Wheat Bran and Psyllium Diets: Effects on N-MethylNitrosourea-Induced Mammary Tumorigenesis in F344 Rats

Leonard A. Cohen, Zhonglin Zhao, Edith A. Zang, Tin T. Wynn, Barbara Simi, Abraham Rivenson*

Background: Experimental and epidemiologic evidence suggests that increased dietary fiber is associated with decreased breast cancer risk. Little is known about the role played by different types of fiber and, particularly, mixtures of soluble and insoluble fibers similar to those consumed by human populations in reducing breast cancer risk. High intake of fiber may suppress bacterial hydrolysis of biliary estrogen conjugates to free (absorbable) estrogens in the colon and thus may decrease the availability of circulating estrogens necessary for the development and growth of breast cancers. Purpose: The purpose of this study was to evaluate the effect of wheat bran (an insoluble fiber) and psyllium (a soluble fiber) alone and in combination on overall estrogen status, on fecal bacterial β-D-glucuronidase (a key diet-responsive estrogen-deconjugating enzyme) activity, and on the induction of mammary tumors in rats treated with N-methylnitrosourea (MNU). Methods: One hundred fifty virgin female F344 rats were fed the NIH-07 diet from 28 days of age until 50 days of age; they were then given a single dose (40 mg/kg of body weight) of MNU by tail vein injection. Three days later, they were randomly assigned to one of five experimental dietary groups (30 animals per group). Soft, white wheat bran (45% dietary fiber content) and psyllium (80% dietary fiber content) were added to a modified (high-fat) American Institute of Nutrition (AIN)-76A diet at the following percents, respectively: 12% + 0% (group 1), 8% + 2% (group 2), 6% + 3% (group 3), 4% + 4% (group 4), and 0% + 6% (group 5). Blood, urine, and feces were collected and analyzed by radioimmunooassay techniques for estrogens. Cecal contents were analyzed for bacterial β-D-glucuronidase activity. After 19 weeks on the experimental diets, the rats were killed, and mammary tumors were counted and classified by histologic type. Cumulative tumor incidence was evaluated by the Kaplan–Meier life-table method and the logrank test. Tumor number was evaluated by the chi-squared test of association, and tumor multiplicity was evaluated by the Mantel–Haenszel chi-squared test. All statistical tests were two-tailed. Results: As the level of psyllium relative to that of wheat bran increased, the total tumor number and multiplicity of mammary adenocarcinomas in rats decreased as a statistically significant linear trend across groups 1-5 (P<.05). Compared with the group given wheat bran alone, the group given the 4:1 (wheat bran:psyllium) combination had maximum protection against mammary tumorigenesis, while the groups given the 4:1 or 2:1 (wheat bran:psyllium) combination or psyllium alone had intermediate protection. No statistically significant differences in circulating estrogens or urinary estrogen excretion patterns were observed among the five experimental groups. Fecal estrogen excretion, however, decreased with increasing levels of psyllium (P<.01), and fecal β-D-glucuronidase activity exhibited a decreasing trend with respect to the increasing psyllium content of the diet across groups 1-5 (P<.01). Conclusions: The addition of a 4%:4% mixture of an insoluble (wheat bran) fiber and a soluble (psyllium) fiber to a high-fat diet provided the maximum tumor-inhibiting effects in this mammary tumor model. Although increasing levels of dietary psyllium were associated with decreased cecal bacterial β-D-glucuronidase activity, these changes were not reflected in decreased circulating levels of tumor-promoting estrogens. Therefore, the mechanism(s) by which mixtures of soluble and insoluble dietary fibers protect against mammary tumorigenesis remains to be clarified. [J Natl Cancer Inst 1996;88:899-907]

