Comparison of the Fecal Microflora of Seventh-Day Adventists with Individuals Consuming a General Diet

Implications Concerning Colonic Carcinoma

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Qualitative and quantitative fecal microflora was studied in a double blind fashion in 28 subjects. Fourteen were Seventh-Day Adventists, who were strict vegetarians, while the remaining 14 subjects were individuals consuming a general western diet. No statistically significant differences were identified in the fecal microflora of the two groups. The bacteriologic analysis included total aerobes and total anaerobes as well as each of the major fecal aerobes and anaerobes. This study seems to indicate that the dietary intake of animal fat and protein does not significantly alter the fecal microflora, a possibility which has previously been suggested as being part of the explanation for the higher incidence of colonic carcinoma in those who consume meat compared with vegetarians. It does not, however, invalidate the concept that dietary animal fat does increase bile acid degradation within the gastrointestinal tract, a factor which has been related to colon cancer. Future studies should be directed at identifying the factors that may be present in the gastrointestinal tracts of vegetarians which modify the ability of their colonic microflora to degrade bile acids, an essential step in the production of intraluminal carcinogens or co-carcinogens.

CURRENT EPIDEMIOLOGIC data have shown that there are striking differences in the incidence of colon cancer in various parts of the world. It has been demonstrated that the occurrence of cancer of the colon is much lower in East Africa, India and Japan than in Western Europe or North America.[5,26] A series of studies of migrants have revealed that these differences are most likely environmental and not genetic.[2,9–12,]-

[19–20,24] Investigations of various environmental influences have frequently linked dietary habits to the development of carcinoma of the colon. Nutritional substances such as fiber, refined carbohydrate, animal fat and protein have all been advanced as being the significant factor responsible for the variance in incidence rates of colonic cancer.[3,8,21,22]

Epidemiologic data have also shown that the incidence of cancer of all types, including carcinoma of the colon, is 30%–40% lower in American Seventh-Day Adventists, who are strict vegetarians, than in the meat consuming general public.[17,23] Further studies have shown that the levels of bile acids, as well as the degradation products and enzymes responsible for the degradation of bile acids in the colonic lumen, are decreased in this group of vegetarians.[18] To date, no randomized study concerning the differences of fecal microflora in Seventh-Day Adventists and the general population has been reported; which is the purpose of this investigation.

Materials and Methods

The study group consisted of 28 individuals employed at the Hinsdale Sanitarium and Hospital, Hinsdale, Illinois. Fourteen were individuals on a general American diet, while 14 were Seventh-Day Adventists who were practicing vegetarians, four of whom had never eaten meat, while two had not eaten meat for 40 years or more, 6 for 20–40 years and two for 10–20 years. Specimens were collected in a double blind fashion and consisted of first morning stools which were voided into plastic bags. These bags were quickly

TABLE 1. *Clinical Features of Different Dietary Groups*

	(General Western Diet) Control	(Vegetarians) Seventh-Day Adventists
Mean age	45	47
Age range	22–66	15–61
Female	10	5
Males	4	9
Mean no. of meals daily	3.0	2.8
Mean no. of bowel movements daily	1.4	1.8

placed into Gas-Pak jars* which provided an anaerobic environment. The specimens were then transported to our research microbiologic laboratory within three hours, where they were promptly processed. Information concerning the dietary habits of the studied subjects was retained by the administrative personnel of the Hinsdale Hospital until the bacteriologic data were completed.

Once in the microbiologic laboratory, each specimen was placed immediately into the anaerobic chamber,† where it was weighed and then mixed for 15 minutes. After thorough mixing, an aliquot was taken to determine the percentage of water. Another aliquot of 11 grams was added to a 99 ml dilution bottle of VPI dilution salts‡ to yield a 10^{-1} dilution. Serial dilutions were then made and plated out on the following media: Bifidobacter medium; Blood agar with vitamin K and hemin; Blood agar with vitamin K and hemin with Vancomycin (7.5 mcg/ml) and Kanamycin (100 mcg/ml); Bacteroides medium (Blood agar with vitamin K and hemin with 2% bile added); Sarcina medium; Veillonella medium; Lactobacillus medium; and Clostrisel agar. These media were then incubated at 35° for 48 hours under anaerobic conditions. After the anaerobic plates had been inoculated, the dilution bottles were removed from the chamber and inoculations were also made on the following media under aerobic conditions: Blood agar, Eosin methylene-blue agar, Bile esculin azide agar, KF Streptococcus agar and Mannitol salt agar. These plates were then incubated for 48 hours at 35° under aerobic conditions with 5% CO_2.

At the end of 48 hours the colonies on all plates were counted and each colony type was subcultured. All colonies growing under anaerobic conditions were suspected of being anaerobes and were subcultured aerobically and anaerobically. Those which failed to grow under aerobic conditions were deemed anaerobes. They were then gram stained and subcultured to pep-

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tone-yeast extract-glucose broth. The broth was incubated for 24–48 hours under anaerobic conditions and a gas chromatographic analysis was made to determine the acid end products formed by the bacteria. Anaerobic bacterial identifications were based on Gram stain, colony morphology and gas chromatographic analysis of acid end products. Aerobes were identified by standard bacteriologic techniques.

Results

The clinical data including age, number of meals and bowel movements each day were similar in both groups and are listed in Table 1. The ratio of females to males in each group, however, was reversed. The mean percentage of fecal water was 73.5% in the controls and 73.3% in the vegetarians.

The total mean aerobic and anaerobic counts as well as counts of bacteria isolated in individuals in each group are presented in Table 2. In general, the individual total aerobic and anerobic counts varied over a wide but similar range in both the vegetarians and controls. The mean values of aerobes and anaerobes, however, are very similar in both groups. The log_{10} mean total aerobic count was 9.0 in the control group and 8.9 in the vegetarians, while the total anaerobic log_{10} mean count was 11.2 in the control group and 11.0 in the vegetarians.

