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The Effect of Bran on Dimethylhydrazine-Induced Colon Carcinogenesis in the Rat (40115)

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The putative role of dietary fiber in preventing colon cancer has generated a great deal of interest and controversy (1, 2). Epidemiological studies indicate that large bowel diseases, particularly colon cancer, have a characteristic geographic distribution. Low incidences of colon cancer are found where the population consumes a diet high in fiber derived from natural, unprocessed foods. On the other hand, the highest incidences occur in areas where people consume highly refined diets low in fiber and high in fats.

There has been a paucity of animal studies designed to test whether or not dietary fibers indeed have a protective role in chemically-induced colon carcinogenesis. Dimethylhydrazine (DMH), a potent carcinogen with an organotropism for the colonic mucosa of the rat, provides a reliable animal model for such studies (3, 4). The present preliminary study utilized this model to determine whether incorporating wheat bran, a model dietary fiber, into the diet of rats influenced the production of colonic tumors.

Materials and methods. Male Sprague–Dawley albino rats obtained from Charles River Breeding Laboratories, Inc. (approx. 200 g body weight) were used. All animals were housed two or three together in plastic boxes lined with clean hardwood chip bedding (Betta Chip), changed twice weekly, and were allowed free access to food (ground Wayne Lab-Blox or 20% bran diet) and tap water. Ten rats each were randomly assigned to one of four groups: Group I—Control; Group II—30 mg/kg DMH; Group III—20% Bran; and Group IV—Bran and DMH.

The bran diet was prepared in 20 kg batches by mixing ordinary wheat bran (Agway, Inc.) into the standard diet at a concentration of 20%. Although bran diluted the components of the standard diet, note must be made that wheat bran contains 14% protein, 3% fat, and numerous vitamins and minerals as well as 11% *crude* fiber. According to Southgate (5), bran contains 42% *dietary* fiber, itself composed of 7.6% cellulosic polysaccharides, 2.9% lignin, and 31% noncellulosic polysaccharides. The *crude* fiber content of the standard diet was 3.2%, whereas addition of 20% bran increased the *crude* fiber content to 4.5%.

Symmetrical 1,2-dimethylhydrazine dihydrochloride (DMH, 97% pure) obtained from Aldrich Chemical Co. was dissolved in 0.9% NaCl solution (15 mg/ml total salt), and given weekly by stomach tube at 30 mg/kg body weight for a total of 10 doses during the first 9 weeks of the study. The oral route of administration was chosen in this study to parallel more closely the exposure of man to possible dietary carcinogens. The two remaining groups not receiving DMH were similarly given 0.9% NaCl solution alone.

Individual body weights were recorded weekly, and daily food consumption was calculated for each rat thrice weekly. Intestinal transit times and fecal output were estimated during the 16th, 17th and 20th weeks of the study. For this purpose, rats were individually housed in metabolism cages for a 1-week acclimation period, and were allowed free access to food and water. A 2% suspension of carmine dye in 0.9% NaCl solution was then given orally by stomach tube (5 ml/kg body weight) to each rat in Groups I and III to serve as a fecal marker. Beginning 11 hr after intubation of the dye, the feces were observed hourly, and the first appearance of the red dye in the fecal pellets was recorded. Fecal output was estimated in all groups as the total wet weight of feces collected in a 24-hr period from each rat.

The rats were killed at 21 weeks by an overdose of ether, 12 weeks after the last dose of DMH. The intestinal tract was opened longitudinally and carefully examined for polypoid or sessile tumors. Raised areas of

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the colonic mucosa, especially near Peyer's patches, which were not obvious as neoplasms grossly were all sampled for microscopic confirmation. In addition, a representative number of the colonic and duodenal tumors were fixed in cold 70% ethanol-10% buffered formalin–glacial acetic acid (20:2:1) or 10% neutral buffered formalin, and embedded in paraffin. Sections 4 μm thick were stained with H&E and PAS for histopathological examination.

Statistics. Data was expressed as mean ± SE. All statistics were done using Student's *t* test.

Results. Gross observations. Rats from all groups were generally normal in appearance and behavior throughout the 21-week study. Melena was seen around the anus of a few tumor-bearing animals, and one rat from Group IV was found dead at 3 weeks, apparently due to accidental intubation of the DMH solution into the lungs.

