Effects of Chronic Dietary Beer and Ethanol Consumption on Experimental Colonic Carcinogenesis by Azoxymethane in Rats

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ABSTRACT

Epidemiological studies have shown an association between consumption of alcoholic beverages, particularly beer, and carcinoma of the large bowel, especially the rectum. We studied the effects of chronic dietary beer and ethanol consumption on experimental colonic carcinogenesis, fecal bile acid and neutral sterol levels, fecal bacterial flora, and colonic epithelial DNA synthesis. Ten-week-old male Fischer 344 rats were pair fed throughout the study with Lieber-DeCarli-type liquid diets providing comparable total carbohydrates, proteins, fats, and calories. The diets provided 23 or 12% of calories as alcohol in beer (Hi-Beer and Lo-Beer groups), 18 or 9% of calories as reagent ethanol (Hi-EtOH and Lo-EtOH groups), or no alcohol (control group). After 3 weeks of dietary acclimatization, 10 weekly s.c. injections of the bowel carcinogen azoxymethane, 7 mg/kg, were given (weeks 1-10). At necropsy in week 26, the high alcohol groups (Hi-Beer and Hi-EtOH) showed a significantly reduced incidence of tumors in the right colon (42 and 46% versus 81% in control, P < 0.01 and P = 0.02) but no effect on left colonic tumorigenesis. In contrast, the low alcohol groups (Lo-Beer and Lo-EtOH) showed a trend toward increased incidence and proportion of tumors in the left colon (incidence of 42 and 35% versus 15% in control, P = 0.06 for Lo-Beer; 28 and 30% of tumors in left colon versus 11%, P = 0.08 and P = 0.07) but no effect on right colonic tumorigenesis. Numbers of right colonic tumors were inversely correlated with alcohol consumption of all rats (r = -0.350, P < 0.001), but left colonic tumors were not correlated. Fecal bile acid and neutral sterol levels, fecal bacterial counts, and colonic epithelial DNA synthesis did not correlate with the effects of alcohol consumption on colonic tumorigenesis. Our findings suggest that: (a) modulation of experimental colonic tumorigenesis by chronic dietary beer and ethanol consumption was due to alcohol rather than other beverage constituents; (b) tumorigenesis in the right and left colon was affected differentially by the levels of alcohol consumption, reflecting complex interactions among the potential mechanisms for alcohol effects in the model used.

INTRODUCTION

Colorectal carcinoma is the second most common cause of cancer deaths in the United States (1). Epidemiological studies have shown the importance of dietary factors, particularly high dietary fat and low fiber, in the pathogenesis of colorectal carcinoma (2-4). In addition, numerous epidemiological studies using a variety of statistical techniques have shown an association between consumption of alcoholic beverages and cancer of the large bowel (5-16). The relationship between beer consumption and rectal cancer has been emphasized in some of these studies (7, 8, 11, 13-16).

Administration to rodents of AOM or its parent compound dimethylhydrazine provides widely used and useful experimental models of large bowel carcinogenesis (17-20). Studies of the effects of chronic dietary ethanol consumption in these models have produced conflicting results (21-25). In one study with dimethylhydrazine, increased tumorigenesis occurred in the distal 5 cm of the large bowel (21). On the other hand, another study with the same carcinogen showed no effect of chronic dietary ethanol or beer consumption on large bowel carcinogenesis (22). Because of these conflicting results, we studied the effects of chronic dietary beer and ethanol consumption on experimental large bowel carcinogenesis in the azoxymethane-treated rat model. We also assessed fecal bile acid and neutral sterol levels, fecal bacterial flora, and colonic epithelial DNA synthesis to attempt to define the mechanisms of alcohol effects.

MATERIALS AND METHODS

Animals and Housing. Guidelines for the care and use of laboratory animals promulgated by the Division of Comparative Medicine of The Johns Hopkins Medical Institutions were followed. Ten-week-old male Fischer 344 rats weighing 170-210 g were obtained from Harlan-Sprague Dawley, Indianapolis, IN. The rats were distributed on the basis of weight into five experimental groups, each with 26 rats. The rats were housed singly in plastic cages with wood chip bedding in animal quarters with controlled temperature (21-22°C), humidity (30-50%), and light (12-h cycles).

