Stability of Normal Human Fecal Flora During a Chemically Defined, Low Residue Liquid Diet

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Ten adult male volunteers (medical students) subsisted for seven days upon a chemically defined, low residue liquid diet, and consumed 1200–1800 calories per day. All stools were collected; three were cultured within the hour—a prediet stool, one collected on the seventh day, and a postdiet stool. Specimens were diluted anaerobically, and anaerobic cultures were streaked upon plates of prereduced agar media and incubated in Brewer jars. During the low residue diet, total fecal mass was relatively small and each subject passed only two or three stools. The mean reduction in daily fecal output was 70%. Mean counts of total aerobes were 10^7/gm throughout the study, and mean counts of total anaerobes were 10^{10}/gm. There was no overgrowth by opportunistic bacteria or fungi. The low residue food did not alter fecal flora; there was neither disappearance nor reduction of any bacterial group.

CHEMICALLY DEFINED, low residue diets may be used clinically to provide complete nutritional maintenance of patients in need of a liquid dietary regimen of high nutritional value.[5] Defined diets offer nutritional support in gastrointestinal disorders such as chronic inflammatory bowel disease, short bowel syndrome following substantial resection of the small intestine and malabsorption syndromes. Elemental diets may also be used preoperatively to provide nutrition to patients scheduled for colonic surgery, postoperatively after gastrointestinal surgery when low residue foods are desirable, and in the management of problems of mastication and fecal incontinence.

Employing chemically defined diets as the sole source of nutrition, Winitz and colleagues[7] found that the weight of volunteers remained stable and noted no biochemical abnormalities. They also reported[6] a 70–80% decrease in stool excretion and a decrease in the concentration and types of fecal bacteria. Both Attebery[1] and Glotzer[3] and their colleagues have confirmed the reduction of fecal volume during elemental, low residue diets, but have not demonstrated a decrease in the concentration and types of fecal bacteria. We have employed a similar defined low residue diet, and have assayed its effect upon the fecal flora of normal adults.

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Methods

Ten healthy male medical students ranging in age from 23 to 31 years (mean age ± standard deviation = 25.9 ± 3.0 years) participated in this study. Each maintained himself on a chemically defined, low residue diet (W-T Low Residue Food®) for seven days. Four to six 300 calorie packets (320–480 gm) furnished dextrin as the carbohydrate, crystalline L-amino acids, vitamins, minerals and a source of essential fat, but lacked protein, starch, indigestible material and additional bulk. Each subject was allowed water *ad libitum* while on the low residue diet. No other foods were allowed.

Three stools from each subject were cultured for quantitation and identification of microflora. The first stool was collected the day before the subject began the defined diet, the second was taken on the seventh day of the defined diet, and the third was obtained three days after resumption of the subject's regular diet. In addition, all stools passed were collected, weighed, and examined for color, odor and consistency. A weighed sample of each was dried overnight in a vacuum oven at 60 C in tared pans so that the dry weight could be obtained and the percentage solids calculated.

The subjects received no medication in the week prior to, or during the diet period. Each took one 5 mg bisacodyl NF tablet (Dulcolax®) on the evening before starting the defined diet to facilitate cleaning the bowel of residue remaining from the normal diet, and with the first feeding of the de-

fined died, two 10 grain refined charcoal tablets as a marker for gross and microscopic observation. Intestinal transit time during a chemically defined, low residue diet has been estimated at five to seven days.[1]

All stools were collected in 11.5 × 13 inch plastic bags. Those to be cultured were processed within 30 minutes of excretion. Each was kneaded in the plastic collection bag to provide a homogeneous specimen; a 0.1 gm sample was transferred to a tared screw cap tube and emulsified in 9.9 ml fluid thioglycollate medium with the aid of a vortex mixer. Serial 100-fold dilutions were then continued under a stream of O_2-free CO_2 in prereduced fluid thioglycollate medium in rubber stoppered tubes. All dilutions were plated aerobically employing blood agar medium (5% human blood in brain-heart infusion agar containing 4% agar), MacConkey agar, mannitol salt agar, cornmeal agar, and Sabouraud agar. These plates were examined after 24 and 48 hours, except cornmeal agar and Sabouraud agar which were inspected after 10 days.