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Epidemiologic evidence provides support, with some exceptions (1,2), for the hypothesis that dietary fiber or compounds of plant origin associated with dietary fiber may be protective against breast cancer (3-7). Moreover, studies by Cohen et al. (8) and Arts et al. (9) have directly demonstrated that supplemental wheat bran suppressed the development of chemically induced rat mammary tumors. Several mechanisms have been proposed by which dietary fiber may exert its protective effects (10). Primary among these mechanisms is the concept that fiber exerts its preventive effects on breast cancer by altering the enterohepatic recirculation of absorbable estrogens (11). Studies (12-14) have shown that women consuming high levels of fiber exhibit (a) decreased urinary excretion of conjugated (nonabsorbable) estrogens, (b) decreased serum estrogens including estrone sulfate (E\textsubscript{1S}), the principal circulating form of estrogen, and (c) enhanced fecal elimination of estrogens. On the basis of these and other findings, it has been proposed (11) that the observed alterations in estrogen disposition were a consequence of the growth-suppressing effect of fiber on deconjugating (β-D-glucuronidase-producing and sulfatase-producing) colonic bacteria. By suppressing bacterial hydrolysis of biliary estrogen conjugates to free (absorbable) estrogens in the colon, high fiber intake was hypothesized to decrease the availability of circulating estrogens necessary for breast cancer growth and development (13).

Based largely on earlier epidemiologic studies linking low fiber intake to increased colon cancer risk, Lanza et al. (15) and several government agencies (16-20) have proposed that total dietary fiber intake (currently, 10-12 g/2000 calories) should be increased to 25 g or more and should come from a broad variety of food sources for the promotion of normal bowel health and for optimal protection against colon cancer. However, with the exception of the study by Alabaster et al. (21) in a rat colon tumor model system, no studies have been reported concerning the influence on experimental cancer development of mixtures of soluble and insoluble fibers, similar to those found in the U.S. diet.

The purpose of our study, therefore, was to assess the effects of mixtures of two fiber-rich isolates—soft, white wheat bran (which is largely insoluble) and psyllium (a common source of soluble fiber derived from the husk of the plant Plantago ovata)—on N-methyl/3-nitrosourea (MNU)-induced mammary tumor development in rats. In addition, by comparing serum, urinary, and fecal estrogen levels with cecal bacterial activity, we tested the hypothesis that mixtures of wheat bran and psyllium selectively alter the enterohepatic recirculation of estrogens.

Materials and Methods

Animal Care and Adherence to Guidelines

Animals received proper care and maintenance in accord with institutional guidelines. Three rats were housed together in a polyethylene cage that contained hardwood shavings and was covered with a filter top. The animal room was controlled for temperature (24 °C ± 2 °C), light (12-hour cycle), and humidity (50%). Diets were provided in powdered form, and tap water was provided ad libitum. Stainless-steel "J"-type powder feeders were used to prevent scattering of food.

Protocol for Experimental Tumor Induction

One hundred fifty female inbred F344 rats (starting age, 28 days old; Charles River Breeding Laboratories, North Wilmington, MA) were maintained on the standard Open Formula Rat and Mouse Ration (NHI-077) diet (4.5% fat, 23.5% protein, 50% carbohydrate, and 4.5% fiber) (Zeigler Bros., Gardners, PA) (22) until 50 days of age. All rats were then assigned to one of five groups of 30 animals each by recognized randomization procedures (23) to equalize initial weight. At 50 days of age, all rats received a single dose (40 mg/kg of body weight) of MNU (Ash Stevens Inc., Detroit, MI) by tail vein injection. MNU was dissolved in a few drops of 3% acetic acid and diluted with distilled water to give a stock solution of 10 mg of MNU/mL, which was administered within 24 hours of preparation (24).

