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# Human Fecal Flora: Variation in Bacterial Composition Within Individuals and a Possible Effect of Emotional Stress

L. V. HOLDEMAN,* I. J. GOOD, AND W. E. C. MOORE

*Anaerobe Laboratory* and Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061*

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Data are presented on the distribution of 101 bacterial species and subspecies among 1,442 isolates from 25 fecal specimens from three men on: (i) their normal diet and normal living conditions, (ii) normal living conditions but eating the controlled metabolic diet designed for use in the Skylab simulation and missions, and (iii) the Skylab diet in simulated Skylab (isolation) conditions. These bacteria represent the most numerous kinds in the fecal flora. Analyses of the kinds of bacteria from each astronaut during the 5-month period showed more variation in the composition of the flora among the individual astronauts than among the eight or nine samples from each person. This observation indicates that the variations in fecal flora reported previously, but based on the study of only one specimen from each person, more certainly reflect real differences (and not daily variation) in the types of bacteria maintained by individual people. The proportions of the predominant fecal species in the astronauts were similar to those reported earlier from a Japanese-Hawaiian population and were generally insensitive to changes from the normal North American diet to the Skylab diet; only two of the most common species were affected by changes in diet. However, one of the predominant species *(Bacteroides fragilis* subsp. *thetaiotaomicron)* appeared to be affected during confinement of the men in the Skylab test chamber. Evidence is presented suggesting that an anger stress situation may have been responsible for the increase of this species simultaneously in all of the subjects studied. Phenotypic characteristics of some of the less common isolates are given. The statistical analyses used in interpretation of the results are discussed.

This is the second of a series of reports concerning human fecal bacterial flora and the factors that may, or may not, affect the flora. In a previous work (11), we reported the kinds of bacteria predominant in the feces of 20 normal individuals, each sampled once. Work on the National Aeronautics and Space Administration's Skylab Medical Experiments Altitude Test (SMEAT) provided an opportunity for us to examine the bacterial flora of several fecal samples from each of three individuals under normal and test (controlled balanced diet and isolation environment) conditions, which should enable us to determine how well a single specimen (as examined previously) represents the typical flora of an individual and to evaluate the effects of the controlled, balanced, Skylab diet and/or an isolation environment on the qualitative and quantitative composition of the flora. In addition, we hope to ascertain whether or not the floras from the normal Japanese-Hawaiian population studied previously could be considered the same as or similar to the bacterial flora of normal North Americans.

The fecal microbiology reported here was one of many physiological studies on the three SMEAT crew members. A comprehensive description of the conditions and parameters of the SMEAT experimentation is published elsewhere (12).

## MATERIALS AND METHODS

**Subjects.** The Caucasian male astronauts are called "R" (Commander, age 35), "T" (Scientist-Pilot, age 43), and "B" (Pilot, age 35).

**Fecal specimens.** To estimate the normal fecal flora of the individuals, fecal specimens were analyzed from each man on days 43, 28, and 1 before they started eating the Skylab diet and while they were working on flight training and living at home. To estimate the effect of the Skylab diet per se on the flora, a fourth sample was obtained from each man 22 days after the Skylab diet had been initiated but while the men were still living at home. Three samples, obtained after 15, 30, and 56 days of confine-

ment in the test chamber while the Skylab diet was still being consumed, were studied to evaluate the combined effect of Skylab diet and environment on the fecal flora. An eighth specimen was obtained from each man 14 days after egress from the chamber and return to normal living conditions, except that the Skylab diet was still being consumed. A ninth specimen was obtained from one of the men (T) 24 days after he returned to his normal diet and normal living conditions.

**Chamber conditions.** In the chamber, the three men were under reduced atmospheric pressure (5 lb/in$^2$) and an increased oxygen concentration of 70% oxygen-30% nitrogen. The men had no direct or personal contact with the outside environment. Materials were exchanged through air locks and there was strict adherence to the dietary regimen.

**Skylab diet.** The astronaut diet contained a considerable variety of foods, which were prepared for long-term storage. All foods were canned, frozen, irradiated, or dehydrated. The actual menu items differed for each man at each meal but in general represented the variety and types of food that would probably be consumed normally. Details of the diets can be found in the complete report of the SMEAT studies (12).

**Bacteriological studies.** Fecal specimens were collected in plastic bags with no precautions to exclude air and were transported to the laboratory with as little delay as possible. However, at times up to several hours elapsed before processing could be initiated. On receipt in the laboratory, each plastic bag was thoroughly flushed with oxygen-free carbon dioxide. The specimen was thoroughly mixed and cultured exactly as described previously (11) by the same persons who processed the specimens in the earlier study.

After overnight incubation, the rumen fluid-glucose-cellobiose agar roll-tube cultures were transported by air (in the passenger cabin) at ambient temperature from Houston, Tex., to Blacksburg, Va., for reincubation. After 5 days' total incubation time, 55 colonies were picked in a randomized manner from each specimen. Cultural counts, microscopic counts, determination of the percentage of dry matter of the fecal specimens, and the identification of all isolates were each performed as described previously (7, 8, 11).

**Statistical analyses. (i) Cultural and microscopic counts.** The cultural counts were compared with the direct microscopic clump counts (DMCC) by using a paired $t$ test. This test was made more accurate by means of a square-root transformation, which makes the distribution of the counts more closely normal as can be seen by plotting the data.

**(ii) Analyses of variance of predominant species.** Because the observed data indicated that the distribution of the species was neither normal nor Poisson, the statistical analyses of the data in Table 4 were performed on a transformation of the values to give approximately normal distributions with approximately equal variances. This transformed value is equal to the square root of [(observed value/2.1) + (3/8)]. This transformation was used because the ratio of the variance to the mean for the normal diet days is 2.1, and it results in a more conservative test than would be produced with the observed values. By using [$\sqrt{(x + 3/8)}$], one obtains a known normalizing and variance-standardizing transformation for Poisson variables (1) for which the variance is, of course, equal to the mean (also see Appendix [b]).

## RESULTS

**Cultural and microscopic counts.** The cultural counts and DMCC per gram of dry matter of the 25 specimens are given in Table 1. The average values for the 25 specimens were: dry matter, 31%; DMCC, $4.08 \times 10^{11}$/g of dry matter (standard deviation = $2.73 \times 10^{11}$); cultural count, $2.56 \times 10^{11}$/g of dry matter (standard deviation = $1.14 \times 10^{11}$); for an average cultural recovery of 63.2% of the bacterial cells observed microscopically. The paired $t$ test, using a square-root transformation, gives a single tail-area probability of 0.008 ($t$ = 2.566 with 24 df). (Without the square-root transformation, $t$ = 2.717, and hence a single tail-area probability of about 0.006. The single tail is justified because it is more likely in advance that the cultural count would be lower than the DMCC.) Therefore, it is probable that the cultural count is low. Neither the DMCC nor the cultural counts depend significantly on the treatments tested.

**Composition of the flora.** The 101 different species and subspecies observed among 1,442 isolates from all 25 specimens are listed, in descending order of their frequency (rank order), in Table 2. These 101 species account for 95 to 98% of the cultivable flora as determined by two different analyses: (i) by the sum of the frequencies calculated by formula $H_9$ of Good (4), and (ii) by the coverage as determined by "1 - number of species observed once/total number of isolates" (also see Appendix [a]).

**Comparison of astronaut normal flora with that of the Japanese-Hawaiian population.** The frequencies of bacterial species from the three astronauts (three or four samples each) living under normal conditions are compared with those previously reported from the Japanese-Hawaiian population (single fecal specimens from each of 20 individuals) in Table 3. In general, the rank order of species bears similarity to that for the Japanese-Hawaiian population, but, because of the large person-to-person variation noted in both groups, one cannot determine with certainty whether the three astronauts and the Japanese-Hawaiians belong to the same population with respect to their fecal flora.

**Analyses of variance of predominant species.** The numbers of isolates of predominant

TABLE 1. *Microscopic and cultural counts per gram of dry matter from 25 fecal specimens*

| Subject | Parameter | Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal diet | | | Skylab diet | | | | | Normal diet |
| | | Ambulatory | | | | In chamber | | | Ambulatory | |
| | | May 10[a] | May 25 | June 22 | July 20 | Aug. 9 | Aug. 24 | Sept. 19 | Oct. 4 | Nov. 1 |
| B | % DM[b] | 30.8 | 27.7 | 27.7 | 30.2 | 30.6 | 33.9 | 36.0 | 30.9 | |
| | DMCC[c] | 4.2 | 3.8 | 4.2 | 2.8 | 10.8 | 1.7 | 2.4 | 1.7 | |
| | Cult.[d] | 2.4 | 2.2 | 5.2 | 3.2 | 2.2 | 1.8 | 1.0 | 2.0 | |
| R | % DM | 27.6 | 35.0 | 15.7 | 30.9 | 33.2 | 32.6 | 27.0 | 28.2 | |
| | DMCC | 8.9 | 2.9 | 3.6 | 2.1 | 8.5 | 1.4 | 3.0 | 8.7 | |
| | Cult. | 2.4 | 1.0 | 3.6 | 1.6 | 1.8 | 3.6 | 2.7 | 5.4 | |
| T | % DM | 34.3 | 26.4 | 29.6 | 34.1 | 32.4 | 33.1[e] | 38.6 | 35.7 | 33.7 |
| | DMCC | 5.7 | 3.7 | 1.6 | 2.0 | 5.7 | 1.2 | 1.1 | 6.7 | 3.6 |
| | Cult. | 2.5 | 3.3 | 3.4 | 1.5 | 1.5 | 2.3 | 2.6 | 3.9 | 1.6 |

[a] Sample date ± 1 day. Treatment changes were made as follows: From normal diet to Skylab diet, June 27; from ambulatory to chamber, July 25; from chamber to ambulatory, September 20; from Skylab diet to normal diet, October 8.

[b] Percent dry matter (DM).

[c] Number given times $10^{11}$ = direct microscopic clump count (DMCC) per gram of dry matter. Average DMCC = $4.08 \times 10^{11}$/g of dry matter; standard deviation (s) = $2.73 \times 10^{11}$.

[d] Number given times $10^{11}$ = cultural count per gram of dry matter. Average cultural count = $2.56 \times 10^{11}$/g of dry matter; s = $1.14 \times 10^{11}$.