Diets and Pair-Feeding Technique. Beer from one brewing batch was obtained and analyzed for total proteins, carbohydrate, protein, lipids, and alcohol content with the cooperation of a well-known major brewery. Five isocaloric Lieber-DeCarli-type liquid diets were then formulated by BioServ, Frenchtown, NJ, to provide 23 or 11% of total calories as alcohol from either beer (Hi-Beer and Lo-Beer) or reagent ethanol (Hi-EtOH and Lo-EtOH), and a no-alcohol control. The formulas provided for equivalent percentage of calories from proteins, carbohydrates, or fats. The Hi-Beer diet was prepared for feeding by adding the diet mix to beer; the other four diets included distilled water. Because beer contains a variety of vitamins, minerals, trace elements, and organic compounds in μg to mg amounts per 100 ml (26, 27), an exact match of the five diets was not feasible. Due to a vendor's error in the recipe for the two reagent ethanol diets, which was discovered after the study was completed, less ethanol was provided in the Hi-EtOH and Lo-EtOH diets than was planned. The dietary compositions as used in the study are shown in Table 1.

Pair feeding with graduated feeding tubes (BioServ) was used to control diet consumption. Each pair-feeding group consisted of five rats of similar weight, one from each of the five experimental groups.


The abbreviations used are: AOM, azoxymethane; ODC, ornithine decarboxylase.
sterols with hexane, the aqueous layer of the saponified extract was procedures described previously (28-29). After extraction of the neutral sample was dried to determine the moisture content. The preliminary aliquot of about 200 mg of the ground feces was and in the week prior to necropsy (week 25). The feces were collected daily and frozen at —70°C until analysis. An accurately weighed each experimental group, 3-day fecal specimens were collected in wire-bottom metabolic cages for separation of urine and feces. The collections were obtained 1 week after the final carcinogen dose (week 11) and in the week prior to necropsy (week 25). The feces were collected daily and frozen at ~70°C until analysis.

For analysis, the 3-day collections were milled in a coffee grinder. An accurately weighed aliquot of about 200 mg of the ground feces was used for extraction of bile acids and neutral sterols. A duplicate aliquot of the sample was dried to determine the moisture content. The preliminary saponification and extraction of bile acids were carried out by procedures described previously (28-29). After extraction of the neutral sterols with hexane, the aqueous layer of the saponified extract was acidified with 6 N hydrochloric acid, and the bile acids were extracted with ethyl acetate.

The ethyl acetate extract was washed with water until free of acid. The bile acids were converted to the corresponding methyl esters and subjected to thin-layer chromatography for the separation of the substituted cholanics acids into several classes (30). The bile acid zones were visualized under UV light after spraying the plates with 8-hydroxy-1,3,6-pyrenetrisulfonic acid in methanol. Each bile acid zone was isolated and extracted separately. The bile acids were converted to the corresponding trifluoroacetates and quantitated by gas-liquid chromatography as described previously (31, 32).

The hexane extract was evaporated under nitrogen and the residue was reconstituted to 0.1 ml with the same solvent. Two equal aliquots of this solution were plated on two separate silica gel G plates. The neutral sterols on one of the plates were treated with about 30 μl of 1% bromine in chloroform to convert cholesterol to the corresponding dibromide in order to affect the separation of cholesterol from cholesterol. The plates were developed with ether: heptane (55:45) and sprayed with Rhodamine 6G to locate the sterol zones under UV light. A standard sterile mixture containing cholesterol, cholestanol, coprostanol, and coprostanone was processed side-by-side with each batch of samples. The sterols were extracted from the zones corresponding to each of the standards and quantified by gas liquid chromatography as reported previously (31, 32).

Results were expressed as μg/g dry weight, μg/g wet weight, μg/day, and μg/day/kg body weight for statistical analysis. The findings were similar and the results for μg/g wet weight are presented.

Fecal Bacterial Flora Procedures. In six rats from each experimental group, fresh fecal specimens defecated directly into preweighed tubes containing precluded Schaedler’s broth medium (BBL, Cocksaysville, MD) were obtained at two times: after 3 weeks of dietary acclimatization and before first carcinogen injection (week A3), and one week after last AOM injection (week 11). The tubes were weighed and the weight of the fecal pellets was calculated. The fecal pellets were dispersed by vortexing the liquid media. Samples were serially diluted and plated in duplicate onto tryptcain-free agar with 5% sheep blood (BBL) and MacConkey’s agar plates (BBL) for quantitation of total aerobic bacteria and aerobic/facultative gram-negative bacteria, respectively. Plates were incubated for 48 h at 37°C. For quantitation of total anaerobic bacteria and Bacteroides species, samples were plated in duplicate onto plates with tryptcain-soy agar with 5% sheep blood (BBL) and plates
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with Columbia agar base (BBL) with 5% sheep blood, gentamicin (10 
µg/ml), and vancomycin (8 µg/ml), respectively. These plates were 
incubated anaerobically in a Gas Pak system (BBL) at 37°C for 48 h.
Following incubation, plates containing between 30–300 colony form-
ing units were counted, the duplicate dilutions averaged, and the results 
expressed as colony forming units per gram wet weight of feces.