Dietary Fiber Supplements and Administration of Specified Diets

A certified lot of the soft, white wheat bran was supplied by the American Association of Cereal Chemists, St. Paul, MN. Before being packed and stored, the bran was processed through an enzyme-deactivation steamer system. The major components of soft, white wheat bran are as follows: total carbohydrate, 63.8%; fat, 6.8%; protein, 16.0%; ash, 6.5%; and moisture, 6.0%. Total dietary fiber was 45.0%, neutral detergent fiber was 30.0%, starch was 16.0%, and pectin was 2.0%; 100% of the particles passed through an American Association of Cereal Chemists’ 80 mesh screen (0.212 mm ± 10 μm). The above analyses were conducted for the American Association of Cereal Chemists by Medallion Laboratories, St. Paul, MN. Psyllium husk fiber (U.S. Pharmacopeial Convention, 22nd ed.) was obtained from J. B. Laboratories, Inc., Holland, MI. The major components of psyllium are as follows: total carbohydrate, 80%; fat, 2%; protein, 5%; moisture, 2%; and total dietary fiber, 80%.

Diets were prepared in 15-kg lots in our kitchen and were stored at 4 °C until use. The diets were formulated to account for the carbohydrate, protein, and fat contributed by the psyllium and soft, white wheat bran. The diets provided approximately 18 calories per day as fat (40% of total calories). Rats were fed three times per week, and feeders were removed and washed after each feeding.

Three days after MNU administration, the rats were transferred to one of five different experimental diets (Table 1). The diets were formulated in our kitchen on the basis of a modification of the American Institute of Nutrition (AIN)-76A diet (25) (Table 1). The standard AIN-76A diet has 5% fat, 20% protein, 65% carbohydrate, and 5% fiber. All ingredients were obtained from Dyets Inc., Bethlehem, PA. The rats remained on these diets for the duration of the experiment (19 weeks).

Observation Schedule

At weekly intervals, beginning 4 weeks after MNU injection, each rat was weighed, and the location of palpable tumor(s) and the date they were found were recorded.

Collection of Urine and Feces

Approximately 18 weeks after the administration of MNU, six (20%) of 30 rats from each group were placed in metabolism cages, and their urine and feces were collected over a 24-hour period. Urine (typically, 10-15 mL every 24 hours) was collected on dry ice in 50-mL conical centrifuge tubes and then thawed and centrifuged at 2000g for 20 minutes at 4 °C. The clear supernatant was collected and stored at −20 °C until use. Fecal pellets were collected, placed in tightly capped 20-mL scintillation vials, and stored at −20 °C until analysis.

Necropsy, Serum and Cecal Collection, and Histopathology

Approximately 19 weeks after MNU administration, the experiment was terminated. Blood was drawn from the rats by heart puncture under ketamine anesthesia between 11 AM and 1 PM in order to minimize variation in hormone levels (26). The blood was collected in evacuated, sterile AutoSep tubes (Terumo
Medical, Elkton, MD). Serum and red blood cells were then separated by centrifugation at 200g for 20 minutes at 4 °C. Serum was stored at -20 °C until it was assayed. Blood was taken during metestrus, diestrus, and late estrus (not proestrus). Rats were then killed by carbon dioxide inhalation, and mammary tumors (classified as palpable or nonpalpable but grossly visible) were excised, fixed in 10% buffered formalin, embedded in paraffin blocks, and stained with hematoxylin—eosin for histologic examination. At necropsy, the ceca were excised, tied off at the open end, and placed in vials flushed with N2 to maintain anaerobic conditions. The vials were then sealed and stored at 4 °C until use. For assay, the vials containing the ceca and the empty vials were weighed, and the weight of the cecal contents was determined by difference. The cecal contents were placed on aluminum foil under a steady stream of a mixture of N2, O2, and CO2 gases. The cecal contents were then suspended in phosphate-buffered saline (pH 7.0), vortexed, and then centrifuged at 600 rpm for 5 minutes (4°C). The supernatant was filtered through Celite (Aldrich Chemical Co., Milwaukee, WI). Following enzymatic hydrolysis, the mixture was extracted two times with 50 mL diethyl ether to obtain free estrone (E1). The ether phase was then dried under N2 gas, and the residue was assayed by RIA for E1 by use of the RIA kit (Wein Laboratories Inc.). The E1 assay is highly specific (cross-reactivity with 17β-estradiol [E2] and estriol [E3] <0.025%) and sensitive (10 pg/mL detectable). Radioactive aliquots were counted in a liquid scintillation counter (LKB; Wallac Inc., Gaithersburg, MD), and RIA data were processed by use of a computer program obtained from Robert Maciel Associates Inc., Arlington, MA. The method used was a modification of that described by Adlercreutz et al. (29). Urinary estrogens are present primarily as water-soluble conjugates. Hence, a deconjugation step is required prior to RIA. For E1 and E2, 0.3 mL of urine was added to 0.7 mL of H2O2, and the mixture was then centrifuged at 2000 U P-glucuronidase activity per sample, was then vortex-mixed and incubated overnight in a shaker bath at 37 °C. It was then extracted twice with 5.0 mL diethyl ether. The deconjugated estrogens present in the ether fraction were dried under N2 gas, and the residue was assayed by RIA for E1 by use of the RIA kit (Wein Laboratories Inc.). The E1 and E2 kits are highly specific (cross-reactivity with E1 or E2 or E3 <0.025%) and sensitive (10 pg/mL detectable). All extractions were done in duplicate. The data were expressed as picograms per milliliter of E1S (as E1) after adjustment for extraction efficiency.

