Serogrouping of *Bacteroides fragilis* subsp. *fragilis* by the Agglutination Test

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The agglutination technique was used to establish a serological classification scheme for 98 strains of *Bacteroides fragilis* subsp. *fragilis* isolated from clinical specimens and normal human feces. Absorbed antisera were prepared to seven strains of *B. fragilis* subsp. *fragilis*. These seven absorbed antisera were species as well as subspecies specific and provided the basis of the serological classification scheme. This scheme was composed of 21 serogroups; seven of these serogroups contained only one group component, whereas the other 14 serogroups were composed of more than one component. There was a total of 45 serological patterns. This serological scheme may be used for the serological classification of strains of *B. fragilis* subsp. *fragilis* and to study the epidemiology of this organism.

Although the intestinal tract of man is the normal habitat of *Bacteroides fragilis* subsp. *fragilis*, this organism is the least common of the five subspecies of *B. fragilis* in human feces (15). However, *B. fragilis* subsp. *fragilis* represents 70% of 684 strains of *B. fragilis* isolated from clinical specimens in our laboratory over a 5-year period. The low frequency of this organism in human feces contrasted to the high frequency in clinical specimens indicates that *B. fragilis* subsp. *fragilis* may be the most pathogenic of the five subspecies of *B. fragilis*.

B. fragilis, and more specifically *B. fragilis* subsp. *fragilis*, has been well documented as the causative agent in various types of human infection (2, 3, 7, 8, 13, 14, 23), but little serological information of this organism was available previously. Eggerth and Gagnon (6) were the first to study *B. fragilis* with the agglutination technique; they concluded that serological classification was doubtful since agglutinating antisera were strain specific. Weiss and Rettger (20) also provided little evidence that a serological classification was possible. However, others indicated that serotypes existed in *B. fragilis* subsp. *fragilis* (21, 22) and Beerens et al. (1) described three serogroups, E₁, E₂, and E₁E₂, which occurred in *B. fragilis*.

Studies on the immune response in humans to *B. fragilis* subsp. *fragilis* infection (2, 3, 13) provided serological evidence that an extensive serological study of *B. fragilis* subsp. *fragilis* was indicated. For example, the agglutination of certain strains of *B. fragilis* subsp. *fragilis* with antisera from different patients indicated serologically related strains; minimal or no re-

action of other strains with heterologous antisera indicated the existence of more than one serogroup within this subspecies. Therefore, the purpose of this study was to develop a serological classification scheme for *B. fragilis* subsp. *fragilis*. With the agglutination technique, 21 serogroups of *B. fragilis* subsp. *fragilis* are described. Certain serogroups were further divided into subserogroups.

MATERIALS AND METHODS

Strains. *B. fragilis* subsp. *fragilis* (85 strains) isolated from clinical specimens in our laboratory were included in the serogrouping study. The sources from which *B. fragilis* subsp. *fragilis* strains were isolated included: blood, wounds (stab, stump, heel, neck, abdomen), abscesses (rectal, peritoneal, groin, flank), swabs (perineal), drainage (abdominal, sump tube, cyst, bladder), fluids (peritoneal, thoracentesis), abdominal cavity, lung cavity, cervix, labia, uterus, suture site, and fistula.

Additional clinical isolates included: *B. fragilis* subsp. *thetaiotaomicron* (twenty-four strains), *B. fragilis* subsp. *distasonis* (fourteen strains), *B. fragilis* subsp. *vulgatus* (twelve strains), *Bacteroides melaninogenicus* subsp. *melaninogenicus* (one strain), *B. melaninogenicus* subsp. *intermedius* (one strain), *B. melaninogenicus* subsp. *asaccharolyticus* (one strain), *Bacteroides oralis* (one strain), *Bacteroides biacutus* (one strain), *Bacteroides* group PS (proteolytic, saccharolytic) (11) (one strain), and *Bacteroides clostridiiformis* subsp. *clostridiiformis* (one strain).

A few isolates of *B. fragilis* subsp. *fragilis* (four strains) were obtained from normal human feces.