The types of aerobic and anaerobic bacteria isolated were very similar in both groups with the exception that clostridia were isolated in 11 vegetarians and in only 6 controls, while peptostreptococcus was isolated in four vegetarians and in 8 of the controls. Sarcina was isolated in only two of the vegetarians while veillonella was found in four vegetarians and in only one of the controls. Analysis of the bacteriologic data by the Wilcoxon Rank Sum Test shows no statistical difference between the two groups at the $p < .05$ level, although a rough comparison does show a slight increase in total anaerobic counts in the control groups (general western diet).

Discussion

Studies concerning the relationship of diet to the development of colon carcinoma show a strong correlation with dietary animal fat and protein and a poor correlation with vegetable fiber.[7] Most recently it has also been noted that the incidence of colon cancer correlates well with the intake of animal protein, but poorly with plant protein, which would seem to indicate that amino acid metabolites are probably not a major factor in the etiology of colon cancer.[13] Considering these observations, one is led to believe that the amount of animal fat in the diet of westerners is the main dietary

factor responsible for their increased incidence of colon cancer.

Proponents of the dietary fat hypothesis of colonic carcinoma suggest that dietary fat causes an increased secretion of bile acids and therefore, an increased concentration of these acids with the lumen of the colon. They further suggest that certain colonic bacteria can produce carcinogens or co-carcinogens from these bile acids.[13,25] One mechanism advanced by which this might be accomplished is the aromatization of the bile acid nucleus to cyclopentaphenanthrene, a compound which has been shown to be carcinogenic in mice.[4] The reactions necessary for this conversion have all been shown to be producible by human colonic microflora, in particular by *Bacteroides fragilis* and *Clostridia paraputrificum*.[16] Advocates of the dietary fat hypothesis also believe that the oral intake of animal fat modifies the colonic microflora; in that it increases the number and types of certain anaerobes and increases their ability to degrade bile acids.[6,13,25] Working along these lines, investigators have shown that the amount of fecal bile acids and degradation products are decreased in groups from areas where the dietary intake of animal fat and the incidence of colon cancer are low.[13,16,25] They have also shown that these groups have lower bacteroides and total anaerobic counts and that these anaerobes are less active metabolic degraders of bile salts.[1,13–14,16,25]

Because of these considerations, much interest has been generated in the fecal microflora, bile acids and their degradation products in the stool of groups with different incidence rates of colonic cancer, as well as different dietary habits. In view of this, one would expect that American Seventh-Day Adventists, vegetarians with a decreased incidence of carcinoma of the colon, would have decreased numbers of anaerobes in their stools. Reddy and Wynder have shown that this group does have decreased bile acids and degradation products in their feces.[18] However, our data in this study show that there are no significant differences in types or numbers of bacteria in their stools compared with the control group. Our data, therefore, seem to contradict one of the points of the dietary fat hypothesis of colonic carcinoma; that is, that dietary animal fat is responsible for differences in anaerobic fecal counts. However, it does not invalidate the concept that dietary animal fat modifies the colonic microflora in some other way. As previously stated Reddy and Wynder have shown that bile acids and degradation products are decreased in the feces of Seventh-Day Adventists.[18] Considering that the total numbers of bacteria are not changed, one might conclude that the ability of the microflora of the Adventists to degrade bile acids is decreased or that conversely the ability of the

TABLE 2. *Fecal Microflora in Seventh-Day Adventists and Control Meat Eaters*

	Controls		Vegetarians	
	Number of Subjects Isolated	Mean ± S.E.	Number of Subjects Isolated	Mean ± S.E.
Total aerobic	13	9.0 ± .2	12	8.9 ± .3
Escherichia coli	13	7.5 ± .2	13	7.4 ± .3
Streptococcus faecalis	14	6.2 ± .2	13	6.8 ± .2
Enterobacter agglomerans	1	6.1		
Klebsiella pneumoniae	1	5.9	2	6.6 ± .1
Edwardsiella			1	7.8
Enterobacter aerogenes			1	7.0
Enterobacter cloacae			1	6.1
Total anaerobic	14	11.2 ± .1	13	11.0 ± .1
Bacteroides	11	9.6 ± .3	10	9.4 ± .3
Bacteroides fragilis	11	10.3 ± .2	12	10.4 ± .1
Bifidobacterium	12	9.4 ± .2	12	9.7 ± .1
Fusobacterium	4	9.6 ± .6	4	9.4 ± .3
Clostridia	6	8.6 ± .3	11	8.4 ± .3
Veillonella	1	8.0	4	6.8 ± .1
Eubacterium	7	9.1 ± .3	3	8.8 ± .6
Sarcina	0		2	7.0 ± .8
Peptostreptococcus	8	9.6 ± .3	4	9.1 ± .3
Propionibacterium	4	8.8 ± .6	2	9.8 ± 1.1
Acidaminococcus	3	9.5 ± .1	2	8.2 ± 1.0
Peptococcus	1	9.2	1	9.3
Lactobacillus	1	10.1		

($\log_{10}$ mean ± standard error of the mean organisms/gram dry weight)

microflora of individuals on a general western diet is increased. Indeed, it has been shown that subculture of oral strains of bacteroides (only 5% of which can degrade bile acids) on bile salt containing media caused the percentages of bile-salt degrading organisms to be increased.[15] Therefore, it might be said that dietary animal fat or, for that matter, anything which increases fecal bile acid concentration causes an increase in the ability of colonic microflora to degrade bile acids.

Acknowledgments

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