**Urinary Estrogen Analysis**

The method used was a modification of that described by Adlercreutz et al. (29). Urinary estrogens are present primarily as water-soluble conjugates. Hence, a deconjugation step is required prior to RIA. For E1 and E2, 0.3 mL of urine was added to 0.7 mL of H2O2 for E1, 0.06 mL of urine was brought up to 1 mL with H2O. After vortex mixing, 0.2 mL of β-g-glucuronidase (type H-5 from *H. pomatia*) was added to the urine sample. The reaction solution, containing 2000 U β-glucuronidase activity per sample, was then vortex-mixed and incubated overnight in a shaker bath at 37 °C. It was then extracted twice with 5.0 mL diethyl ether. The deconjugated estrogens present in the ether fraction were dried, and the residue was used for RIA of E1, E2, and E3 with the commercially available kits (Wein Laboratories Inc.). The E1 and E2 kits are highly specific (cross-reactivity with E1 or E2 or E3 <0.025%) and sensitive (10 pg/mL detectable). All extractions were done in duplicate. The data were expressed as picograms of estrogen per milliliter of creatinine to account for variations in total 24-hour urinary output.

**Assay of Unconjugated Fecal Estrogens**

The method used was based on that of Adlercreutz and Järvenpää (30). Approximately 70%-80% of rat fecal estrogens are present in the unconjugated form (7). Fecal pellets were weighed (wet weight) and then freeze-dried. Following lyophilization, the pellets were weighed again (dry weight) and then ground to a powder with a mortar and pestle, and to 0.25 g of powdered feces was added 5 mL of 70% methanol. The mixture was sonicated for 10 minutes, vortex-mixed for 1 minute, and centrifuged at 20000 g at 20 °C overnight to precipitate lipids and protein. The mixture was centrifuged at 25000 g for extraction efficiency.
for 10 minutes at 4 °C, and the methanol layer was dried under N₂ gas until approximately 2 mL remained. Free estrogens were extracted with ether after 1 mL (pH 4.2) acetate buffer was added to the mixture. The ether layer was then decanted and dried under N₂ gas.

The dried residue was brought up to 70% methanol and passed through a DEAE-Sephadex A-25 column (acetate form). The column was eluted with 1.0, 1.0, and 6.0 mL of 70% methanol. The eluates were combined in a 16 x 100-mm glass test tube and dried under N₂ gas. The resulting residue was dissolved in 1 mL ethanol and vortex-mixed. The solution was transferred to three different tubes for E₅, E₄, and E₂ assay. The tubes were dried again and then assayed by RIA. Data were expressed as nanograms per gram of feces or nanograms per 24 hours of fecal collection.