Strains obtained from D. Danielsson, Central County Hospital, Orebro, Sweden included: three strains of *B. fragilis* subsp. *fragilis* isolated from

blood (1226-72), abscess (1227-72), or fistula (1344-72); two strains of *B. fragilis* subsp. *thetaiotaomicron*; and one strain of *B. fragilis* subsp. *distasonis*. Strains obtained from L. V. Holdeman, Anaerobe Laboratory, Virginia Polytechnic Institute (VPI), Blacksburg, included eight strains of *B. fragilis* subsp. *thetaiotaomicron* and three strains of *B. fragilis* subsp. *distasonis*. Nine strains of *B. fragilis* subsp. *fragilis* obtained from L. V. Holdeman included: 184-76 (←VPI 2550←Finegold, Los Angeles, Calif.; ischiorectal abscess); 185-76 (←VPI 2633←Balows, Lexington, Ky.; abdominal wound); 186-76 (←VPI 6057B←Tucson, Ariz.; buttock drainage); 187-76 (←VPI 6123←Corning, N. Y.; abdominal incision drainage); 188-76 (←VPI 6859B←New Haven, Conn.; blood); 189-76 (←VPI 0479←Prévot 1048A, Paris, France; liver abscess); 190-76 (←VPI 2393←Beerens 12, Lille, France); 191-76 (←VPI 5650←Wilmington, N.C.; blood); and 192-76 (←VPI 2553←Sonnenwirth, EN-2, St. Louis, Mo.←NCTC 9343; appendiceal abscess).

Identification. The biochemical tests used for identification of anaerobic strains were those described previously (12). Biochemical media used for identification were prerduced, anaerobically sterilized (Scott Laboratories, Fiskeville, R.I.). Taxonomic schemes described by Holdeman and Moore (11) were used to classify all anaerobes. Tests for acid production from mannitol, rhamnose, and trehalose, indole production, and bile stimulation were repeated on all *B. fragilis* strains at the end of the serological study. Metabolic end products were analyzed by the gas chromatograph as described by Holdeman and Moore (11).

Antigen preparation. For immunization of rabbits and preparation of the agglutination antigen, test strains were inoculated onto reduced blood agar plates consisting of brain heart infusion agar (Difco) supplemented with 5% sheep blood, 0.5% yeast extract, hemin (0.0005%), and menadione (0.00005%). These plates were used within 2 h or stored in an anaerobic atmosphere and used within 8 h. The reduced blood agar plates were cross-streaked and incubated at 35 C for 48 h in an anaerobe chamber containing an atmosphere of 80% nitrogen, 10% hydrogen, and 10% CO₂. After incubation, the organisms were harvested in 0.85% sterile saline (PSS) which had been Seitz filtered and autoclaved (2). The cells were treated with formalin and then washed with sterile PSS as described previously (12). For use as vaccines, the cells were diluted in sterile PSS to a density of approximately 2×10^9 organisms/ml (24% transmittance at 610 nm in a Coleman spectrophotometer, model 6A). For use in agglutination tests, the cells were standardized in PSS to a turbidity of a McFarland no. 7 standard diluted 1:2 (1 + 1). Merthiolate was added to both antigen preparations to a final concentration of 1:5,000. Purity of the antigens and killing efficacy of the formalin were determined by a subculture on aerobic and anaerobic blood agar plates. The antigens were stored at 4 C and were stable for at least 6 months.

Preparation of antisera. Young, male New Zealand albino rabbits weighing 3 to 4 kg each were

bled before injection; these preimmune sera were used as controls to establish the absence of antibody in the rabbits against the test strains. Formalin-, but not heat-, treated cells were used to immunize rabbits. The immunization schedule described by Lambe (12) was used to prepare the immune sera.

The animals were bled from the heart 1 week after the last injection. Merthiolate was added to each antiserum to a final concentration of 1:5,000. The antisera were stored at -50 C. Before use, antisera were equilibrated to room temperature and then inactivated at 56 C for 30 min.