Body weights and food consumption. The mean body weights (Table I) of rats in Groups II and IV were lower ($P < 0.05$) than Control (Group I) at 5 weeks. This trend was more pronounced at 10 weeks ($P < 0.01$–0.001), and may reflect the toxicity of DMH. Following cessation of DMH treatment, body weight gains in both groups given DMH equalled or exceeded that of the Control group. The reason for the depressed growth rate in rats given the 20% bran diet is not known at this time, but may have been due to adsorption to the fiber of trace elements essential for growth (6). Food consumption varied from day to day within and between groups, but overall average daily food consumption of the standard diet was 28 g/rat compared to 34 g/rat for the 20% bran diet.

Intestinal transit time and fecal output. The transit time-50% for rats given the standard diet was 18 hr compared to ≤11 hr for rats given the 20% bran diet. Mean transit time was also decreased ($P < 0.05$) in the same group. Rats in both groups on the 20% bran diet excreted more feces ($P < 0.05$) than those fed the standard diet (Table II). The average increase in fecal output was greater than the amount of bran in the diet, and so was not simply the result of indigestable fiber in the 20% bran diet.

Tumor incidence and distribution. (Table III.) Gastrointestinal tumors were not found in rats belonging to Groups I and III. Rats given DMH alone had a 100% (10/10) incidence of grossly observable colonic tumors with a mean of 6.4 tumors/rat and a range of 3–12 per rat. There was a slight decrease in the incidence of colonic tumors in rats fed 20% bran and given DMH (67%, 6/9), and a lower ($P < 0.001$) mean of 1.8 tumors/rat/group with a range of 0–5 per rat (Table III). Also, the number of colonic tumors found in rats that had tumors in this

TABLE II. INTESTINAL TRANSIT TIMES AND MEAN FECAL OUTPUT.

Group	Transit time-50%[a] (hr)	Mean transit time (hr)	Fecal output[c] (g)
Control	18	18.6 ± 1.43[b]	6.0 ± 0.68
DMH			7.7 ± 1.06
Bran	≤11	13.9 ± 1.16*	10.0 ± 1.33*
Bran & DMH			9.6 ± 1.09*

[a] First appearance of red-colored fecal pellets in 50% of the rats in each group.

[b] Transit time in one rat was >24 hr; arbitrarily estimated to be 25 hr.

[c] Wet weight/24 hr.

* Statistically different ($P < 0.05$) from Control group.

TABLE I. MEAN BODY WEIGHTS (g) OF RATS GIVEN BRAN, DMH, OR BRAN AND DMH.

	Weeks on Test					
Group[a]	0	2	5	10	15	20
Control	192 ± 4	297 ± 4	406 ± 6	499 ± 6	567 ± 9	585 ± 15
DMH	192 ± 3	291 ± 5	378 ± 10*	451 ± 13**	534 ± 15	581 ± 16
Bran	201 ± 4	293 ± 5	392 ± 9	474 ± 12	530 ± 20	563 ± 26
Bran & DMH	201 ± 3	289 ± 6	372 ± 14*	440 ± 13***	507 ± 12**	527 ± 21*

[a] Statistical differences from Control group.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

TABLE III. EFFECTS OF BRAN ON DIMETHYLHYDRAZINE-INDUCED INTESTINAL TUMORS IN RATS.

	Colonic tumors		Duodenal tumors		Cecal tumors	
Group	Incidence	No./Rat[a]	Incidence	No./Rat[a]	Incidence	No./Rat[a]
DMH	10/10 (100%)	6.4 ± 0.88 (3–12)	8/10 (80%)	1.3 ± 0.33 (0–3)	1/10 (10%)	0.2 ± 0.20 (0–2)
Bran and DMH	6/9 (67%)	1.8 ± 0.62*[b] (0–5)	8/9 (89%)	2.2 ± 0.55 (0–5)	2/9 (22%)	0.2 ± 0.15 (0–1)

[a] Mean ± SE for group (range).
[b] Mean number of tumors/rat with tumors was 2.7 ± 0.67 ($P < 0.01$).
* Statistically different ($P < 0.001$) from DMH group.

group was 2.7 tumors/rat, still lower ($P < 0.01$) than that found in Group II. However, the size (mean 3.7 mm diam, range 1–10 mm) and distribution (mean 10.7 cm proximal to anorectal junction) of the colonic tumors were similar between Groups II and IV.