**Cecal β-δ-Glucuronidase Assay**

The cecal β-δ-glucuronidase assay was based on the method described by Kulkami and Reddy (31). The cecal contents were weighed, mixed into a slurry in 0.1 M phosphate buffer, sonicated on ice for 30 seconds, and then centrifuged at 40g for 15 minutes at 4 °C. Supernatant (1 mL) was added to a 15-mL tube, sonicated on ice, and centrifuged at 26,185g for 20 minutes at 4 °C. The supernatant was saved, and 0.1 mL was added to a 16 x 125-mm glass test tube. To each tube was added 0.8 mL of 0.1 M phosphate buffer and 0.1 mL of phenolphthalein-β-δ-glucuronide, and the reaction mixture was then mixed and incubated at 37 °C for 30 minutes. The reaction was stopped by the addition of 2.5 mL of alkaline glycine buffer (pH 10.25). After dilution of the sample by the addition of 2.5 mL of distilled H₂O, the reaction mixture was allowed to stand for 10 minutes at room temperature. Free phenolphthalein was then measured by absorption spectrophotometry at 540 nm. β-δ-Glucuronidase activity was expressed as micrograms of phenolphthalein liberated per hour per gram of cecal content or as micrograms of phenolphthalein liberated per hour per gram of total cecal content.

**Statistical Analysis**

Tumor-free survival was estimated separately for each group by the Kaplan–Meier product-limit estimate for censored data (32). The survival distributions for the five groups were then compared by the logrank test (33,34). The purpose of the analysis was to test the null hypothesis that the survival distributions of all groups were equal. In addition to the overall test of significance, pairwise comparisons between groups were made.

Tumor incidence (expressed as the percentage of tumor-bearing animals) was compared among the groups by the chi-squared test of association. Overall dose-related associations between tumor incidence across groups 1-5 were tested by linear regression analysis.

**Results**

**Tumor Yields**

As the ratio of wheat bran to psyllium decreased, there was an overall decline in mammary tumor yields. Maximal protection was seen in MNU-treated rats fed a diet that included supplemental wheat bran–psyllium at a ratio of 1:1, and moderate protection was observed in MNU-treated rats fed diets containing supplemental wheat bran–psyllium at 4:1 and 2:1 or psyllium alone, when compared with the group fed wheat bran. When assessed in terms of percent tumor incidence, there was a borderline statistically significant negative trend (P<.08) (Table 2). When tumor yields were assessed in terms of tumor multiplicity or total number of tumors, a statistically significant linear trend (P<.05) was found across groups 1-5. The effect of psyllium was most evident when adenocarcinomas only were included in the analysis (Fig. 1). Tumor latency (i.e., time to development of first tumor) was not significantly delayed in group 4 compared with that seen in the other four treatment groups (Fig. 2).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet</th>
<th>No. of rats with tumor/total No. of rats‡</th>
<th>%</th>
<th>Total No. of tumors§</th>
<th>No. of rats with tumor/total No. of rats‡</th>
<th>%</th>
<th>Total No. of tumors§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AIN-76A + 12% wheat bran</td>
<td>24/30</td>
<td>80</td>
<td>83</td>
<td>16/30</td>
<td>53</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>AIN-76A + 8% wheat bran + 2% psyllium</td>
<td>27/30</td>
<td>90</td>
<td>58</td>
<td>18/30</td>
<td>60</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>AIN-76A + 6% wheat bran + 3% psyllium</td>
<td>24/30</td>
<td>80</td>
<td>62</td>
<td>12/30</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>AIN-76A + 4% wheat bran + 4% psyllium</td>
<td>20/30</td>
<td>67</td>
<td>54</td>
<td>9/30</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>AIN-76A + 6% psyllium</td>
<td>25/30</td>
<td>83</td>
<td>59</td>
<td>13/30</td>
<td>43</td>
<td>18</td>
</tr>
</tbody>
</table>

*Tumor incidence: All pairwise comparisons were not statistically significant when adjusted for multiple comparisons. The unadjusted comparison of group 2 and group 4 was statistically significant for both all tumors and adenocarcinoma only (P = .0286 and P = .0195, respectively). Armitage's test for linear trends across groups, P<.08 for adenocarcinoma only.*

†Tumor number, for all tumors: When unadjusted for multiple comparisons, group 1 versus group 2, P<.053; group 1 versus group 4, P<.0132; and group 1 versus group 5, P<.0439. When adjusted, all pairwise comparisons were not statistically significant by chi-squared test.

