. uncatus*	−	−	−	−	−	−	+	−	−	−	−	+	−	+	+	+	+	−	−	−
9	*B. tumidus*	−	−	−	−	+	−	−	−	−	−	+	−	−	+	+	+	+	−	−	+
10	*B. ovatus*	−	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
11	*B. vescus*	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+	+	−	−	−
12	*B. convexus*	−	+	−	−	−	−	−	−	+	+	+	−	+	+	+	+	+	+	−	+
13	*B. exiguus*	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	+	±	−	−
14	*B. inaequalis*	+	−	−	−	−	+	+	−	+	−	−	−	+	+	+	+	−	−	+	+
15	*B. insolitus*	+	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+
16	*B. varius*	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+
17	*B. coagulans*	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
18	*B. siccus*	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+

On blood agar and on glucose agar, the colonies are 1 to 1.5 mm. in diameter; they are elevated and dry and difficult to emulsify. In infusion and in glucose broth, growth occurs as a powdery sediment with a clear supernatant fluid.

Fermentation reactions: Gas in small amounts is formed from peptone. Acid, but no additional gas, is formed from levulose only.

Gelatin is not liquefied. Milk is not changed. Lead acetate is blackened. Indol is not formed. Phenol red and brom cresol purple are decolorized in the presence of meat infusion.

The chief biological characteristics of the above 18 species of Bacteroides are given in abbreviated form in table 2.

ATTEMPTED SEROLOGICAL CLASSIFICATION OF THE GRAM-NEGATIVE BACTEROIDES

We immunized 33 rabbits with different strains of Gram-negative Bacteroides, hoping that agglutination reactions would aid in the identification and classification of these organisms. We were unable to secure agglutinating sera of high potency, even after 15 to 18 semi-weekly injections; most of the sera agglutinated the homologous organisms in dilutions of 1:50 to 1:100.

To our disappointment, we found that these sera were strain specific rather than species specific; a serum rarely agglutinated any organism but the homologous one; when a serum did agglutinate another strain, absorption of the serum with the heterologous organism failed to remove the major agglutinin.

For example, we immunized 9 rabbits with 9 different strains of *Bacteroides vulgatus*, the commonest organism in our series. Each serum was tested against the 9 strains. In 5 cases, only the homologous strain was agglutinated. The other 4 sera each agglutinated one other strain besides the homologous one. These 4 sera were each absorbed twice by a heavy suspension of the heterologous agglutinable strain. Such absorbed sera still agglutinated the homologous strains though they now failed to agglutinate the heterologous ones.

In the same way, we prepared 4 sera against 4 strains of *Bacteroides distasonis*, and sera against 3 strains each of *Bacteroides thetaiotaomicron*, *Bacteroides variabilis*, and *Bacteroides uniformis*. In all cases the sera were strain specific.

DISCUSSION

Distaso (1912) has described two species of Gram-negative fecal Bacteroides that we did find in our stool cultures: *Bacteroides bullosus* and *Bacteroides variegatus*. His descriptions are given briefly as follows:

Bacteroides bullosus is a small rectangular bacillus with bi-polar staining. Young cultures show spherical or oblong forms that stain uniformly, also long slender rods with a thick enlargement at one end or the middle. Occasionally these forms bifurcate at one end. The organism is motile. Acid and abundant gas are produced from glucose. No action on lactose, sucrose, gelatin, or milk. Indol is not formed.

Bacteroides variegatus is sometimes a short bacillus, sometimes very long and flexuous. It is motile. It contains Gram-positive granules. Glucose and lactose are fermented without gas. Milk is coagulated. Indol is formed. Gelatin is not liquefied.

Bergey's Manual also classifies Distaso's *B. laevis* (1912), *B. liquefaciens* (1911), and *B. rigidus* (1911) in the genus Bacteroides. This must be an error, as Distaso clearly states that these three species are *facultative* anaerobes.

We did not meet any of the three species of Gram-negative non-spore-bearing anaerobes described by Tissier as occurring in the stools of infants. These three species are:

Coccobacillus anaerobius-perfoetens (1900). Description of this species is very meager; there are no data on indol production, liquefaction of gelatin, or action on lactose or sucrose. It is oval in shape, occurs single, in pairs or short chains, and produces acid and gas from glucose.

Coccobacillus preacutus (1908). This is a coccobacillus with long fine pointed extremities, the total length being 5 to 10 micra. It is rapidly motile. There is acid and abundant gas from glucose. No indol. No action on milk, gelatin, lactose, or sucrose.

Bacillus capilosus (1908). Appears as a large curved bacillus or tangled filaments. Non-motile. No indol. No action on gelatin, milk, lactose, or sucrose. Acid but no gas from glucose.

Harris (1901–1905) has described an interesting organism, *Bacillus mortiferus*, that obviously is related to our second group of Gram-negative Bacteroides because it produces gas from proteins without the addition of sugar. This was isolated from a liver abscess. It did not grow on ordinary media, the addition of blood, serum, or ascitic or hydrocele fluid being necessary.

SUMMARY AND CONCLUSIONS

1. In 91 per cent of our series of 60 stools of adults, the predominating organisms were obligate anaerobes of the genus Bacteroides. In the remaining 9 per cent, the aerobes were more numerous than the anaerobes. Over half of the stool cultures yielded 90 per cent or more of anaerobes.

2. The Gram-positive and the Gram-negative Bacteroides differ, biologically, in many respects. This justifies grouping and considering them separately. As our work with the Gram-positive group is incomplete, it will be reported at a later time.

3. The Gram-negative Bacteroides have been studied and classified. Two species, already described by Distaso, have been studied in greater detail. In addition, sixteen new species are described.

4. Agglutinating sera prepared against the Gram-negative Bacteroides are strain specific and are therefore of little or no value in the identification and classification of species.

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