Absorption of antisera. Absorptions were made by using antiserum that was diluted 1:5 with sterile PSS. To determine the minimal amount of antigen necessary to achieve the desired absorption, a series of tubes were set up. Each tube contained either 0.1, 0.15, 0.2, or 0.3 ml of packed cells per 5.0 ml of a 1:5 dilution of antiserum. The antiserum-cell suspensions were mixed and incubated in a 37 C water bath for 1 h followed by overnight incubation at 4 C. The suspensions were gently mixed and then centrifuged at 3,500 rpm for 20 min. The absorbed antiserum was removed, centrifuged again, and then stored in a screw-capped test tube at 4 C.

Agglutination. Agglutination tests were performed in glass test tubes (10 by 75 mm) containing 0.5 ml of antiserum serially diluted in sterile PSS starting at a dilution of 1:10. An equal volume of antigen was added to 0.5 ml of the diluted antiserum. The final reported titer was the dilution of antiserum before addition of antigen. Agglutination tubes were incubated overnight at 45 C; the tubes were shaken and the reactions were graded with the naked eye on a scale of 4+ to negative: 4+, heavy clumping; 3+, moderate clumping; 2+, light clumping; 1+, questionable clumping; and negative, no reaction. The highest antiserum dilution yielding a 2+ reaction was considered positive. Using absorbed antisera, an agglutination titer of 1:20 or greater was considered significant (16).

RESULTS

Biochemical identification. All strains of anaerobic bacteria were identified according to the taxonomic schemes of Holdeman and Moore (11). *B. fragilis* subsp. *fragilis* produced acid from fructose, glucose, lactose, maltose, mannose, raffinose, starch, sucrose, and xylose. No acid was produced from arabinose, cellobiose, aesculin, mannitol, rhamnose, or trehalose. Aesculin and starch were hydrolyzed. Indole was not produced and nitrate was not reduced. Gelatin was not digested. A clot was formed from milk. Bile stimulated the growth of 86 of the 89 strains. The three strains that were inhibited by bile (20%) were isolated from the same patient; one of these strains grew in bile in the VPI Anaerobe Laboratory. Volatile acids that were produced included acetic, isobutyric, and isovaleric acids.

Antisera. The homologous agglutination titers produced by the immunization schedule

used for the classification of *B. fragilis* subsp. *fragilis* were comparable to the titers reported by others (1, 4, 9, 22). From preliminary agglutination studies of 67 strains of *B. fragilis* subsp. *fragilis* and 12 *B. fragilis* subsp. *fragilis* antisera (unpublished data), seven antisera were selected that produced minimal cross-reactions with the other six immunizing strains (Table 1). Absorption of 1.0 ml of antiserum diluted 1:5 in sterile saline (0.85%) with a quantity of 0.2 ml of packed cells was sufficient to produce monospecific antisera. Each antiserum was monospecific in that it reacted with the homologous antigen but failed to react with the six heterologous antigens. These seven monospecific antisera were coded 1 through 7. This absorption method of using monospecific antisera was used for the classification of *Propionibacterium acnes* (16).

Agglutination. For a reproducibility study of the agglutination tests, duplicate titers on different days were performed on 40 strains chosen at random. Titers showed that 82.5% of such duplicate tests were identical. No duplicate test varied more than one-tube dilution. The 95% confidence limits, as determined by binomial function, for those strains showing no difference in titer upon retesting was 66 to 92%.

Strains of *B. fragilis* subsp. *fragilis* (98 strains) were agglutinated by one or more of the seven antisera (Table 2). Many organisms (32 strains) reacted with one antiserum specifically; these strains comprised serogroups I through VII. The remaining 66 strains (67%) reacted with two or more of the absorbed antisera; these strains made up serogroups VIII through XXI.