The incidence of duodenal tumors was similar between the two dietary groups. The number of duodenal tumors in Group IV was slightly higher (2.2 tumors/rat/group), but not significantly different from Group II (1.3 tumors/rat/group). The size (mean 5.7 mm diam, range 1–14 mm) and distribution (mean 2.4 cm distal to pyloric junction) of these tumors were similar in both groups.

In addition, two tumors were found in the cecum of one rat from Group II, and one cecal tumor was found in two rats from Group IV.

Histopathology. In general, random and representative tumors from Groups II and IV were subjected to histopathologic examination. Polypoid adenomas, adenocarcinomas, and mucinous adenocarcinomas were noted in agreement with earlier reports (7). No attempt was made to characterize histologically every tumor counted grossly. One interesting feature, though, was a striking absence of PAS-positive material in the polypoid adenomas which correlated with the relative absence of mature goblet cells.

Discussion. There is a group of diseases characteristic of Western civilization which has been linked to fiber-depleted diets. These diseases include diverticular disease of the colon, appendicitis, hiatus hernia, hemorrhoids, varicose veins, and cancer of the colon and rectum (1). Burkitt suggests that the occurrance of bowel cancer and fiber depletion is a "reasonably well-supported hypothesis", but little experimental data in animals is available to support or reject this hypothesis. Dietary fibers are known to affect the bacterial flora of the gut (8), and effects on intestinal transit time and fecal output are well documented (9).

In the present experiment, and in keeping with previous reports in man, dietary bran decreased intestinal transit time and significantly increased fecal output. The time allowable for bacterial activation of postulated cocarcinogens would therefore be reduced, and the greater fecal bulk would tend to dilute the carcinogen, as well as bile salts, the latter recently implicated as cocarcinogens (10, 11). These effects were associated with a reduction in the incidence and number of colonic tumors in rats, suggesting that this dietary fiber provides partial protection against chemically-induced colon carcinogenesis.

The number and incidence of duodenal tumors were similar between dietary groups. The close proximity of these tumors to the opening of the common bile duct suggested that an active metabolite of DMH was being excreted directly into the bile, or that the high local concentration of bile salts was acting to promote tumorigenesis in this area. It should be mentioned that the mechanism of carcinogenesis of DMH has a significant systemic component (12). The active metabolite of DMH, methylazoxymethanol, can reach the epithelial cells of susceptible mucosa via the systemic circulation independent of the lumenal contents of the gut. The fact that bran probably exerts its major physiological effects in the colon may explain the absence of any reduction in the mean number of duodenal tumors in rats given the bran diet.

The results of this study are in agreement with Wilson *et al.* (13) who noted that bran reduced the incidence of colon tumors induced by DMH in rats fed diets high in beef fat or corn oil. On the other hand, Ward *et*

al. (14) found that while alphacel (refined cellulose) had little effect on the number of colon tumors, small intestinal tumors were reduced by addition of high levels of this fiber to the diet. The differences in the mode of administration of DMH and the type of fiber used are probably the major reasons for this discrepancy. Cellulose is only one chemically-defined component of dietary fiber, while bran is a substance with complex nutritional and chemical characteristics (15). The modest increase in *crude fiber* of the 20% bran diet should not obscure the fact that the *dietary fiber* content is qualitatively different on a much greater scale.

Summary. The putative role of dietary fiber was investigated in dimethylhydrazine (DMH)-induced colon carcinogenesis in rats. Four groups of 10 male Sprague–Dawley rats each were used: Group I—Control; Group II—30 mg/kg DMH; Group III—20% bran; and Group IV—Bran and DMH. Intestinal transit times were decreased and fecal outputs were increased in rats given the 20% bran diet. In Group II, all 10 rats had tumors with a mean of 6.4 tumors/rat whereas in Group IV, 6 of 9 rats (67%) had tumors with a mean of only 1.8 tumors/rat/group and 2.7 tumors/rat which had tumors. The size and distribution of these tumors were similar in both groups. Bran in the diet had little effect on the incidence, number, and distribution of duodenal tumors.

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