[e] Estimated value from average for subject T. There was insufficient sample for direct determination.

species from the 25 individual astronaut fecal specimens and the significance of the variation are given in Table 4. The values given for the number of isolates of a species are the actual numbers of isolates from each specimen. For convenience in the statistical analyses, no correction was made for the variation among these totals. Results of analysis of variance obtained with the transformed data were similar to those obtained using the untransformed data, which shows that the analysis is robust relative to the statistical model.

Of the 15 predominant species analyzed, 7 showed significant person-person variation, 4 showed significant treatment-treatment variation, and 1 showed person-treatment interaction. No significant variation was noted with 6 of the 15 species analyzed.

## DISCUSSION

**Cultural and microscopic counts.** In the present study, the average cultural recovery was 63% of the DMCC, whereas in the study reported previously (11) it was 93% of the DMCC. The only known procedural difference between the two studies was a delay in transporting the specimens to the laboratory in the present study. Delays were unavoidable because the men were in different locations at the Manned Space Flight Center during the non-chamber phase of the study and because of logistics for taking materials through the air locks during the chamber portion of the study. The average percentage of dry matter was 10% higher in the present specimens than in those examined previously, but the water content was still 61.4 to 84.3% (Table 1). Although we do not know the effect of the moisture content on the viability of the bacteria, we postulate that the prolonged exposure to oxygen was the major factor affecting the viable bacterial count.

**Comparison of astronaut normal flora with that of the Japanese-Hawaiian population.** Gross examination of the data in Table 3 shows that there is a general similarity of major bacterial types found in the normal samples from the astronauts and in the Japanese-Hawaiian population. The variation of the bacterial flora among the individual astronauts is also shown in Table 3. To determine whether the astronauts' flora differed from that of the Japanese-Hawaiians, we compared the relative frequency of species (in rank order, with "1" being the most common) in the normal samples from the astronauts with the rank order of species in each of the Japanese-Hawaiians. When the rank order of species obtained from the normal diet specimens from each astronaut was compared with the 20 rank orders of species from the individual Japanese-Hawaiian specimens, no two specimens were alike. Indeed, even if one compared only the three most numerous species in each sample, or from one individual, no two people or specimens had the same species in the same rank order. If one compared

Table 2. *Relative frequency of bacterial species of the fecal flora of 3 North American men, each sampled 8 or 9 times during a 5-month period, on normal diet, astronaut diet, and in land-based Skylab chamber (1442 isolates)*

| Rank | No. isol. | Count[a] | % of flora[b] | Organism(s) |
|---|---|---|---|---|
| | | (x $10^{10}$) | | |
| 1 | 171 | 3.04(.23) | 11.8(.90) | *Bacteroides fragilis* ss. *vulgatus* |
| 2 | 144 | 2.56(.21) | 9.9(.83) | *Eubacterium aerofaciens* |
| 3 | 129 | 2.29(.20) | 8.9(.78) | *Bacteroides fragilis* ss. *thetaiotaomicron* |
| 4 | 96 | 1.70(.17) | 6.6(.68) | *Peptostreptococcus productus* II |
| 5 | 87 | 1.54(.17) | 6.0(.64) | *Bacteroides fragilis* ss. *distasonis* |
| 6 | 64 | 1.13(.14) | 4.4(.55) | *Fusobacterium prausnitzii* |
| | | (x $10^9$) | | |
| 7 | 51 | 8.95(1.3) | 3.5(.49) | *Coprococcus eutactus* |
| 8 | 44 | 7.70(1.2) | 3.0(.45) | *Eubacterium aerofaciens* III |
| 9 | 41 | 7.17(1.1) | 2.8(.44) | *Peptostreptococcus productus* I |
| 10 | 40 | 6.99(1.1) | 2.7(.43) | *Ruminococcus bromii* |
| 11 | 39 | 6.81(1.1) | 2.6(.43) | *Bifidobacterium adolescentis* |
| 12-13[c] | 33 | 5.74(1.0) | 2.2(.39) | *Gemmiger formicilis*, *Bifidobacterium longum* |
| 14 | 31 | 5.38(1.0) | 2.1(.38) | *Eubacterium siraeum* |
| 15 | 27 | 4.66(.09) | 1.8(.35) | *Ruminococcus torques* |
| 16 | 25 | 4.31(.09) | 1.7(.34) | *Eubacterium rectale* III-H |
| 17-18 | 24 | 4.13(.09) | 1.6(.33) | *Eubacterium rectale* IV, *Eubacterium eligens* |
| 19 | 22 | 3.77(.08) | 1.5(.32) | *Bacteroides eggerthii* |
| 20 | 21 | 3.59(.08) | 1.4(.31) | *Clostridium leptum* |
| 21 | 19 | 3.24(.08) | 1.3(.29) | *Bacteroides fragilis* ss. a |
| 22 | 18 | 3.06(.07) | 1.2(.29) | *Eubacterium biforme* |
| 23 | 14 | 2.35(.06) | .91(.25) | *Bifidobacterium infantis* |
| 24 | 13 | 2.17(.06) | .84(.24) | *Eubacterium rectale* III-F |
| 25-26 | 9 | 1.46(.05) | .57(.20) | *Coprococcus comes*, *Bacteroides capillosus* |
| 27-31 | 8 | 1.29(.05) | .50(.18) | *Ruminococcus albus*, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum* I, *Fusobacterium russii* |
| 32-35 | 7 | 1.11(.04) | .43(.17) | *Ruminococcus obeum*, *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmannii* |
| | | (x $10^8$) | | |
| 36-37 | 6 | 9.39(.40) | .36(.16) | *Ruminococcus callidus*, *Butyrivibrio crossotus* |
| 38-41 | 5 | 7.67(.36) | .30(.14) | *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides fragilis* ss. *fragilis*, *Bacteroides* AR |
| 42-47 | 4 | 5.96(.32) | .23(.12) | *Coprococcus catus*, *Eubacterium hadrum*, *E. cylindroides*, *E. ruminantium*, *Eubacterium* CH-1, *Staphylococcus epidermidis* |
| 48-57 | 3 | 4.29(.27) | .17(.10) | *Peptostreptococcus* BL, *Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides* L, *Fusobacterium mortiferum* I, *F. naviforme*, *Clostridium innocuum*, *C. ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens* |
| 58-73 | 2 | 2.68(.21) | .10(.08) | *Ruminococcus* AT, *Peptococcus* AU-1, *Eubacterium* AG, -AK, -AL, -AL-1, -AN; *Bacteroides fragilis* ss. *ovatus*, -ss. d, -ss. f; *Bacteroides* L-1, L-5; *Fusobacterium nucleatum*, *F. mortiferum*, *Escherichia coli*, *Streptococcus morbillorum* |
| 74-101 | 1 | 1.19(.13) | .05(.05) | *Peptococcus magnus*, *Peptococcus* G, -AU-2; *Streptococcus intermedius*, *Ruminococcus lactaris*, *Ruminococcus* CO, *Gemmiger* X, *Coprococcus* BH, -CC; *Eubacterium tenue*, *Eubacterium ramulus*, *Eubacterium* AE, -AG-H, -AG-M, -AJ, -BW-1; *Bacteroides clostridiiformis* ss. *clostridiiformis*, *B. coagulans*, *B. oralis*, *B. ruminicola* ss. *brevis*, -ss. *ruminicola*, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides* L-4, -W-1; *Fusobacterium* H, *Lactobacillus* G, *Succinivibrio* A |

[a] The estimated count per gram of fecal dry matter (the standard deviation of the estimate is given in parentheses).

[b] The percentage of the fecal population (the standard deviation of the estimate is given in parentheses).

[c] Where two rank numbers are listed, each organism cited was detected with equal frequency.

Table 3. *Frequency of bacterial species in feces of different individuals under normal conditions*

**Person B — Three normal specimens** (20 J-H[a])

| No. isol. | Species | Isol. / persons |
|---|---|---|
| 30 | *E. aerofaciens* | 70/13 |
| 20 | *B. fragilis* ss. *vulgatus* | 140/19 |
| 18 | *P. productus* II | 51/17 |
| 11 | *B. fragilis* ss. *thetaiotaomicron* | 52/16 |
| 8 | *E. rectale* III-H | 5/2 |
| 7 | *F. prausnitzii* | 80/14 |
| 7 | *C. eutactus* | 5/3 |
| 6 | *R. torques* | 9/5 |
| 5 | *P. productus* I | 39/15 |
| 5 | *B. fragilis* ss. a | 26/9 |
| 5 | *B. fragilis* ss. *distasonis* | 28/11 |
| 3 | *G. formicilis* | 21/8 |
| 3 | *Ruminococcus obeum* | 14/9 |
| 3 | *B. eggerthii* | 1/1 |
| 3 | *B. adolescentis* | 75/14 |
| 3 | *L. leichmannii* | 6/5 |
| 2 | *R. albus* | 13/6 |
| 2 | *R. callidus* | 5/3 |
| 2 | *R. flavefaciens* | 0/0 |
| 2 | *E. formicigenerans* | 4/4 |
| 2 | *Eubacterium hadrum* | 0/0 |
| 2 | *Bacteroides* L-1 | 1/1 |
| 2 | *B. longum* | 22/7 |
| 2 | *B. capillosus* | 2/2 |
| 1 | *C. comes* | 6/4 |
| 1 | *P. magnus* | 0/0 |
| 1 | *Peptostrep.* BL | 0/0 |
| 1 | *E. aerofaciens* III | 29/9 |
| 1 | *E. hallii* | 1/1 |
| 1 | *E. rectale* II | 27/11 |
| 1 | *Eubacterium* AG | 2/2 |
| 1 | *Eubacterium* AG-M | 0/0 |
| 1 | *B. coagulans* | 0/0 |
| 1 | *B. fragilis* ss. *fragilis* | 8/4 |
| 1 | *Bacteroides* AR | 0/0 |
| 1 | *F. naviforme* | 0/0 |
| 1 | *F. russii* | 4/2 |