For anaerobic culture, plates of blood agar medium and Rogosa SL agar were prereduced by storage for at least 48 hours in a flexible plastic anaerobic glove box containing an environment of 5% CO_2, 10% H_2, and 85% N_2. Reduced plates were removed from the glove box in sealed Gas-Pak® jars so that they would be exposed to room air only during inoculation. The inoculum was spread on the agar surface, and anaerobic plates were immediately incubated in Brewer jars. These were evacuated and filled with a mixture of 95% H_2 and 5% CO_2 three times before being clamped off. In the interim, plates in partially filled Brewer jars were kept under a stream of O_2-free CO_2 until the jar could be sealed, evacuated, and gassed. All anaerobic plates were incubated for at least four days before being examined and subcultured. All media were incubated at 37 C, except for cornmeal agar and Sabouraud agar at room temperature.

Reduced brain-heart infusion agar roll streak tubes were inoculated from each specimen under a stream of O_2-free CO_2, but did not yield total counts of anaerobes different from those obtained from plate cultures. In addition, tubes of brain-heart infusion broth, fluid thioglycollate medium, and cooked meat medium were inoculated from the initial dilution of each specimen. These allowed both aerobic and anaerobic bacteria to grow, and were studied as a check on the organisms isolated from surface cultures. The growth of species on appropriate selective media also served as an internal check. Aerobic isolates were streaked to eosin-methylene blue agar to identify coliforms, and were subcultured to heart infusion broth, from which Gram-stained smears were prepared. Standard methods were used to identify Gram-negative enteric bacteria. Isolates from anaerobic plates were streaked to a blood agar plate for aerobic incubation to verify that the isolate was an anaerobe.

All bacterial counts were transformed to logarithms to the base 10 for ease of statistical analysis. Mean differences between treatments were examined by the *t* test. The criterion for random distribution between two treatment groups was selected as a probability (P) equal to or greater than 0.05 with 18 degrees of freedom ($t < 2.101$).

Results

Each subject passed only two or three stools during the seven days of the defined diet. Table 1 indicates that the mean daily output of feces was diminished 70% during this period. This difference was statistically significant ($P < 0.001$). The mean daily output after resumption of a regular diet by each subject was not significantly different from output initially. This table also shows that the mean percentage of fecal solids was not altered significantly during the period of the defined diet. Although the mean body weight of subjects decreased three pounds during the diet and increased subsequently, these changes are not significantly different, and represent intestinal bulk lost during the low residue diet period and regained subsequently.

Observation of stools for color, odor and consistency indicated that these properties generally remained unchanged. However, three subjects, who passed liquid stool on the sixth and seventh day of the defined diet, experienced neither pain, discomfort nor cramping. The initial and sometimes the second stool of the diet period was noted to be darker or to have dark patches due to the charcoal used as a marker. This was confirmed by microscopic examination.

Quantitative bacteriological studies indicated that the change to the chemically defined, low residue diet caused no alteration of fecal flora. Table 2 summarizes the means for total aerobes and anaerobes, and for the several groups of aerobic and anaerobic bacteria. Although mean values for some bacterial groups were lower during the defined diet, none of the differences between pre-diet and diet means and pre-diet and post-diet means was statistically significant. Bacteroides were the predominant microorganisms in feces, and were followed in descending order of concentration by lactose-fermentating coliforms (*Escherichia* and *Enterobacter*), the fusobacteria, anaerobic lactobacilli (*Bifidobacterium*), other anaerobic Gram-positive rods, and streptococci. The clostridia, veillonellae, and peptostreptococci were minor anaerobic constituents, and staphylo-

TABLE 1. *Influence of a Chemically Defined, Low Residue Liquid Diet on 10 Healthy Adult Males*