Serological classification. The terms serogroup and subserogroup, which we used to sero-

logically classify the *B. fragilis* subsp. *fragilis* strains, was patterned after the terminology used for the serological classification of *Shigella* (5). *B. fragilis* subsp. *fragilis*, like *Shigella*, is nonmotile; therefore, no flagella antigens were considered for the serological classification of this organism. The antigenic component that was common to all strains within the same serogroup of *B. fragilis* subsp. *fragilis* was called the group component, whereas the antigenic component that was common to strains within a particular subserogroup only was called the subgroup component (Table 3). These antigenic components were designated by arabic numbers (1, 2, etc.) Consequently, serogroup I was composed of four strains with group component 1 only, whereas serogroup XI subserogroup b was composed of group components 2 and 7 and subgroup component 3. The serological classification scheme was composed of 21 serogroups; seven of these serogroups contained one antigenic component only, the other 14 serogroups contained multiple group components or group and subgroup components. Thus, there was a total of 45 serological patterns. The 98 strains of *B. fragilis* subsp. *fragilis* included isolates from various geographic locations within the United States (Georgia, California, Kentucky, Arizona, New York, Connecticut, and North Carolina), as well as from certain foreign countries (Sweden, England, and France). It was of interest to note that the strains from foreign culture collections fit into this classification scheme: strain 192-76 (NCTC 9343) contained all seven of the antigenic components; strain 190-76 (Beerens 12) contained group component 7 only; strain 189-76 (Prevot 1048A) contained group components 3 and 5, and subgroup component 7; 1226-72

TABLE 1. Agglutination of *Bacteroides fragilis* subsp. *fragilis* antisera before and after absorption

Immu- nizing strain	Antisera												
	Anti-289-71		Anti- ^a 322- 72A	Anti-586-71		Anti-531-71A		Anti-988-72B		Anti-270-72A		Anti-898-72	
	Unab- sorbed	Absorbed with 988- 72B	Unab- sorbed	Unab- sorbed	Absorbed with 270- 72A	Unab- sorbed	Absorbed with 270- 72A	Unab- sorbed	Absorbed with 531- 71A	Unab- sorbed	Absorbed with 898- 72	Unab- sorbed	Absorbed with 586- 71
289-71	640 ^b	320	— ^c	—	—	—	—	20	—	—	—	—	—
322-72A	—	—	320	—	—	—	—	—	—	—	—	—	—
586-71	—	—	—	1,280	320	80	—	40	—	20	—	80	—
531-71A	—	—	—	20	—	320	160	80	—	—	—	20	—
988-72B	80	—	—	20	—	—	—	320	80	—	—	—	—
270-72A	20	—	—	40	—	40	—	40	—	1,280	1,280	20	—
898-72	—	—	—	20	—	—	—	—	—	20	—	160	80

^a Antiserum required no absorption.

^b Reciprocal of the antibody titer.

^c No agglutination in antiserum diluted 1:20 or greater.

TABLE 2. Agglutination results of 98 strains of *B. fragilis* subsp. *fragilis* using seven absorbed *B. fragilis* subsp. *fragilis* antisera