**Person R — Three normal specimens** (20 J-H)

| No. isol. | Species | Isol. / persons |
|---|---|---|
| 23 | *E. aerofaciens* | 70/13 |
| 20 | *R. bromii* | 17/9 |
| 17 | *B. fragilis* ss. *vulgatus* | 140/19 |
| 11 | *B. adolescentis* | 75/14 |
| 11 | *B. longum* | 22/7 |
| 10 | *B. fragilis* ss. *distasonis* | 28/11 |
| 7 | *P. productus* II | 51/17 |
| 6 | *B. fragilis* ss. *thetaiotaomicron* | 52/16 |
| 5 | *F. prausnitzii* | 80/14 |
| 4 | *Eubacterium* CH-1 | 0/0 |
| 4 | *Ruminococcus obeum* | 14/9[b] |
| 3 | *E. rectale* III-H | 5/2 |
| 3 | *P. productus* I | 39/15 |
| 3 | *R. albus* | 13/6 |
| 3 | *B. praeacutus* | 0/0 |
| 2 | *R. torques* | 9/5 |
| 2 | *E. aerofaciens* III | 70/13 |
| 2 | *E. formicigenerans* | 4/4 |
| 2 | *Eubacterium* AL-1 | 0/0 |
| 2 | *B. capillosus* | 2/2 |
| 2 | *Bacteroides* L | 4/3 |
| 2 | *F. russii* | 4/2 |
| 2 | *L. leichmannii* | 6/5 |
| 1 | *C. catus* | 6/3 |
| 1 | *G. formicilis* | 21/8 |
| 1 | *Ruminococcus lactaris* | 0/0 |
| 1 | *Ruminococcus* AT | 0/0 |
| 1 | *E. limosum* | 1/1 |
| 1 | *E. rectale* III-F | 10/6 |
| 1 | *Eubacterium siraeum* | 0/0 |
| 1 | *B. fragilis* ss. f | 3/3 |
| 1 | *B. oralis* | 0/0 |
| 1 | *Bacteroides* L-4 | 0/0 |
| 1 | *Bacteroides* L-5 | 0/0 |
| 1 | *F. mortiferum* I | 0/0 |
| 1 | *Clostridium leptum* | 0/0 |
| 1 | *B. infantis* | 18/4 |
| 1 | *Lactobacillus* G | 0/0 |
| 1 | *S. epidermidis* | 2/2 |

**Person T — Four normal specimens** (20 J-H)

| No. isol. | Species | Isol. / persons |
|---|---|---|
| 35 | *B. fragilis* ss. *vulgatus* | 140/19 |
| 29 | *P. productus* II | 51/17[b] |
| 16 | *F. prausnitzii* | 80/14 |
| 14 | *E. biforme* | 38/10 |
| 14 | *E. rectale* IV | 23/6 |
| 13 | *B. fragilis* ss. *thetaiotaomicron* | 52/16 |
| 12 | *C. eutactus* | 5/3 |
| 11 | *E. eligens* | 43/12 |
| 10 | *B. fragilis* ss. *distasonis* | 28/11 |
| 8 | *E. rectale* III-H | 5/2 |
| 7 | *B. eggerthii* | 1/1 |
| 6 | *Butyrivibrio crossotus* | 0/0 |
| 5 | *C. comes* | 6/4 |
| 5 | *G. formicilis* | 21/8 |
| 5 | *P. productus* I | 39/15 |
| 4 | *R. bromii* | 17/9 |
| 4 | *E. aerofaciens* III | 29/9 |
| 3 | *E. aerofaciens* | 70/13 |
| 2 | *R. callidus* | 5/3 |
| 2 | *R. torques* | 9/5 |
| 2 | *Peptococcus* AU-1 | 0/0 |
| 2 | *E. formicigenerans* | 4/4 |
| 2 | *Eubacterium hadrum* | 0/0 |
| 2 | *F. russii* | 4/2 |
| 2 | *C. ramosum* | 3/1 |
| 2 | *P. acnes* | 3/2 |
| 1 | *C. catus* | 6/3 |
| 1 | *R. flavefaciens* | 0/0 |
| 1 | *Ruminococcus* CO | 0/0 |
| 1 | *E. hallii* | 1/1 |
| 1 | *E. rectale* II | 27/11 |
| 1 | *E. ruminantium* | 2/2 |
| 1 | *E. tenue* | 0/0 |
| 1 | *E. ventriosum* I | 0/0 |
| 1 | *Eubacterium siraeum* | 0/0 |
| 1 | *Eubacterium* AE | 1/1 |
| 1 | *Eubacterium* AJ | 0/0 |
| 1 | *Eubacterium* AK | 4/3 |
| 1 | *Eubacterium* AL | 0/0 |
| 1 | *B. fragilis* ss. *fragilis* | 8/4 |
| 1 | *B. fragilis* ss. d | 5/4 |
| 1 | *B. ruminicola* ss. *brevis* | 2/1 |
| 1 | *B.* " ss. *ruminicola* | 0/0 |
| 1 | *Bacteroides* AR | 0/0 |
| 1 | *Succinivibrio* A | 0/0 |
| 1 | *F. naviforme* | 0/0 |
| 1 | *F. nucleatum* | 0/0 |
| 1 | *Fusobacterium* H | 0/0 |
| 1 | *B. longum* | 22/7 |
| 1 | *L. leichmannii* | 6/5 |
| 1 | *S. epidermidis* | 2/2 |

[a] Number of isolates/number of persons from which this species was isolated in the Japanese-Hawaiian population of 20 people (11).

[b] *Ruminococcus obeum* was not differentiated from *Peptostreptococcus productus* II in our report of the Japanese-Hawaiian population (11).

TABLE 4. *Numbers of isolates of predominant species from individual specimens*

| Species | Treatment | | | | | | | | | $P^a$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal diet | | | Astronaut diet | | | Astronaut diet in chamber | | | |
| | B[b] | R | T | B | R | T | B | R | T | |
| *B. fragilis* subsp. *vulgatus* | 5 10 5 | 10 4 3 | 11 6 8 10 | 7 3 | 1 3 | 12 7 | 5 4 6 | 8 6 3 | 14 17 3 | *P |
| *E. aerofaciens* | 14 8 8 | 10 13 - | - 3 - - | 15 16 | 2 4 | - 1 | 15 13 12 | 1 3 1 | 2 2 1 | ***P |
| *B. fragilis* subsp. *thetaiotaomicron* | 4 5 2 | 4 1 1 | 3 4 4 2 | 1 4 | 2 3 | 7 5 | 5 19 1 | 7 12 4 | 6 13 10 | *T |
| *P. productus* II | 3 2 13 | 5 1 1 | 3 13 8 5 | 7 - | 5 5 | 6 5 | 1 - - | 1 - 2 | 2 1 7 | *T |
| *B. fragilis* subsp. *distasonis* | 1 2 2 | 2 2 6 | 2 3 1 4 | - 5 | 4 4 | 5 5 | 1 5 2 | 5 5 4 | 6 8 3 | NS |
| *F. prausnitzii* | - 2 5 | 3 1 1 | 1 3 4 8 | 3 3 | 2 7 | - 5 | - 2 3 | 1 2 5 | - 2 1 | NS |
| *C. eutactus* | 3 - 4 | - - - | 2 1 4 5 | 6 1 | - - | 3 7 | 11 - - | - - - | - 2 2 | *P |
| *E aerofaciens* III | - 1 - | 1 1 - | 1 2 1 - | 1 - | 6 4 | 5 - | - - - | 6 5 2 | 3 1 4 | **P*T |
| *P. productus* I | 2 2 1 | 1 2 - | 1 - 3 1 | 2 2 | 6 2 | - 1 | 4 1 1 | 1 4 1 | 3 - - | NS |
| *R. bromii* | - - - | 1 1 18 | - - 4 - | 1 - | 1 3 | - - | - - - | 4 1 6 | - - - | *P |
| *B. adolescentis* | 2 - 1 | 3 8 - | - - - - | - 1 | 2 - | 1 - | 2 3 2 | 3 7 - | - 4 - | NS |
| *G. formicilis* | - - 3 | 1 - - | 2 1 2 - | - - | 2 2 | 1 2 | 4 1 2 | 2 1 3 | - 2 2 | NS |
| *B. longum* | 1 1 - | 3 7 1 | - - - 1 | - - | 1 - | - - | - 7 3 | 2 4 2 | - - - | *P |
| *E siraeum* | - - - | - - 1 | - - - 1 | - - | 6 7 | - 1 | - - - | 7 7 1 | - - - | ***P**TI |
| *R. torques* | 1 3 2 | - - 2 | - - - 2 | 2 - | 1 1 | - 1 | 2 1 2 | 2 1 - | 2 - 2 | NS |
| Total no. of isolates | 50 55 61 | 54 61 47 | 49 58 56 79 | 66 54 | 59 56 | 53 57 | 57 72 50 | 55 66 51 | 55 66 55 | |

[a] *, Significant at 0.05 level; **, significant at 0.01 level; ***, significant at 0.001 level; NS, not significant; P, person; T, treatment; I, interaction.
[b] Person tested.

only the most numerous two species found in any one individual, only astronaut B and Japanese-Hawaiian specimens 10 and 22 were the same, with *Eubacterium aerofaciens* and *Bacteroides fragilis* subsp. *vulgatus* having the same rank order (Table 3; 11). The several next most frequent species were dissimilar. Therefore, because of the large person-to-person variation in the flora among both populations, we cannot say that the astronauts and the Japanese-Hawaiians do, or do not, belong to the same population with respect to their fecal flora. In these comparisons, the greatest similarity was seen in isolates from the multiple samples from individual astronauts.

**Person-to-person variation.** To determine whether the apparent person-to-person variation in composition of the flora was real or only reflected small sample sizes or simply large daily variation within individuals, an analysis of variance was performed on the 15 most numerous species in the astronaut population (Table 4). For a number of species, the frequency was reasonably constant between different specimens and also between different men. For a number of other predominant species, variation between men was significantly greater than variation between different samples within men. Indeed, *Coprococcus eutactus* did not appear at all in subject R, and this is clearly very strong evidence by itself that the flora differs from one individual to another. Therefore, these analyses indicate that there are statistical differences in the composition of the flora of the three men. Additionally, it appears virtually certain that the differences observed previously in the composition of the flora of different individuals, each sampled only once, do reflect a real difference in the floras that different persons tend to maintain and do not result from sampling problems or wide daily variation within each individual.