‡Number of tumor-bearing rats/total number of rats at risk (n = 30). Since no group had 100% incidence, the numerator is always less than the denominator.

§Number of tumors per group was assessed by chi-squared analysis, whereas multiplicity was assessed by Armitage's test.

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The histopathologic profile of the mammary tumors induced by MNU included intraductal and invasive papillary carcinoma with desmoplastic features and fibroadenoma with ductal carcinoma in situ. The latter category appeared as a fibroadenoma with small clusters of malignant epithelial cells confined to the intraductal space.

**Estrogens**

Single-point serum or urinary conjugated estrogen levels did not vary in any consistent way with the wheat bran/psyllium ratio in the diet or with tumor yields (Table 3).

Fecal estrogen excretion profiles exhibited a statistically significant overall linear decrease as psyllium levels increased (Table 3) for all estrogens. Fecal estrogen excretion was highest in the wheat bran group and lowest in the psyllium group. To account for differences in fecal output, we assessed fecal estrogens in terms of both nanograms of estrogen excreted every 24 hours and nanograms excreted per gram of fecal dry weight. Although differences among groups were more pronounced when expressed in terms of 24-hour output, similar trends were observed regardless of how the data were expressed. As previous studies (9,13) noted, the concentration of estrogen was about 500 times higher in urine and feces than in serum.

**Cecal β-D-Glucuronidase**

To account for differences in cecal mass (Fig. 3) among the treatment groups, β-D-glucuronidase activity was expressed per gram of cecal mass and per total grams of cecal mass. Regardless of the mode of expression, a statistically significant linear decrease in β-D-glucuronidase activity was found as the relative proportion of psyllium to wheat bran increased (Fig. 3). The highest levels were consistently found in the wheat bran group and the lowest levels in the psyllium group. These results indicate that psyllium is a very effective suppressor of β-D-glucuronidase-producing cecal bacteria.

**Cecal and Fecal Weights**

Cecal and fecal wet weights varied inversely as the psyllium content of the diet increased (Table 3). Cecal wet weights in-
Table 3. Blood, urine, and fecal estrogen profiles as a function of diet*

<table>
<thead>
<tr>
<th>Diet supplement</th>
<th>Strain supplement</th>
<th>Urinary estrogens†</th>
<th>Fecal estrogens‡</th>
<th>Weight of fecal and cecal contents§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran, %</td>
<td>Psyllium, %</td>
<td>Estrone sulfate</td>
<td>Estrone</td>
<td>17β-Estradiol</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>177 ± 16</td>
<td>92 ± 13</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>164 ± 21</td>
<td>83 ± 9</td>
<td>76 ± 18</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>171 ± 34</td>
<td>85 ± 14</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>172 ± 29</td>
<td>96 ± 15</td>
<td>61 ± 15</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>201 ± 25</td>
<td>97 ± 7</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>Serum estrogens†</td>
<td>Estrone sulfate</td>
<td>177 ± 16</td>
<td>92 ± 13</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>64 ± 9</td>
<td>76 ± 18</td>
<td>61 ± 11</td>
<td>61 ± 15</td>
</tr>
<tr>
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<td>17β-Estradiol</td>
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</table>

*Five rats were randomly selected from group 1, and six rats each were randomly selected from groups 2-5. Blood, urine, feces, and cecal contents analyzed were obtained from the same rats.

†Values = means ± standard deviation, pg/mL. Estrone sulfate (as estrone) after adjustment for extraction efficiency (70%). Analysis of variance, not statistically significant (NS). Test for linear trend across groups by regression analysis, NS.