DNA Labeling and Necropsy Procedures. The rats were killed 16 
weeks after the last injection of azoxymethane (week 26). One hour 
before sacrifice in the early afternoon, seven pair-fed groups with one 
rat from each experimental group were given a single i.p. injection of 
tritiated thymidine (0.5 µCi/kg, approximately 80 Ci/mmol, New Eng-
land Nuclear, Boston, MA). The rats were killed by decapitation. The 
hepatic and splenic flexures of the colon were marked by suture ties in 
the mesentry. The entire gastrointestinal tract was removed and 
opened. The large bowel and anus along with the distal 5 cm of the ileum were pinned on a paraffin block along with a segment represent-
ing the esophagus through the proximal jejunum and a segment of mid 
jejunum. Any tumors not included in these standard segments were 
also submitted for fixation with notation of the anatomic location. 
Following fixation in Hollande fixative and washing, the specimens 
were reexamined for the presence of any grossly visible tumors. The 
tumor-containing segments were traced on the record sheet for the rat 
and the position of tumors diagrammed. All tumors and any suspicious 
lesions were submitted for histopathological examination. The lymph-
oid nodules of the right and left colon were submitted in all rats.

For autoradiography, specimens of grossly normal mid right and 
mid left colon were submitted for histological processing with careful 
orientation. The histological slides were coated with NTB-2 emulsion 
(Kodak, Rochester, NY), incubated for 2 weeks at 4°C, and developed.
Well-oriented crypts showing the lumen from the surface to the base 
were analyzed morphometrically for total number of cells and position 
of labeled cells. For analysis of positions of labeled cells, the total 
number of cells in each crypt was corrected to 50. The positions of 
labeled cells in the upper, middle, and lower third of the crypt epithe-
loid were expressed as a percentage of all cells which were labeled 
(labeling index) and as the percentage of total labeled cells.

Statistical Analysis. All rats were included for analysis. Five died of 
tumor complications before sacrifice (one in the Hi-EtOH in week 21, 
one in the Lo-EtOH group in week 24, and three in the control group 
in weeks 24 and 25). Incidences and proportions of tumors in the 
various segments of the gastrointestinal tract were compared by Fisher 
exact test. The ecum, ascending, and transverse colon were considered 
together as right colon. Differences between means were calculated by 
two-tailed t tests for paired and unpaired data, Wilcoxon-matched pairs 
signed-ranks test, and Mann-Whitney U test. For the studies of con-
comitant variation, correlation was assessed by calculation of Pearson 
product moment correlation coefficient and the nonparametric Spear-
man rank correlation coefficient (r). Stepwise multiple regression anal-
ysis (34) was used to assess the multiple correlations with tumor 
outcome, and only those parameters which remained statistically sig-
nificant by F test were considered acceptable. Data Analysis Interactive 
System (DAISY) professional statistical software (Rainbow Comput-
ing, Northridge, CA) was used.

RESULTS

Dietary Intake and Body Weight. Caloric intake was similar in 
all five experimental groups (Fig. 1), as a result of pair 
feeding of liquid diets providing comparable calories. Intake of 
diet fell following the first carcinogen injection and then showed 
a slow decline throughout the remainder of the study. Ethanol 
consumption (Fig. 2) reflected the intake of liquid diet and the dietary ethanol content (see 
Table 1).

Fig. 1. Caloric intake during the study. Caloric intake was similar in all five 
experimental groups as a result of pair-feeding liquid diets which provided 
comparable calories (see Table 1).

Fig. 2. Ethanol consumption during the study. The ethanol consumption of 
each group reflected the intake of liquid diet and the dietary ethanol content (see 
Table 1).