The analyses of variance were performed independently on the 15 most frequent species. Therefore, one or more of the differences detected could have occurred by chance alone. However, the statistical differences in the concentrations of *E. aerofaciens, C. eutactus, E. aerofaciens* II, *Ruminococcus bromii*, and *Bifidobacterium longum* are also similar to the differences among the specimens from different individuals reported previously (11). It also appears that the species responded independently of each other to person, treatment, or person-treatment interaction.

**Effect of diet change on the flora.** The analyses of data shown in Table 4 also indicate that some species were sensitive to a change from the astronauts' normal diet to the Skylab diet, whereas other intestinal species were apparently indifferent to the dietary change.

The response of *E. aerofaciens* III to treatment appeared to be related to the diet rather than to conditions in the chamber. The propor-

tions of this species increased noticeably in two of the men and reached 10.2 and 10.9% of the isolates from single specimens in one of the men (B) while on the Skylab diet. However, this bacterial species constituted 14.5 and 11.1% of the isolates from two individuals of the Japanese-Hawaiian population on their normal diets; therefore, it is unlikely that its increase was caused by unique properties of the Skylab diet per se.

*Eubacterium siraeum* also responded to the change of diet in one (R) of the two astronauts in which it was present when on normal diet. This species was not detected in the Japanese-Hawaiian population. Therefore, the significance of its response to the Skylab diet in one of the men is not known.

**Effect of conditions in the test chamber on the flora.** One species *(B. fragilis* subsp. *thetaiotaomicron)* was apparently affected by environmental changes in the test chamber. Changes in the proportion of this species in all three men are illustrated in Fig. 1. For a more analytical approach to the significance of this simultaneous increase in all three astronauts, see Appendix (c) and (f). Figure 2 shows the corresponding graphs for *Peptostreptococcus productus* II, but the effect illustrated in Fig. 2 may be mere coincidence, as is shown in Appendix (c), which takes into account the specific selection of the species.

When the increase in the proportion of *B.*

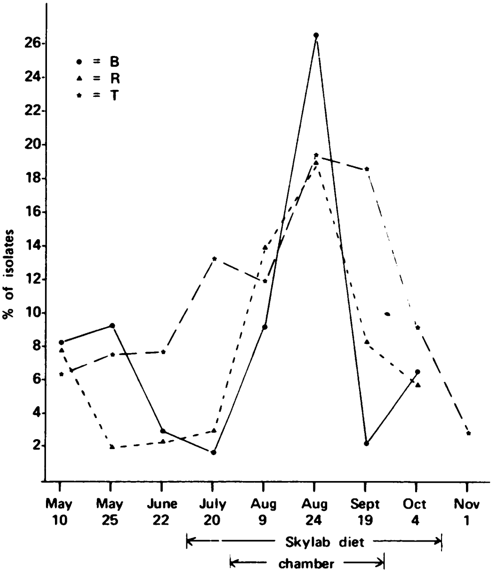


FIG. 1. *Percentage of isolates, from different fecal specimens, that were B. fragilis subsp. thetaiotaomicron.*

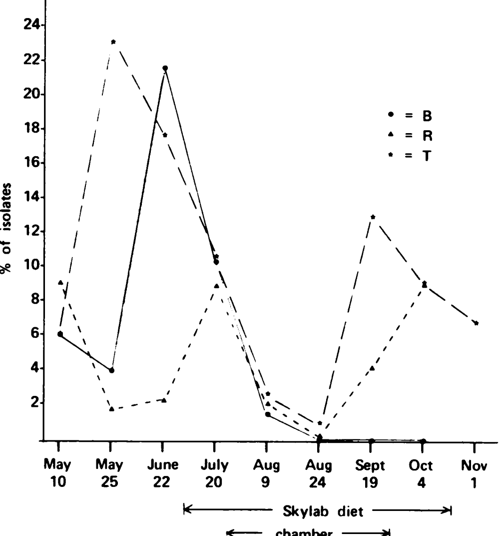


FIG. 2. *Percentage of isolates, from different fecal specimens, that were P. productus II.*

*fragilis* subsp. *thetaiotaomicron* in the chamber was first noted, we wondered whether it represented an increase in the strains normally maintained by each man or whether there was "cross contamination" due to the confined and isolated conditions in the chamber. In preliminary testing we had noted that the strains of *B. fragilis* subsp. *thetaiotaomicron* isolated from one astronaut were resistant to tetracycline at test levels (17) and those from the other two astronauts were susceptible. Therefore, representative strains of this species from each man were tested by broth disk analyses (17) for their resistance to tetracycline. Throughout the trial, the tested strains from one man were resistant and those from the other two men were susceptible, indicating that there was a proliferation of each individual's own strains rather than cross contamination between the men in close confinement.

The rise in the proportion of *B. fragilis* subsp. *thetaiotaomicron* did not correlate with chamber conditions per se, since there was a simultaneous decrease in this species while the men were still in the chamber. The only factor that appeared to correspond with both the simultaneous increases and decreases in the proportion of this species is that the proportion increased in response to an anger stress situation that developed during the test and decreased as that situation was reconciled. This would indeed be a very hypothetical and tenuous surmise if a similar change in flora had not been independently noted by another investigator and also by

us in two other studies. Because of the potential significance of this observation, and of the various inherent problems in defining "stress," we wish to pursue this point in some detail and cite three other apparently similar cases.

**Effect of stress on the flora.** Soon after we observed the high level of *B. fragilis* subsp. *thetaiotaomicron* in the three men simultaneously, we observed a similar high value from one of five other persons who were participating in a different diet study (Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, G109, p. 44). At the time (March, 1973) that this person had a high level of *B. fragilis* subsp. *thetaiotaomicron* (Table 5), she was experiencing a strong personality conflict with her co-workers and was under consideration for dismissal. Subsequently the problems resolved and the proportion of this species was lower in later specimens. The statistical significance of 11 strains of *B. fragilis* subsp. *thetaiotaomicron* of 57 isolates in the second specimen (3/5/73) can be described as having a tail-area probability of 1:16, as presented in more detail in the Appendix (d).

Coincident observations of the high proportion of *B. fragilis* subsp. *thetaiotaomicron* in some specimens from the astronauts and in one specimen from the person in the diet study suggested that stress might influence the proportion of this species in fecal specimens. To test this hypothesis, specimens were obtained from graduate students on the day of their preliminary oral examinations (a stress time) and again 2 weeks later. There was no significant difference in the proportion of *B. fragilis* subsp. *thetaiotaomicron* isolated from the specimens obtained the day of examination and those obtained later (Table 6), and the relative numbers of the isolates of this species from the graduate students were within the range observed for most people. Clearly, stress per se did not increase the levels of *B. fragilis* subsp. *thetaiotaomicron* in these individuals. We realized, in retrospect, that the stress experienced by the students was of a different nature from that of the astronauts and of the person on the diet study. It appeared that an anger stress situation might be the correlating factor. The cases cited below are in support of this hypothesis.

TABLE 5. *Proportion of B. fragilis subsp. thetaiotaomicron in fecal specimens from one individual*[a]

| Date | No. of *B. fragilis* subsp. *thetaiotaomicron*/total no. of isolates | % of isolates |
|---|---|---|
| 1/10/73 | 0/60 | 0.0 |
| 3/05/73 | 11/57 | 19.4 |
| 5/22/73 | 6/59 | 10.2 |
| 9/17/73 | 6/57 | 10.5 |

[a] Only normal diet is considered in this table.

TABLE 6. *Proportion of B. fragilis subsp. thetaiotaomicron in fecal samples from three graduate students*

| Subject | No. of isolates of *B. fragilis* subsp. *thetaiotaomicron*/total no. of isolates | |
|---|---|---|
| | Day of exam | Ca. 2 weeks later |
| S-09 | 2/53 | 2/52 |
| S-10 | 1/61 | 0/58 |
| S-11 | 3/63 | Not tested |

Recently we found high levels of *B. fragilis* subsp. *thetaiotaomicron* in the intestinal tract of a 19-year-old-woman who was shot by her husband who, in turn, committed suicide. In this person the proportions of this species were: ascending colon, $^{17}/_{51}$ (33%); transverse colon, $^{8}/_{50}$ (16%); descending colon, $^{4}/_{57}$ (7%); rectum, $^{5}/_{55}$ (9%). Since the proportions of observed species in these different areas of the tract were remarkably uniform in other individuals (Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, D57, p. 61), the high proportion of *B. fragilis* subsp. *thetaiotaomicron* in the ascending colon of this woman suggests a rather rapid response to probable anger or fear stress for a period previous to death. The statistical significance of the high level in the ascending colon can be described as having a single-tailed probability of 1:37 (details of the analysis are presented in Appendix [e]).

More recently, Bruce Maier, University of Missouri, in an independent study, observed a continual increase in the proportion of *B. fragilis* subsp. *thetaiotaomicron* in feces from 12 individuals who were fed a monotonous diet for 60 days (personal communication). These individuals became extremely angry and argumentative with the test supervisors concerning the conditions of the test and the requirement to consume the exact same menu day after day. The rise in *B. fragilis* subsp. *thetaiotaomicron* was accentuated as the experiment continued through a change in diet from low meat to high meat. Dr. Maier is preparing a detailed report of the microbiology of this study for publication.

Because anger stress appeared to be a common factor in any specimens in which we noted a high relative frequency of *B. fragilis* subsp. *thetaiotaomicron,* we investigated in more detail the conditions in the NASA SMEAT trial. An understandable anger stress situation apparently did exist during the study, especially for one astronaut (T) who felt his diet had been

miscalculated (which it proved to be; he lost 19 lbs. during the study), and because of equipment failure and misunderstandings between the astronauts and the chamber support personnel. Stress aspects of this study are described in the SPT report (16): "... At no time was an angry or irritable word exchanged between crew members.... The most obviously stressful aspects of the test were those times when it appeared that data were being lost after considerable effort on the part of the crew to gather it [sic], especially if it had involved difficulty on their part. Examples of this were: continued runs with faulty gear which was apparently not being repaired; obviously erroneous lab data such as the polyethylene glycol [one capsule was taken at each meal during the study and used as a non-absorbable fecal recovery standard for nutrient balance] when many hours were expended counting pills to check that the material was correctly consumed at each meal (a nuisance in itself); or seeing incorrect data repeating that had been previously corrected by the subjects. Also stressful was the impression that arose from some situations that the crew were being used as experimental animals rather than participating investigators in an experiment. Some [outside] investigators were never able to accept or tolerate any view of the situation other than investigator-subject. There was unquestionably some polarization of 'we' (the crew) against 'them' (the outside world)...."