‡Values = means ± standard deviation, ng/mg of creatinine. Analysis of variance comparing diet groups, NS. Test for linear trend across groups by regression analysis, NS.

§Values = means ± standard deviation, ng/g of fecal dry weight. Test for linear trend across groups by regression analysis statistically significant at P < .05 for all estrogens. Group 2 differs from groups 4 and 5 for 17β-estradiol and total estrogens (P < .01). Groups 1 and 2 differ from groups 4 and 5 for 17β-estradiol and total estrogens (P < .05). Groups 1 and 2 differ from group 5 for estrone (P < .05). Group 1 (P < .01) and groups 3 and 4 (P < .05) differ from group 5 for estriol. All P values were based on analysis of variance followed by Tukey's adjustment for multiple comparisons.

‖Values = means ± standard deviation, g; 24-hour collection.

¶Test for linear trend by regression analysis, NS.

#Test for linear trend by regression analysis, P < .0001.

Fig. 3. Cecal β-glucuronidase activity plotted as a function of diet group. There was a statistically significant, overall dose-related negative trend across groups 1-5 by linear regression analysis (P < .01). Values are means ± standard deviation (n = 6). The standard deviation for group 5 was 60.
increased significantly across groups 1-5, most likely as a result of the high water-retention properties of psyllium while residing in the cecum. The opposite was the case with fecal wet and dry weights, although the trend toward lower weights in the psyllium-containing groups did not achieve statistical significance. The lower fecal weight in the psyllium-containing groups may be attributed to increased reabsorption of water from psyllium-containing feces in the large bowel and to the greater fermentability of psyllium compared with wheat bran. The percentage of fecal water in all five groups was similar; it averaged 25% and varied from a low of 16% to a high of 34%.

Animal Weight Gains

Rats fed wheat bran alone (group 1) gained weight to a statistically significantly greater extent than rats in groups 4 and 5 (Fig. 4).

Small Group Sample Sizes

In Table 3, none of the three one-way analyses of variance were statistically significant, perhaps because of the small group sample sizes. In an effort to determine the power of these comparisons, we noted that groups 2 and 5 differed the most across all three serum estrogen variables. Power analyses were simplified and based on just this two-group comparison with Student's t test (corrected for type I error to resemble a Tukey's post hoc comparison). For E1S, the probability of detecting a 37-unit (201–164) difference between the two groups on the basis of the given sample sizes and standard deviations (power) was found to be less than 45%; 11 rats in each group are needed for the power to be greater than 80%. Similarly, for E1, the power to detect a 15-unit difference was less than 50%, and 10 rats are needed in each group to achieve at least 80% power. For E2, a 15-unit difference has less than 15% power, so that more than 25 rats are needed in each group to attain 80% power based on the given standard deviations. In terms of how large a difference would have been statistically significant (i.e., to find statistical significance 80% of the time), we would need to see a 55-unit difference in E1S levels between the two groups, a 20-unit difference for E1 and a 35-unit difference for E2 (based on given sample sizes and standard deviations).

Discussion

To our knowledge, this is the first study to demonstrate the inhibitory effects of mixtures of soluble and insoluble fibers in an experimental mammary tumor model. Previous studies conducted by us (8) and other investigators (9) have demonstrated that diets supplemented with wheat bran inhibit MNU-induced mammary tumor development. In these studies, the inhibitory effect of fiber was most evident in rats fed diets high in fat (8).

It is of interest that the effect of fiber was most pronounced when assessed in terms of mammary adenocarcinoma. When fibroadenomas with ductal carcinomas in situ were included in the analysis, the effect was less dramatic. Since ductal carcinomas in situ are considered to be part of a morphologic continuum leading from an original initiating event to a fully developed carcinoma (39), this suggests that one effect of fiber may be to act at the clonal expansion stage of the carcinogenic process.