Sero-group	Sub-sero-group	Strain no.	Absorbed antisera (strain no.)						
			1 (289-71)	2 (322-72A)	3 (586-71)	4 (531-71A)	5 (988-72B)	6 (270-72A)	7 (898-72)
I	—	289-71	320	— ^a	— ^a	— ^a	— ^a	— ^a	— ^a
		413-72	40	—	—	—	—	—	—
		411-72A	20	—	—	—	—	—	—
		662-71	40	—	—	—	—	—	—
II	—	322-72A	— ^a	320	—	—	—	—	—
		950-70A	—	20	—	—	—	—	—
		565-71	—	20	—	—	—	—	—
		566-71	—	20	—	—	—	—	—
		442-72B	—	40	—	—	—	—	—
		1586-74	—	80	—	—	—	—	—
		1271-74	—	20	—	—	—	—	—
III	—	586-71	—	—	320	—	—	—	—
		946-72A	—	—	20	—	—	—	—
		863-72	—	—	20	—	—	—	—
		80-72B	—	—	20	—	—	—	—
		850-72A	—	—	40	—	—	—	—
IV	—	531-71A	—	—	—	160	—	—	—
		3-72A	—	—	—	80	—	—	—
V	—	988-72B	—	—	—	—	80	—	—
		380-72	—	—	—	—	20	—	—
VI	—	270-72A	—	—	—	—	—	1280	—
		800-72B	—	—	—	—	—	40	—
		357-72	—	—	—	—	—	160	—
		186-76	—	—	—	—	—	20	—
VII	—	898-72	—	—	—	—	—	—	80
		1227-72	—	—	—	—	—	—	80
		449-71C	—	—	—	—	—	—	40
		1280-73A	—	—	—	—	—	—	40
		1361-73C	—	—	—	—	—	—	40
		1361-73A	—	—	—	—	—	—	40
		1162-74	—	—	—	—	—	—	20
		190-76	—	—	—	—	—	—	40
VIII	a	513-72	20	—	20	20	—	—	—
	b	414-72	20	—	40	—	40	—	—
	c	168-72	40	—	40	—	80	160	—
	d	488-72F	20	—	40	—	—	20	20
	e	694-71B	40	—	20	40	—	20	80
IX	a	141-74	40	—	—	40	—	—	—
X	a	1279-73B	20	—	—	—	—	—	80
		1589-74	20	—	—	—	—	—	40
XI	a	424-72	—	20	—	—	—	—	40
		1270-74	—	40	—	—	—	—	20
	b	1269-74	—	20	40	—	—	—	40
XII	a	76-72	—	—	20	—	40	—	—
		473-72A	—	—	20	—	20	—	—
	b	974-72	—	—	40	20	40	—	—
	c	375-72	—	20	40	40	20	—	—
		185-76	—	80	40	20	20	—	—
	d	189-76	—	—	80	—	20	—	80
XIII	a	50-72F	40	—	—	40	20	—	—
		423-72C	20	—	—	40	20	—	—
	b	958-72A	20	—	—	20	20	40	—
	c	786-72	—	—	—	80	80	20	40
XIV	a	337-75	—	—	—	20	—	—	40
		338-75	—	—	—	20	—	—	40
XV	a	1587-74	—	—	—	—	40	—	20
XVI	a	1588-74	—	—	—	—	—	20	40
	b	275-72	—	20	—	—	—	20	40
	c	191-76	—	20	—	—	40	40	40
XVII	a	1226-72	20	80	—	—	—	20	—
	b	1344-72	20	40	40	80	—	80	—
		184-76	20	40	20	40	—	20	—
	c	196-72C	160	20	—	40	—	320	20

TABLE 2—Continued

Sero-group	Sub-sero-group	Strain no.	Absorbed antisera (strain no.)						
			1 (289-71)	2 (322-72A)	3 (586-71)	4 (531-71A)	5 (988-72B)	6 (270-72A)	7 (898-72)
XVIII	d	658-71A	40	20	—	80	20	20	40
		559-71C	80	20	—	40	40	20	80
		188-76	20	20	—	20	80	80	80
	a	625-71A	80	—	20	20	20	40	—
		447-71A	40	—	20	20	80	40	—
		1179-72A	40	—	40	40	80	20	—
		515-72	40	—	20	20	40	20	—
	b	1-74	40	—	40	40	160	—	40
XIX		2-74	40	—	20	40	160	—	40
		580-71	20	—	20	20	20	—	160
	c	593-71	80	—	20	40	80	160	20
		183-72	80	—	80	20	40	160	80
		1215-72A	320	—	40	160	40	20	40
	a	1598-74	—	20	—	20	40	—	40
		23-74A	—	40	—	40	80	—	40
		23-74B	—	80	—	40	80	—	80
		913-70	—	20	—	40	40	—	40
		207-72A	—	20	—	80	40	—	40
XX	b	295-72D	—	20	20	20	20	—	80
	c	34-74B	—	80	—	80	80	40	80
		664-71D	—	20	—	40	20	40	160
	a	445-71	—	—	160	—	20	20	20
XXI	b	164-72	—	40	20	—	20	20	20
	c	824-73	20	40	20	—	40	40	80
	a	371-74	80	40	20	40	40	—	—
		514-72	20	20	40	20	40	—	—
	b	507-72A	80	40	40	20	20	20	—
		609-72B	160	20	80	80	20	80	—
		101-72B	20	40	80	40	40	80	—
	d	136-72	40	20	80	20	20	—	20
		814-72A	80	40	80	80	80	160	160
		700-72A	80	20	40	40	20	20	20
		109-72	80	40	40	40	40	80	160
		187-76	20	40	20	80	40	40	80
		192-76	40	40	40	20	20	40	40

^a —, ≤10.