The flight surgeon verified that an anger stress situation did exist and indicated (personal communication) that crew discussions with responsible "outside" personnel, in which problems were recognized and assurances were given of problem solution, "if not by the end of the test, at least before Skylab actually flew," served to reassure the crew during the later phase of the test that their personal dedication was not a wasted effort. The decrease in the proportion of *B. fragilis* subsp. *thetaiotaomicron* in the feces of the astronauts appeared to coincide with the time that these assurances were given, except for subject T, whose diet was insufficient and who believed the balance trials that had established his dietary allowance had been incorrectly interpreted.

The effect of emotional stress on the intestinal health of astronauts in the NASA Manned Space Flight program was recognized as a serious consideration (13). Anger or fright, and depression, reportedly produce distinct effects upon the colon and upper gastrointestinal tract. Emotional stress affects epinephrine and gastrin secretion which, in turn, affect intestinal motility, intestinal blood flow, gastric and colonic mucin secretion, nutrient and bile derivative absorption, and possibly bile secretion. These changes are known to be related to colonic irritability and colitis, as well as to gastric and duodenal ulcers (2, 3, 9, 15). To the many among us who have experienced ulcers or colitis, it would be surprising if some important members of the colonic flora were not affected by physiological changes that result from stress due to anger or emotional conflict.

A significance test for the hypothesis that an anger stress situation tends to produce a high count for the species *B. fragilis* subsp. *thetaiotaomicron* shows a composite tail-area probability of 1:3,000 (also see Appendix [f]).

When the proportion of *B. fragilis* subsp. *thetaiotamicron* increased in the astronauts (Fig. 1), the proportion of *P. productus* II decreased (Fig. 2). There was no obvious in vitro interrelationship between the strains of these two species when cross streaked on agar plates. Thus, they appear to have responded independently to changes in the host intestinal physiology that may have occurred during anger stress. Concurrent low proportions of *P. productus* II were not observed in the two other anger stress cases we have examined. This observation and the simultaneous occurrence of relatively high proportions of both species in astronaut T on July 20 and September 19 (Fig. 1 and 2) further indicate that the responses of *B. fragilis* subsp. *thetaiotaomicron* and *P. productus* II are independent.

Currently there is no evidence concerning any possible medical significance of the changes in fecal flora observed during the SMEAT trials.

**Identification of fecal isolates.** Many strains isolated in the studies of human fecal flora do not have characteristics that correspond to those of any described species. Although it is legal to describe a new species on the basis of a single strain, we believe that this practice has caused much taxonomic confusion. Therefore we prefer not to assign full taxonomic recognition to those less frequent species of which we have only a few strains. However, we are proposing full taxonomic recognition for those species for which we have at least nine isolates from at least six different persons. Descriptions of eight new species (*Desulfomonas pigra*, *Clostridium leptum*, *Eubacterium hadrum*, *Eubacterium ramulus*, *Eubacterium siraeum*, *Ruminococcus lactaris*, *Ruminococcus obeum*, and *Butyrivibrio crossotus*) that are cited in the work will be published elsewhere (W. E. C. Moore, J. L. Johnson, and L. V.

Holdeman, Int. J. Syst. Bacteriol., in press). Characteristics of other recently described species cited in this publication can be found in references 5, 7, and 10.

Also cited in this study are (i) phenotypic groups within recognized species and (ii) species for which we have too few strains to warrant full taxonomic recognition. Criteria used to differentiate among these strains are presented below.

**(i) New phenotypic groups within recognized species.** Among some species, the recognition of subspecies or biotypes on the basis of distinct clusters of strains within the species has proved to be of value. In *B. fragilis*, subspecies designated on this basis have since been shown to be genetically distinct entities (6) that should merit species rank. In this work we have recognized distinct clusters within species. Some were described previously (11). The clusters of strains within species that were determined in the present study, but not described previously, are given below.

*Fusobacterium mortiferum* I: The biochemical activities of this organism are similar to those described for *F. mortiferum*. However, no growth is produced on the surface of blood agar (BAP) or supplemented brain heart infusion agar plates incubated in anaerobe jars, cellular morphology is generally uniform, and little or no gas and no hydrogen is produced. Known strains of *F. mortiferum* usually exhibit extreme pleomorphism, especially from growth on BAP, and produce copious gas with more than 2% hydrogen in the head space.

*Eubacterium ventriosum* I: The species *E. ventriosum* and *E. ruminantium* have similar acid products. We have differentiated *E. ruminantium* from *E. ventriosum* on the basis of hydrogen production in at least small amounts by strains of *E. ventriosum*. *E. ventriosum* appears to contain three groups of strains which we currently refer to as *E. ventriosum*, *E. ventriosum* I, and *E. ventriosum* II. These groups are differentiated according to the characteristics listed in Table 7.

**(ii) Characteristics of strains that do not belong to described species.** We have assigned letter designations within the appropriate genus to those strains that do not have characteristics of any described species and of which we have only a few representatives. Some of these were described previously (11). Characteristics of other infrequently isolated species are given in Tables 8 and 9 and in the descriptions below.

None of these species grew aerobically on BAP, grew in a candle jar, produced detectable catalase, or reduced nitrate. Unless specifically stated below, they did not grow in 6.5% NaCl, produce propionate from threonine, or utilize lactate. Unless otherwise noted, subsurface colony descriptions are from rumen fluid-glucose-cellobiose agar cultures and surface colony descriptions are from cultures on BAP incubated in an anaerobe jar and from cultures on brain heart infusion agar roll streak tubes (8).

TABLE 7. *Differential reactions of Eubacterium ventriosum subgroups*

| Substrate | Subgroup[a] | | |
|---|---|---|---|
| | *E. ventriosum* | *E. ventriosum* I | *E. ventriosum* II |
| Amygdalin | – | –A | A |
| Arabinose | – | –A | – |
| Cellobiose | – | A | w |
| Mannose | A– | –w | A |
| Melibiose | – | Aw | – |

[a] A, Acid reaction, pH below 5.5; –, negative reaction; w, weak reaction, pH between 5.5 and 6.0.

TABLE 8. *Differential reactions of some fermentative, sucrose-negative, indole-positive species of the genus Bacteroides*

| Test | *Bacteroides* sp.[a] | | | | |
|---|---|---|---|---|---|
| | *B. eggerthii* | *Bacteroides* AR | *B. splanchnicus* | *Bacteroides* W-1 | *Bacteroides* W-2 |
| Arabinose | Aw | – | A | A | A |
| Cellobiose | Aw | –w | – | – | A |
| Glycogen | A– | –A | –A | A | – |
| Maltose | A | wA | – | A | A |
| Mannose | A | wA | A | A | – |
| Rhamnose | Aw | –w | – | – | A |
| Starch pH | A | v | – | Aw | A |
| Starch hydrolysis | + | v | – | + | + |
| Gelatin | v | + | v | + | – |
| Hydrogen | 1 | 1 | 2,3 | 2,4 | – |
| Bile growth | 3,1 | 4,1 | 4 | 4 | –1 |

[a] A, Acid reaction, pH below 5.5; w, weak reaction, pH between 5.5 and 6.0; –, negative reaction; +, positive reaction. Numbers, hydrogen production on "– to 4+" scale; growth in bile on "– to 4+" scale.

**Coprococcus.** *Coprococcus* BH: Subsurface colonies are 1 mm and translucent with a rhizoid center. Surface colonies are 0.5 to 2 mm, buff, flat with opaque or spotted centers or raised with entire to slightly irregular edges. Glucose broth (peptone-yeast extract-glucose [PYG]) cultures are turbid with white flocculent sediment and a pH of 4.4 to 4.9 in 5 days. Acetylmethylcarbinol is not produced. Lactate

is converted to acetate and butyrate. Threonine is converted to propionate. Growth is enhanced by Tween 80 and by heme. Moderate growth is sometimes produced in the absence of fermentable carbohydrate. Production of formic acid, fermentation of melezitose, and hydrolysis of starch differentiate this species from *C. comes*. Fermentation of arabinose, mannitol, and sorbitol differentiate this species from *C. eutactus*.

*Coprococcus* CC: Subsurface colonies are 0.2 to 0.5 mm, lenticular to raspberry shaped, translucent to transparent. Surface colonies are 0.5 to 1 mm, circular, entire, convex, translucent to opaque, glistening, and white to yellow. PYG cultures have smooth white sediment without turbidity and a pH of 5.8 to 5.9 in 1 to 5 days. The pH in fructose or inositol broth is 4.6 to 5.4 in 5 days. Production of indole and fermentation of fructose, glucose, and inositol differentiate this species from others in the genus.

**Gemmiger.** *Gemmiger* X: Subsurface colonies are 0.5 mm, tan, lenticular, and translucent to opaque. No growth was produced on the surface of BAP or brain heart infusion agar plates incubated in anaerobe jars. In sweet E (8) agar streak tubes, surface colonies were 2.0 mm, fried-egg shaped, opaque, white, and smooth. Maltose broth cultures have ropy sediment without turbidity in 4 days. Growth is usually best in maltose and occasionally is moderate in cellobiose, arabinose, or another carbohydrate broth. However, most carbohydrate media have little or no growth. Little or no growth was produced in 6.5% NaCl. This species appears to differ from *Gemmiger formicilis* in producing poorer growth. Growth is not stimulated by rumen fluid or Tween 80, and no detectable formic acid is produced. The cellular morphology of the two species is similar.

**Peptococcus.** *Peptococcus* G: Subsurface colonies are 0.5 mm, white, translucent and lenticular. Surface colonies are pinpoint to 1 mm, circular, entire, convex, glistening, and white. PYG cultures are turbid with a smooth sediment and a pH of 5.0 to 5.4 in 4 to 5 days. The carbohydrates fermented and production of isobutyric and isovaleric acids differentiate this species from others in *Peptococcus* or *Peptostreptococcus*.