Differences in body weight gain were noted among the groups fed wheat bran and psyllium. The reason for these differences is unclear, since the diets were formulated to be isonutrient with respect to one another. One possibility is that availability of nutrients in wheat bran was greater than that in psyllium. Nonetheless, the overall differences in animal weights among the treatment groups, though small, could account in part for the differences observed in tumor yields.

The mechanism(s) by which fiber may inhibit breast cancer have been reviewed (10,40). They include reduction of circulating tumor-promoting estrogens via suppression of bacterial β-d-glucuronidase activity in the colon and cecum (11); direct binding of estrogens in the colon by fiber (8); or the presence of isoflavonoids (10), phytates (41), and protease inhibitors (42) in fiber, all of which have demonstrated anticancer properties. In
this study, we tested the hypothesis that tumor inhibition is the direct consequence of the ability of dietary fiber to alter the enterohepatic recirculation of estrogens. According to this hypothesis, a decrease in circulating estrogens, particularly E$_2$/S, the most abundant and presumably diet-responsive circulating estrogen (43,44), coupled with a decrease in $\beta$-d-glucuronidase and a concomitant increase in estrogen excretion, would be expected in the group fed 4% wheat bran-4% psyllium compared with the group fed wheat bran alone. While increasing levels of psyllium did indeed suppress $\beta$-d-glucuronidase activity (as shown previously by Roberts-Andersen et al. (45)), there was no concomitant change in circulating or urinary estrogen concentrations.

It should be noted that, since serum estrogen measures in this study represent one point in time, in contrast to urinary and fecal measurements, which represent 24-hour clearance levels, the possibility exists that changes in serum levels actually did occur at earlier points in the experiment but were not detected. This possibility could be readily tested by cannulation followed by longitudinal assays during the course of a feeding study. In this manner, transient alterations in circulating estrogens, imposed on circadian and estrus rhythms, could possibly be detected. Also worth noting is the fact that, because of the relatively small sample size (n = 6), there may have been insufficient power to detect differences between groups. Future studies with larger sample sizes will address this problem.

Precisely how mixtures of soluble and insoluble fibers interact physiologically in the gut is poorly understood. Soluble fibers such as psyllium are known to alter the emulsification and lipolysis of fat, interact with sterols, readily form gels with water, and are susceptible to bacterial fermentation to short-chain fatty acids such as butyrate (46). Also, it is interesting that, in the case of psyllium, when compared with cellulose, fecal aerobes have been reported to increase with respect to anaerobes, colonic pH is decreased, and $\beta$-d-glucuronidase is suppressed (45). On the other hand, wheat bran, an insoluble fiber, does not readily undergo fermentation and, consequently, does not lower the pH of the gut: however, it does bind estrogens (8) and also suppresses $\beta$-d-glucuronidase activity (45). Psyllium, therefore, appears to share certain properties with insoluble fiber; i.e., it can cause a suppression of bacterial $\beta$-d-glucuronidase and an increase in the relative numbers of fecal aerobes. Psyllium also has properties normally associated with soluble fibers, i.e., high water retention, fermentability, and lowering of colonic pH. On the basis of our results, psyllium exerts a suppressing effect on $\beta$-d-glucuronidase activity in a dose-related manner. Nonetheless, although addition of psyllium to wheat bran clearly modulates the microbial ecology of the cecum, the relationship, if any, between bacterial $\beta$-d-glucuronidase activity and mammary tumor development remains to be clarified.

It is of interest that our results are in close accord with those reported by Alabaster et al. (21) in the dimethylhydrazine-induced colon tumor model. Since the incidence of and rates of mortality from breast and colon cancers are concordant in many populations (47-51), the possibility may be considered that a common environmental factor is involved in the cause and prevention of both diseases. Minor constituents present in wheat bran and/or psyllium, including phytates (41), protease inhibitors (42), and isoflavonoids (Adlercreutz H: unpublished data), may, therefore, merit more scrutiny in the future.

References

(24) Chan PC, Head JF, Cohen LA, Wynder EL. Influence of dietary fat on the induction of mammary tumors by N-nitrosomethylurea: associated hor-