(Swedish strain) contained group components 1, 2, and 6; 1227-76 (Swedish strain) contained group component 7 only; and 1344-72 (Swedish strain) contained group components 1, 2, and 6 and subgroup components 3 and 4. None of the 98 strains of *B. fragilis* subsp. *fragilis* could be classified into more than one serogroup by this method.

Each of the seven absorbed antisera failed to give a positive agglutination with *B. fragilis* subsp. *thetaiotaomicron* (34 strains), *B. fragilis* subsp. *distasonis* (18 strains), *B. fragilis* subsp. *vulgatus* (12 strains); and one strain each of *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, *B. melaninogenicus* subsp. *asaccharolyticus*, *B. oralis*, *B. biacutus*, *Bacteroides* group PS, and *B. clostridiiformis* subsp. *clostridiiformis*.

Heat resistance of the antigenic components was tested. Cell suspensions in 0.85% sterile saline of each of the seven immunizing strains of *B. fragilis* subsp. *fragilis* were heated for 60 min at 100 C. The agglutination test was per-

TABLE 3. Agglutination results with 98 strains of *Bacteriodes fragilis* subsp. *fragilis* with seven absorbed antisera

Sero-group	Subsero-group	No. of strains	Group component	Subgroup component
I	— ^a	4	1	—
II	—	7	2	—
III	—	5	3	—
IV	—	2	4	—
V	—	2	5	—
VI	—	4	6	—
VII	—	8	7	—
VIII	a	1	1, 3	4
	b	1	1, 3	5
	c	1	1, 3	5, 6
	d	1	1, 3	6, 7
	e	1	1, 3	4, 6, 7
IX	a	1	1, 4	—
X	a	2	1, 7	—
XI	a	2	2, 7	—
	b	1	2, 7	3
XII	a	2	3, 5	—
	b	1	3, 5	4
	c	2	3, 5	2, 4
	d	1	3, 5	7
XIII	a	2	4, 5	1
	b	1	4, 5	1, 6
	c	1	4, 5	6, 7
XIV	a	2	4, 7	—
XV	a	1	5, 7	—
XVI	a	1	6, 7	—
	b	1	6, 7	2
	c	1	6, 7	2, 5
XVII	a	1	1, 2, 6	—
	b	2	1, 2, 6	3, 4
	c	1	1, 2, 6	4, 7
	d	3	1, 2, 6	4, 5, 7
XVIII	a	4	1, 3, 4, 5	6
	b	3	1, 3, 4, 5	7
	c	3	1, 3, 4, 5	6, 7
XIX	a	5	2, 4, 5, 7	—
	b	1	2, 4, 5, 7	3
	c	2	2, 4, 5, 7	6
XX	a	1	3, 5, 6, 7	—
	b	1	3, 5, 6, 7	2
	c	1	3, 5, 6, 7	1, 2
XXI	a	2	1, 2, 3, 4, 5	—
	b	3	1, 2, 3, 4, 5	6
	c	1	1, 2, 3, 4, 5	7
	d	5	1, 2, 3, 4, 5	6, 7

^a —, None.

formed with the heat-treated cells as well as with unheated cells. The heat-treated cells of all seven strains showed agglutination patterns that corresponded to the patterns of the unheated cells.

DISCUSSION

The important contribution of this study was the development of a serological classification scheme of *B. fragilis* subsp. *fragilis* based on the agglutination test; this scheme was com-

posed of seven serogroups that contained only one group component, and 14 other serogroups that contained multiple group components or group and subgroup components. There were 45 possible serological patterns. Certain serogroups were rare in that they contained only one strain; other serogroups contained as many as 8 to 11 strains. This serogrouping scheme is the most extensive serological classification of *B. fragilis* subsp. *fragilis* described thus far. Two features were unique to our system: the use of absorbed antisera, which were monospecific with respect to the immunizing antigens, and the use of a larger number of antisera (seven) than that used by other workers (1, 17-19). All 98 strains of *B. fragilis* subsp. *fragilis* used in this study were classified by our serological scheme.