*Peptococcus* AU-1: Subsurface colonies are 0.5 mm, lenticular, and translucent. Surface colonies are 0.5 to 1 mm, circular, entire, low convex, opaque, and grayish white. PYG cultures have a granular sediment with no turbidity in 4 days; the pH is 5.8 to 6.2. This species differs from *P. magnus* in that longer chains of cells are produced, milk is digested, and growth is not stimulated by Tween 80.

*Peptococcus* AU-2: Subsurface colonies are 0.2 mm, white, lenticular, and translucent. Surface colonies are punctiform to 0.5 mm, circular, entire, low convex, semi-opaque, white, glistening, and smooth. PYG cultures have moderate growth with little turbidity, flocculent sediment, and a pH of 5.9 to 6.0 in 2 days. This species is differentiated from *Peptostreptococcus micros*, which it closely resembles, by its production of hydrogen and trace amounts of isobutyric, *n*-butyric, and isovaleric acids.

**Peptostreptococcus.** *Peptostreptococcus* BL: Subsurface colonies are 0.5 to 1 mm, lenticular, and off-white. No growth is produced on the surface of BAP or brain heart infusion agar plates incubated in anaerobe jars. Surface colonies on sweet E streak tubes are punctiform to 1 mm in diameter, circular, entire, raised, and translucent to opaque. PYG cultures have faint to moderate turbidity and no change in pH after 5 days. This indole-positive species differs from *Peptococcus asaccharolyticus* in morphology and arrangement of cells and by digesting gelatin and sometimes milk. Growth is not stimulated by Tween 80.

**Ruminococcus.** *Ruminococcus* AT: Subsurface colonies are 1 mm, lenticular, translucent, and white. Surface colonies are 1 to 2 mm, circular, entire, convex, opaque, glistening, and smooth. PYG cultures have slight turbidity with ropy sediment and a pH of 4.9 to 5.2 in 5 days. This species differs from *Ruminococcus* AQ described earlier (11) by fermenting salicin and trehalose.

*Ruminococcus* CO: Subsurface colonies are 0.1 to 2 mm, tan to brown, lenticular, translucent, and sometimes have a diffuse edge. Surface colonies are 1 to 2 mm, low convex to fried-egg shaped, circular, entire, smooth, and glistening. PYG cultures have slight turbidity with a mucoid or crumby sediment and a pH of 4.6 to 5.4 in 5 days. Growth is sometimes enhanced with Tween 80 or rumen fluid. This species differs from *R. lactaris* by the production of acid from sucrose and trehalose.

**Lactobacillus.** *Lactobacillus* G: Subsurface colonies are 0.5 mm, lenticular, and translucent to opaque. Surface colonies are punctiform to 1 mm, circular, entire, convex, translucent to opaque, smooth, and glistening. PYG cultures have moderate turbidity with smooth white sediment and a pH of 5.2 to 5.9 in 4 to 5 days. Hydrolysis of esculin and occasional fermentation of maltose, salicin, rhamnose, or xylose differentiate this species from *L. rogosae*.

**Fusobacterium.** *Fusobacterium* H: Subsurface colonies are 0.5 mm, spherical, fuzzy, and white. Surface colonies are punctiform, circu-

TABLE 9. *Characteristics of less common bacteria of the fecal flora*[a]

| Characteristic | *Cocrococcus* BH FBAL* | *Coprococcus* CC BA(pls) | *Gemmiger* X Ba(pL) | *Peptococcus* G Aibiv(i5) | *Peptococcus* AU-1 a(ls) | *Peptococcus* AU-2 a(pibibls2) | *Peptostrep.* BL (apbls) | *Ruminococcus* AT AF2(ls) | *Ruminococcus* CO AF2(ls) | *Lactobacillus* G Laf | *Fusobacterium* H Bap(ls) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. strains / No. people | 1/1 | 4/4 | 2/2 | 2/2 | 2/1 | 1/1 | 4/3 | 2/1 | 3/2 | 3/3 | 1/1 |
| Adonitol | w | — | - | — | — | — | - | — | — | - | — |
| Amygdalin | w | — | —A | — | — | — | — | — | —w | - | — |
| Arabinose | A | — | w— | Aw | — | — | — | — | - | - | — |
| Cellobiose | A | — | — | — | — | — | — | — | —w | Aw | — |
| Dextrin | A | — | — | w— | — | — | — | — | — | - | — |
| Esculin pH | —w | — | — | Aw | — | — | — | — | — | A- | — |
| Esculin hyd. | + | — | + | + | — | — | — | + | +— | + | — |
| Fructose | A | Aw | — | A | w— | —w | — | A | A | A— | — |
| Galactose | A | — | —A | w | — | — | — | w | A | - | — |
| Glucose | A | wA | w— | A | w— | —w | — | A | A | Aw | — |
| Glycerol | w | — | — | — | — | — | — | — | — | - | — |
| Glycogen | — | — | - | — | — | — | — | — | - | - | — |
| Inositol | — | A | - | — | — | — | — | — | - | - | Aw |
| Inulin | A | — | — | — | — | — | — | — | — | - | — |
| Lactose | A | — | - | —w | — | — | — | w | Aw | w- | — |
| Maltose | A | — | w— | Aw | — | — | — | A | A | -w | — |
| Mannitol | A | — | - | — | — | - | - | — | A— | - | — |
| Mannose | A | — | —w | A | — | w | — | — | A | — | — |
| Melezitose | A | — | - | — | - | — | — | — | — | - | — |
| Melibiose | A | — | - | — | — | — | — | w | - | - | — |
| Raffinose | A | - | - | — | — | — | — | Aw | - | - | — |
| Rhamnose | — | — | — | — | — | — | — | — | - | -w | — |
| Ribose | —w | — | - | A | — | — | — | — | -w | - | — |
| Salicin | A | — | —w | A | — | — | — | wA | -w | A- | — |
| Sorbitol | Aw | - | — | w | - | — | — | — | — | - | — |
| Sorbose | w | - | - | —w | — | — | — | — | — | - | — |
| Starch pH | A | — | - | As | — | — | — | — | — | — | — |
| Starch hyd. | + | — | — | — | — | — | — | — | — | — | — |
| Sucrose | A | — | —A | A— | — | — | — | A | A | - | — |
| Trehalose | — | — | — | A | — | — | - | Aw | A— | - | — |
| Xylose | A | — | v | w— | — | — | - | — | - | w- | — |
| Gelatin dig. | — | — | — | — | + | — | + | — | — | - | — |
| Milk | c | — | — | — | cd | — | —d | c— | c | —c | — |
| Indole | — | + | — | — | — | — | + | — | — | — | — |
| EYA react. | — | - | - | — | — | — | - | — | - | - | - |
| Hemolysis | — | —a | - | — | — | — | - | — | - | — | — |
| Gas | 4 | 2,4 | —1 | 3 | — | — | — | 2,4 | 3 | — | — |
| Hydrogen | 4 | 4 | — | 1 | — | 3 | — | 4 | 4,1 | — | — |
| PY-growth | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | —1 | 2 |
| PY-CHO gr. | 4 | 4 | 2,1 | 3,4 | 2,4 | 2 | 1,2 | 4 | 4 | 4 | 3 |
| PYG-bile gr. | 4 | 1— | 1— | 2,3 | 1 | 2 | —1 | 4 | 4,3 | —1 | — |
| Resaz. red. | + | v | — | + | - | — | — | + | + | —+ | — |
| Pyruvate | Ab | BA | B(A) | A | — | — | — | A | — | AF | Ba |
| Gluconate | — | — | — | — | — | — | — | + | + | — | — |
| Motility | — | — | — | — | — | — | — | — | — | — | — |
| Morphology | | | | | | | | | | | |

10 microns

Table 9. *cont'd.*

| Characteristic | *Eubacterium* AG-H a(bls) | *Eubacterium* AG-M a(pibbpy) | *Eubacterium* AJ Al(F2) | *Eubacterium* AL Lb(as) | *Eubacterium* AL-1 Lb(vsfa) | *Eubacterium* BW-1 ASl | *Eubacterium* CH-1 AFl2(s) | *Bacteroides* L-4 as(pbiv) | *Bacteroides* L-5 A2i44i5(lp) | *Bacteroides* AR Sapl(ibiv) | *Bacteroides* W-1 ASp(biv) | *Succinivibrio* A As |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. strains / No. people | 4/4 | 1/1 | 1/1 | 6/3 | 2/1 | 2/2 | 7/5 | 2/2 | 2/2 | 6/4 | 1/1 | 1/1 |
| Adonitol | - | — | — | — | — | - | - | - | - | w— | w | — |
| Amygdalin | — | — | — | A— | Aw | — | —w | — | — | —w | — | — |
| Arabinose | - | - | — | — | — | Aw | — | - | — | — | A | — |
| Cellobiose | - | - | — | —w | — | — | —s | - | — | —w | — | — |
| Dextrin | — | — | — | — | — | — | w— | - | — | Aw | A | — |
| Esculin pH | - | — | — | w— | w | w | — | - | — | w— | —w | — |
| Esculin hyd. | — | — | — | + | + | v | — | — | — | + | + | — |
| Fructose | w— | - | A | As | wA | Aw | A | - | Aw | w | w | — |
| Galactose | - | - | A | — | wA | Aw | A | - | A | w— | w | — |
| Glucose | — | — | A | As | A | Aw | A | - | A | wA | Aw | — |
| Glycerol | - | — | — | — | - | —w | — | — | - | — | — | — |
| Glycogen | — | - | — | — | - | — | — | - | — | —A | Aw | — |
| Inositol | — | - | — | - | - | - | — | - | - | — | — | — |
| Inulin | — | - | — | — | - | - | — | — | - | — | — | — |
| Lactose | - | - | — | w— | — | —w | A | - | — | Aw | w | — |
| Maltose | — | - | — | —w | — | —w | A | - | — | Aw | A | — |
| Mannitol | - | - | — | — | - | — | — | - | - | — | — | — |
| Mannose | - | - | w— | — | - | w— | —w | - | sw | Aw | Aw | — |
| Melezitose | - | - | — | — | — | — | — | - | — | — | — | — |
| Melibiose | - | - | — | — | - | — | — | - | - | —w | w | — |
| Raffinose | - | - | — | — | - | — | — | - | - | — | — | — |
| Rhamnose | — | - | A | — | - | —A | — | - | — | — | — | — |
| Ribose | - | - | Aw | — | — | w— | —w | - | - | —w | w | — |
| Salicin | - | - | w | A— | A | w- | — | - | — | — | — | — |
| Sorbitol | — | - | — | — | - | —w | A— | - | — | — | — | — |
| Sorbose | - | - | — | — | - | - | — | - | - | — | — | — |
| Starch pH | — | - | — | — | — | — | w— | - | — | Aw | Aw | — |
| Starch hyd. | — | - | — | — | — | — | — | - | — | +— | + | — |
| Sucrose | — | — | — | Aw | A | — | v | - | — | — | — | — |
| Trehalose | — | - | w | —A | - | A— | — | - | — | — | — | — |
| Xylose | - | - | — | — | - | A— | — | - | w— | v | Aw | — |
| Gelatin dig. | — | — | — | — | — | — | — | — | — | + | + | — |
| Milk | — | — | — | — | — | c— | c— | — | — | c | c | — |
| Indole | — | — | — | — | — | — | — | + | — | + | + | — |
| EYA react. | - | - | - | - | - | - | - | - | — | — | — | — |
| Hemolysis | - | - | — | — | — | b— | - | - | - | — | — | — |
| Gas | —1 | —1 | 3 | —2 | 2— | v | 4,2 | — | v | — | — | — |
| Hydrogen | 4 | 4 | 4 | —1 | — | 4 | 3,4 | — | 2,1 | — | 3 | 2 |
| PY-growth | 1— | 1— | 1 | 1— | 1 | 1 | 1 | 1— | 1 | 1,2 | 2,3 | 1 |
| PY-CHO gr. | 2,1 | 2 | 4 | 4,3 | 4 | 4,3 | 4 | 1— | 4 | 4,3 | 3,4 | 2 |
| PYG-bile gr. | — | — | 4 | v | 1 | 3,1 | 3 | —1 | 2— | 4 | 4,2 | —1 |
| Resaz. red. | — | — | + | + | + | + | + | - | +— | — | + | — |
| Pyruvate | — | — | A | a | Ab(F) | AF | AF | — | A | A | ABibiv | AS |
| Gluconate | - | — | — | — | — | — | — | - | + | — | — | — |
| Motility | — | + | — | — | — | — | — | — | — | — | — | + |
| Morphology | | | | | | | | | | | | |