	Mean ± Standard Deviation		
	Pre-diet	Diet	Post-diet
Feces produced, grams wet weight per day	157.5 ± 70.1	43.4 ± 17.6	126.5 ± 55.7
Percentage dry fecal solids	26.3 ± 7.5	25.7 ± 6.7	28.0 ± 9.1
Body weight, lbs.	188 ± 27	185 ± 25	191 ± 22

TABLE 2. *Bacterial Counts of Feces* from 10 Healthy Adult Males*

Microorganism	Pre-diet	Diet	Post-diet
Total aerobes	7.82 ± 0.79	7.67 ± 1.12	7.46 ± 1.32
Total anaerobes	10.41 ± 0.23	10.52 ± 0.33	10.30 ± 0.87
Escherichia	7.56 ± 0.74	7.09 ± 1.52	6.97 ± 1.28
Enterobacter	3.65 ± 2.77	1.28 ± 2.47	2.29 ± 2.67
Streptococcus	4.02 ± 2.90	3.19 ± 2.69	6.08 ± 2.22
Staphylococcus	1.36 ± 2.37	2.22 ± 2.28	1.22 ± 2.00
Lactobacillus	2.81 ± 3.71	1.99 ± 3.35	2.76 ± 3.75
Bacteroides	10.11 ± 0.39	10.39 ± 0.38	10.09 ± 0.90
Fusobacterium	6.91 ± 4.77	6.92 ± 4.56	7.45 ± 4.04
Veillonella	1.25 ± 2.93	1.94 ± 3.85	3.08 ± 4.52
Clostridium	2.74 ± 3.25	1.53 ± 2.38	1.43 ± 3.17
Bifidobacterium	6.92 ± 3.82	6.14 ± 3.36	6.40 ± 4.22
Other anaerobic Gram-positive rods	6.58 ± 4.56	4.73 ± 4.62	2.90 ± 4.48
Peptostreptococcus	1.03 ± 2.92	1.82 ± 3.83	1.12 ± 3.21

* Arithmetic means of $\log_{10}$/gram wet weight ± standard deviation.

cocci and lactobacilli were minor aerobic constituents of the flora. Fungi were not found, and yeasts were isolated only from the diet stool of two subjects (log values of 4.42 and 5.90). There was no overgrowth of opportunistic bacteria or fungi with use of the defined diet. Bacteroides were isolated easily without use of selective media containing antibiotics. Aerobic and anaerobic blood agar plates served as the primary medium for colony counts with the other selective media employed only as a supplementary back-up.

Discussion

The defined low residue diet diminished stool mass and decreased the frequency of stool passage. Concomitantly, there was no marked reduction in bacterial population nor was there disappearance or reduction of particular bacterial groups. In this regard, our findings confirm those of Attebery *et al.*[1] and Glotzer *et al.*[4], but not those of Winitz *et al.*[6] The present study utilized specimens of fresh feces obtained under ideal conditions from ten healthy adults receiving no medication as opposed to specimens obtained from hospitalized patients. This report establishes baseline data of value for future studies of changes in human fecal flora.

Our previous report[2] and that of Nichols and colleagues[4] regarding the effect of preoperative mechanical preparation of the colon by means of enemas, cathartics, and low residue or clear liquid diet have shown reduction in the total fecal mass and either no change in the concentration of any fecal microorganism or reduction in the concentration of coliforms, respectively. Although fecal output was diminished 70% as a result of the low residue diet, it may be calculated from the results of the present study that the total bacterial content of the fecal mass was only lowered from 4.07×10^{12} to 1.44×10^{12}. Thus, any feces remaining in the colon contain such large numbers of bacteria that their potential for infection remains a major hazard following colonic operations. Accordingly, use of defined diet alone would be inadequate for intestinal antisepsis for preoperative preparation of the colon, and should be accompanied by the additional administration of appropriate oral, nonabsorbable antibiotic(s) in the regimen.

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