The antigenic components of the seven *B. fragilis* subsp. *fragilis* strains were heat resistant, and were, therefore, probably polysaccharides rather than proteins. Pulverer and Ko (16) also reported that the agglutinating antigens of *P. acnes* were highly heat resistant.

Previously, several workers studied *B. fragilis* subsp. *fragilis* with the agglutination test, although they obtained only fragmentary serological data. In 1933, Eggerth and Gagnon (6) reported little cross-reactivity among the subspecies of *B. fragilis*, as well as little cross-reactivity within each subspecies. They concluded that the agglutinating sera were strain specific, and therefore, of little value in the serological classification of these anaerobes. The homologous agglutinating titers of 1:50 and 1:100 which were obtained in their studies were low compared with titers as high as 1:1,280 which were produced in our studies. The low titers of Eggerth and Gagnon may account for the small amount of cross-reaction between serologically related strains of *B. fragilis* subsp. *fragilis*.

Henthorne et al. (10) reported three strains of *B. fragilis* that were serologically identical, whereas a fourth strain was serologically distinct, but no mention was made of the particular subspecies of *B. fragilis* that they studied. Weiss and Rettger (20) demonstrated four serogroups that corresponded to the biotypes *Bacteroides vulgatus*, *Bacteroides varius*, *Bacteroides uncatus* and *Bacteroides variabilis*; they classified *B. fragilis* strains under *B. vulgatus*. Our experience has indicated that no cross-reaction occurred between *B. fragilis* subsp. *fragilis* and *B. fragilis* subsp. *distasonis*, *B. fragilis* subsp. *thetaiotaomicron*, or *B. fragilis* subsp. *vulgatus*.

A serological scheme was also reported by Beerens et al. (1), Shinjo et al. (19), and Ro-

mond et al. (17). The major limitations of their studies were the use of unabsorbed reference antisera, as well as the use of only two antisera of *B. fragilis*. As mentioned previously, we used seven absorbed antisera. In the above studies (1, 17, 19), 8 of 23 strains of *B. fragilis* subsp. *thetaiotaomicron* gave a positive agglutination titer with serogroups E₁, E₂, or E₁E₂ (anti-*B. fragilis*). The minor cross-reactions (1:20 or 1:40) of five of the eight positive strains might have disappeared if absorbed antisera had been used. The relatively strong cross-reactions (1:80 and 1:60) of the other three strains of *B. fragilis* subsp. *thetaiotaomicron* with anti-*B. fragilis* subsp. *fragilis* sera are more difficult to explain, since we have not observed this much cross-reaction between these two subspecies of *B. fragilis*. We observed a minor cross-reaction (1:10) between only one of 34 strains of *B. fragilis* subsp. *thetaiotaomicron* and anti-*B. fragilis* subsp. *fragilis* (D. A. Moroz, Masters thesis, Emory Univ., Atlanta, 1975), but this minor cross-reaction disappeared when absorbed antisera were used.

A common E antigen found in 86% of *B. fragilis* strains was associated with the pathogenicity of the organism (1, 17, 19). Similar findings were reported by others (18). We showed a common antigen in 79% of 89 strains of *B. fragilis* subsp. *fragilis* using unabsorbed antisera (Moroz, Master's thesis, 1975). The presence of common antigens made serological classification complicated because of the large number of serogroups that resulted with the unabsorbed antisera. No one particular antigen predominated among 98 strains of *B. fragilis* subsp. *fragilis* examined when absorbed antisera were used.

It was of interest to note that strains from clinical specimens and normal feces, as well as from different geographic locations (this laboratory, as well as a limited number of strains from other laboratories in the United States, Sweden, England, and France), could all be classified by this serological scheme. These findings indicate that this basic serological scheme may be useful in studying the epidemiology of *B. fragilis* subsp. *fragilis* from a variety of sources both in the United States and abroad. New serogroups may be added as additional strains are studied.

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Identification by Lillian V. Holdeman of certain cultures obtained from the Virginia Polytechnic Institute Anaerobe

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