10 microns

lar, slightly erose, low convex, glistening, and off-white. Inositol broth cultures have dense flocculent and ropy sediment with no turbidity and a pH of 5.3 to 5.4 in 4 days. This species is unusual in that it grows well in basal medium with added heme and ferments only inositol.

**Eubacterium.** *Eubacterium* AG-H: Subsurface colonies are 0.2 to 0.5 mm, off-white to tan, transparent to translucent, and may have darkened centers. Surface colonies are punctiform, circular, and transparent. Carbohydrate broth cultures have slight smooth sediment, no turbidity, and no decrease in pH in 2 to 5 days. This species differs from *Eubacterium* AG (11) by producing copious hydrogen.

*Eubacterium* AG-M: Subsurface colonies are 0.5-mm rhizoid spheres. Maltose broth cultures have very faint sediment in 10 days. Poor to moderate growth is produced in 6.5% NaCl. This species differs from *Eubacterium* AG-H in morphology, and it is motile and amphitrichous. It differs from *Eubacterium* AG (11) by motility and hydrogen production.

*Eubacterium* AJ: Subsurface colonies are 0.5 mm, lenticular, and white. Surface colonies are 0.5 to 1 mm, circular, low convex, and semiopaque to opaque, with a granular surface. PYG cultures have heavy turbidity with smooth sediment and a pH of 5.2 to 5.3 in 6 days. This species differs from *E. eligens* by producing copious hydrogen. It differs from *E. formicigenerans* in morphology and failure to ferment (or grow well in) maltose broth.

*Eubacterium* AL: Subsurface colonies are punctiform to 0.5 mm, off-white, lenticular, and translucent. Surface colonies are 1 to 2 mm, circular, entire, convex, and opaque to translucent. PYG cultures have mucoid sediment without turbidity and a pH of 4.7 to 5.7 in 4 to 7 days. In some PYG cultures pH below 6.0 is not obtained, but growth is superior to that obtained with many other carbohydrates or the basal medium. Growth may also be stimulated by Tween 80. This species differs from *E. cylindroides* in morphology and by lack of fermentation of mannose. It differs from *E. tortuosum* in morphology and by the lack of hydrogen production.

*Eubacterium* AL-1: Subsurface colonies are 1 to 2 mm, tan, lenticular, and translucent. Surface colonies are 1 mm, circular, entire to erose, convex, and opaque. PYG cultures have heavy turbidity with smooth white sediment and a pH of 4.4 to 4.8 in 3 to 5 days. Poor growth is sometimes produced in 6.5% NaCl. This species differs from *Eubacterium* AL and *E. cylindroides* in morphology and from *E. tortuosum* in morphology and by the absence of hydrogen production.

*Eubacterium* BW-1: Subsurface colonies are 0.5 mm, lenticular, white, and translucent. Surface colonies are punctiform to 1 mm, circular, entire, convex, translucent, off-white, sometimes with transparent edges, smooth, and glistening. PYG cultures have a dense flocculent sediment with little or no turbidity and a pH of 5.1 to 5.5 in 5 days. Absence of fermentation of mannitol, sorbitol, and xylose differentiate this species from *Eubacterium* AY described previously (11).

*Eubacterium* CH-1: Subsurface colonies are 0.5 to 1 mm, lenticular, multifoliate or rhizoid, white to tan, and semi-opaque. Surface colonies are 1 to 2 mm, circular, entire to erose, convex, and opaque or translucent with dense centers, and off-white in color. PYG cultures have dense flocculent or mucoid sediment and dense turbidity with a pH of 5.0 to 5.3 in 5 to 7 days. Scant growth is produced in noncarbohydrate media, and cells in these cultures are coccoid and arranged in long chains. Elongated cells as well as cocci are produced in the same chains of cells in those carbohydrate media that are attacked. This species resembles *Ruminococcus* species in its requirement for fermentable carbohydrate, acid products, and production of coccoid forms. However, the presence of definite rods within the chains indicates it should be associated with *Eubacterium* species.

**Bacteroides.** *Bacteroides* L-4: Subsurface colonies are 1 mm, tan, and lenticular. No growth is produced on the surface of agar media incubated in anaerobe jars. Scant growth with slight turbidity and no sediment is produced in broth media. Terminal pH in carbohydrate broth is the same as in the basal medium. This

[a] Acids and alcohols produced in PY-carbohydrate (usually glucose or fructose) broth cultures are given below the name of the organism. Those produced from pyruvate are indicated in the table. Capital letters indicate 1 or more meq/100 ml; small letters indicate less than 1 meq/100 ml. Products in parentheses are not uniformly detected. Abbreviations for products: a, acetic acid; b, butyric acid; f, formic acid; ib, isobutyric acid; iv, isovaleric acid; l, lactic acid; p, propionic acid; py, pyruvic acid; s, succinic acid; 2, ethanol; i4, isobutyl alcohol; 4, butyl alcohol; i5, isoamyl alcohol. Abbreviations and symbols for other reactions: A (carbohydrate cultures), acid (below pH 5.5); a (hemolysis), alpha; b (hemolysis), beta; c (milk), curd; +, positive reaction; −, negative reaction; -, no visible growth or only slight growth and negative reaction; w, weak reaction or pH between 5.5 and 6.0; s, growth is stimulated; v, variable reaction; numbers (growth and gas), amount estimated on "− to 4+" scale. Where two reactions are given (e.g., Aw), the first is the more usual and the second is observed less frequently. EYA, Egg yolk agar (modified McClung-Toabe).

species differs from other unreactive *Bacteroides* species in that it produces indole but no hydrogen and fails to coagulate milk.

*Bacteroides* L-5: Subsurface colonies are 0.5 mm, lenticular, or rhizoid. Surface colonies are 0.5 to 2 mm, circular, entire, convex to slightly peaked, translucent, buff, and smooth. PYG cultures are turbid with a flocculent sediment and a pH of 5.2 to 5.5 in 4 to 6 days. This species differs from *Bacteroides* species described previously (11) by the production of acid (pH below 5.5) from glucose and fructose and the production of hydrogen. Growth is stimulated in the presence of fructose, glucose, galactose, and mannose. Hydrogen sulfide is not produced in SIM (BBL) medium.

*Bacteroides* AR: Subsurface colonies are 0.5 to 2 mm, lenticular, translucent, and white. Surface colonies are punctiform to 1 mm in diameter, circular, entire, convex, translucent to opaque, glistening, smooth, and off-white to buff. PYG cultures have moderate to slight turbidity with smooth sediment and a pH of 5.3 to 5.6 in 5 to 8 days. Threonine is converted to propionate. This species is similar to *B. eggerthii* except that arabinose is not fermented and the cells are thinner.

*Bacteroides* W-1: Subsurface colonies are 1-mm, white wooly balls. Surface colonies are 0.5 to 2 mm, circular, entire, low convex, and translucent. PYG cultures have heavy turbidity with smooth white sediment and a pH of 5.2 to 5.9 in 4 to 5 days. Threonine is converted to propionate and lactate is utilized. The fermentative, sucrose-negative, indole-positive species, including *B. splanchnicus, B. eggerthii, Bacteroides* AR, *Bacteroids* W-1, and *Bacteroides* W-2 (11), are differentiated according to the characteristics shown in Table 8.

**Succinivibrio.** *Succinivibrio* A: Subsurface colonies are 0.5 mm, white, and rhizoid. Surface colonies are 0.5 mm, circular, entire, transparent, low convex, smooth, and glistening. PYG cultures have a slight turbidity and slight sediment with no decrease in pH in 4 days. Monotrichous curved cells and production of succinic acid from pyruvate indicate that this species is a member of the genus *Succinivibrio*. Production of large amounts of acetic acid and smaller amounts of propionic acid and butyric acid without a measurable decrease in pH of carbohydrate media differentiate this species from others of the genus.

Variable reactions among all the species described above are listed in Table 9.

## APPENDIX

**(a) Composition of the flora.** Let the number of species represented by $r$ isolates in the sample be denoted by $n_r$, the "frequency of the frequency $r$." It has been shown by Good (4) that the "coverage" of a sample is approximately $1 - n_1/N$, where $N$ is the total number $\Sigma rn_r$ of isolates, and the coverage is the probability that the next isolate sampled will belong to a species that has already occurred. In our problem, as mentioned in Results, we had $N$ = 1,442. Further, we had $n_1 = 28$, so that the estimate $1 - n_1/N$ of the coverage is 98%. But we consider that a species represented by only two or three isolates might, in some cases, be found to correspond to more than one species if the sample were much increased, so that $n_1$ is probably an underestimate of its true expected value. Accordingly, we consider that the formula $H_9$ in Good (4), which is $n_r \propto x^r/r(r + 1)$($x$ close to 1) and which fits well for $r \geq 2$, may well give a more realistic estimate of the coverage. It gives the lower figure of 95% mentioned in Results.

A good fit to the observed values of $n_r$, including $n_1$, can be obtained by Fisher's formula ($H_3$ of reference 4), $n_r = \beta x^r/r$, with $\beta = 24.744$ and $x = 0.98313$, and this hypothesis would again lead to the estimate of 98.3% for the coverage. An even more exact fit to the observed $n_r$ values could of course be obtained by means of the more general hypothesis $H_4$, which contains three parameters. For the determination of the parameters see Sichel (16), but for the purpose of estimating the coverage the use of this hypothesis is unnecessary.

On the available evidence it is impossible to decide whether 98 or 95% is the better estimate of the coverage. This becomes especially clear when it is held in mind that it is unimportant for a smoothing to be good for large values of $r$ for the purpose of estimating the coverage.

**(b) Note on the transformation.** $\sqrt{(x/2.1 + 3/8)}$. The choice of this transformation was based on the frequencies $x$ of isolates (Table 4) corresponding to the eight common species (*B. fragilis* subsp. *vulgatus, E. aerofaciens, B. fragilis* subsp. *thetaiotaomicron, P. productus* II, *B. fragilis* subsp. *distasonis, F. prausnitzii, C. eutactus,* and *P. productus* I). After making the transformation, the following summary statistics were computed for the data as broken down into (i) normal diet, (ii) Skylab diet, not in chamber, and (iii) Skylab diet, in chamber (with the entries 19, 12, and 13 for the period August 24 removed to help with the analysis discussed in section c, below): (i) 79 df, $\bar{x} = 1.3785$, standard deviation (s) = 0.55267; (ii) 47 df, $\bar{x} = 1.4489$, s = 0.53818; (iii) 69 df, $\bar{x} = 1.3188$, s = 0.59860. The three means for the three diets certainly do not differ significantly [all three $t$ values, for comparison of [i] with [ii], [i] with [iii], and [ii] with [iii], are less than 0.25). Bartlett's test for homogeneity of variances gives $\chi^2[2\ df] = 0.758$, so there is certainly no reason to suspect that the variances depend on the diet. The pooled mean is 1.3604 and the pooled variance is 0.32015 (194 df), corresponding to an estimated standard deviation of 0.5658. The pooled variance is used in section c (below).

**(c) An analytical discussion corresponding to Fig. 1 and 2.** The graphical approach of Fig. 1 and 2 could be misleading, so we here discuss the data concerning *B. fragilis* subsp. *thetaiotaomicron* and

*P. productus* II analytically. The frequency counts of isolates for the former, classified both by periods and by person, were:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| B | 4 | 5 | 2 | 1 | 5 | 19 | | 1 | 4 |
| R | 4 | 1 | 1 | 2 | 7 | 12 | | 4 | 3 |
| T | 3 | 4 | 4 | 7 | 6 | 13 | 10 | 5 | 2 |

where the nine columns correspond to the nine periods specified in Fig. 1. Now the totals (shown in the last row of Table 4) were high corresponding to column 6 (August 24), namely 72, 66, and 66; so we begin by replacing the column [19, 12, 13] by [19 × $^{56}/_{72}$, 12 × $^{56}/_{66}$, and 13 × $^{56}/_{66}$]. If we then apply the normalizing transformation $\sqrt{(x/2.1 + 3/8)}$, the data become

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| B | 1.5 | 1.7 | 1.2 | 0.9 | 1.7 | 2.7 | | 0.9 | 1.5 |
| R | 1.5 | 0.9 | 0.9 | 1.2 | 1.9 | 2.29* | | 1.5 | 1.3 |
| T | 1.3 | 1.5 | 1.5 | 1.9 | 1.8 | 2.4 | 2.27* | 1.7 | 1.2 |
| Totals | 4.3 | 4.1 | 3.6 | 4.0 | 5.4 | 7.4 | | 4.7 | 4.5 |

(* The reason for giving two of the entries to two decimal places is to help in the nonparametric test described below. These entries are both regarded as equal to 2.3 for all other purposes.)

By chance, $1/\sqrt{3}$ times the sample standard deviation of the totals 4.3, 4.1, . . ., 4.5 (excluding the total 7.4 for August 24) is smaller than that for individual entries 1.5, 1.7, . . . This in its turn is smaller than the estimated standard deviation, 0.5658, based on 194 df, as found in section b (above). To avoid overstating our thesis we have used the latter estimate. We then regard the seven numbers 4.3, 4.1, 3.6, 4.0, 5.4, 4.7, and 4.5 as normal variables of unknown mean, and standard deviation estimated by 0.5658 × $\sqrt{3}$ = 0.980. If the total on August 24 had the same expectation as that in the other periods, the probability that a difference as large as 7.4 − (4.3 + . . . + 4.5)/7 = 3.029 would be attained would be approximately that of a Student's $t$ equal to 3.029/[0.980 $\sqrt{(1 + 1/7)}$] = 2.891, where the denominator has 194 df. The corresponding single tail-area probability is 1/468. It might be thought that a double tail-area should be used and that a penalty should be paid for the special selection of the species and of the period August 24. The reason for not paying these penalties is explained in section f.

In case the assumptions of approximate normality are not accurate enough, we can also evaluate the significance of the "sixth column" by a nonparametric test. Even after multiplying the three counts in that column by $^{56}/_{72}$, $^{56}/_{66}$, and $^{56}/_{66}$, we find that they give the top three among 25 numbers. The probability of this, if the effect were merely random, would be $^{1}/_{2,300}$, which is even smaller than $^{1}/_{468}$. But 2.29 is only just larger than 2.27 so this nonparametric test overevaluates the statistical significance.

For *P. productus* II, the table of transformed data is

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| B | 1.3 | 1.2 | 2.6 | 1.9 | 0.9 | 0.6 | 0.6 | 0.6 |
| R | 1.7 | 0.9 | 0.9 | 1.7 | 0.9 | 0.6 | 1.2 | 1.7 |
| T | 1.3 | 2.6 | 2.0 | 1.8 | 1.2 | 0.9 | 1.9 | 1.7 |
| Totals | 4.3 | 4.7 | 4.5 | 5.4 | 3.0 | 2.1 | 3.7 | 4.0 |

The difference between the (low) total for August 24 and the mean of the other seven totals is now 4.229 − 2.1 = 2.129 and the corresponding Student $t$ is 2.032, corresponding to a double tail-area probability of about 1/23. Here we do not need to allow for the special selection of the period, but some allowance must be made for the selection of the species, so that the result for *P. productus* II is not striking. (The results are slightly better than this argument suggests because the samples were large for the period August 24.)

**(d) Statistical interpretation of Table 5.** The high value of 11 out of 57 in Table 5 can be evaluated by the same method as in section c (above). After applying the transformation $\sqrt{(x/2.1 + 3/8)}$ we can interpret the deviation 2.4 − (0.6 + 1.8 + 1.8)/3 = 1.0 by regarding 1.0/[0.5658$\sqrt{(1 + 1/3)}$] = 1.53 as being a $t$ distribution in which the denominator has 194 df. This corresponds to a single tail-area probability of 1/16.

**(e) Statistical significance of the ascending colon observation.** Of the observations $^{17}/_{51}$, $^{8}/_{50}$, $^{4}/_{57}$, and $^{5}/_{55}$, the first referred to the ascending colon. For the sake of accuracy we can first replace the frequencies 17, 8, 4, and 5 by 17 × 56/51, 8 × 56/50, 4 × 56/57, and 5 × 56/55 and then apply the transformation $\sqrt{(x/2.1 + 3/8)}$ to them. This gives the numbers 3.04, 2.154, 1.50, and 1.67, and the significance of the difference 3.04 − (2.154 + 1.50 + 1.67)/3 = 1.267 can be measured, as in section d, as that of a $t$ value of 1.267/[0.5658$\sqrt{(1 + 1/3)}$] = 1.939, where the denominator has 194 df. The corresponding single-tailed probability is 1/37.

**(f) Significance of the anger stress hypothesis.** Here we attempt to evaluate the significance of the hypothesis that an anger-stress situation tends to lead to a high count for the species *B. fragilis* subsp. *thetaiotaomicron,* based on the combination of all the observations.

The statistical evaluation of the data cannot logically depend on the chronological order of the observations. Accordingly, it is legitimate to start with the independent observations by Bruce Maier, mentioned in the text. We can regard his observations as implying a prima facie case for the hypothesis under consideration. Had this information been available to us before we analyzed the astronaut data, and had we known of the stressful situation of the astronauts in a period including August 24, we might have expected high counts of *B. fragilis* subsp. *thetaiotaomicron* in that period. This period probably covered most of the time in the chamber. It should be noted that the totals 4.3, 4.1. . . . in section c (above) are largest for the three sample days in this period. For simplicity in the analysis we decided to test the significance of the hypothesis by using just the highest of these three totals. Although this involves a little special selection, we feel that this is compensated by the high totals in the two adjacent sample dates. Accordingly we regard it as justifiable to combine the pieces of evidence of section c, d, and e as if the hypothesis under consideration had been formulated in advance.

The individual single-tailed $P$ values, given in sections c, d, and e, were 1/468, 1/16, and 1/37,

respectively. We combine these by Fisher's method to obtain a composite tail-area probability of 1/3,000.

Thus, the hypothesis appears probable in the light of all the available evidence. It may well be that there is also an effect on some of the other species, but for most of them the samples may be too small for the effect to be noticed.

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