Fig. 3. Body weights during the study. Mean body weights during the initial 
weeks were similar in the experimental groups other than the Hi-EtOH group, 
which lagged behind the other four groups despite pair feeding. From week 15 
onward, mean body weight in the Lo-Beer, Lo-EtOH, and control groups showed 
a gradual decline. By contrast, the Hi-Beer and Hi-EtOH groups, the groups 
found to have the lowest incidences of tumors, remained steady.

two groups found to have the lowest tumor incidences, showed a 
gradual decline.

Tumor Outcome. The anatomic sites of the large bowel tu-
mors in the 26 rats of each experimental group are shown diagrammatically in Fig. 4. The colonic tumor incidences are
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Fig. 4. Diagram of anatomic distributions of large bowel tumors in the 26 rats in each experimental group. The Hi-Beer and Hi-EtOH groups showed fewer tumors in the right and transverse colon than the control group, but no differences from control in the left colon. By contrast, the Lo-Beer and Lo-EtOH groups showed more tumors in the left colon than the control group, but no change in the right and transverse colon.

Fig. 5. Diagram of colonic tumor incidences. The incidence of right and transverse colonic tumors was reduced significantly (+) in the Hi-Beer and Hi-EtOH groups, whereas the Lo-Beer and Lo-EtOH groups showed no significant differences from control for this site. By contrast, the Lo-Beer and Lo-EtOH groups showed a trend toward higher incidences of left colonic tumors than the control group (borderline for statistical significance in the Lo-Beer group). See Table 2 for data on distribution of colonic tumors.

Table 2. Anatomic distribution of colonic tumors

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Right + Transverse</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>Hi-Beer</td>
<td>18</td>
<td>43</td>
</tr>
<tr>
<td>Hi-EtOH</td>
<td>17 (85)</td>
<td>43</td>
</tr>
<tr>
<td>Lo-Beer</td>
<td>3 (15)</td>
<td>37</td>
</tr>
<tr>
<td>Lo-EtOH</td>
<td>21 (72)</td>
<td>37</td>
</tr>
</tbody>
</table>

Numbers of left colon tumors in control were inversely correlated with numbers of right and transverse colon, but not the left colon. Numbers of right colon tumors were inversely correlated with alcohol consumption of all rats (Table 3), suggesting that ethanol played a role in inhibition of tumorigenesis. By contrast, both groups consuming low alcohol diets (Lo-Beer and Lo-EtOH) showed a trend toward increased incidence in the left colon (42 and 35% versus 15% in control, P = 0.064 for lo-Beer), but no difference from control in tumor incidence in the right colon (Figs. 4 and 5). Numbers of left colonic tumors were not correlated significantly with ethanol consumption (Table 3). Thus, tumorigenesis in the right and left colon was affected differentially by alcohol consumption.

The proportion of tumors in the right and left colon (Table 2) showed results similar to those for tumor incidence. The total number of tumors in the Hi-Beer and Hi-EtOH groups was reduced markedly as compared to control (20 and 18 tumors, respectively, versus 45), but not in the Lo-Beer and Lo-EtOH groups (43 and 37 tumors). If there was no differential effect of ethanol on tumorigenesis in the right and left colon, the proportion of tumors in these anatomic sites of the alcohol-fed groups would be similar to the control group. The Hi-EtOH, Lo-Beer, and Lo-EtOH groups, however, showed a shift in the proportion of tumors into the left colon with 2.5 to 2.7 times as many tumors as expected (Table 2). The differences in proportion approached statistical significance in the Lo-Beer and Lo-EtOH groups, the same groups with a trend toward increased incidences of left colonic tumors.

Small bowel and ear tumors were found in only a few rats, and there were no significant differences between experimental groups nor correlation with ethanol consumption.

Fecal Bile Acids and Neutral Sterols (Table 4). The levels in the alcohol-consuming groups and control group were generally not statistically significantly different. A trend toward decreased fecal concentration of both bile acids and neutral sterols toward the end of the study was seen in most experimental groups. Fecal deoxycholic acid concentration in week 11 was inversely correlated with alcohol consumption, but not with the number of colonic tumors (Table 3). By contrast, fecal coprostanone concentration in week 25 was correlated with number of left colonic tumors, but not with alcohol consumption. Thus, fecal bile acids and neutral sterols did not appear to play a major role in the effects of alcohol consumption on colonic tumorigenesis.

Fecal Bacterial Flora (Table 5). The four groups consuming alcohol-containing diets showed no differences from control after 3 weeks of dietary acclimatization (week A3). By contrast, after completion of the course of carcinogen injection (week 11), the Hi-Beer, Hi-EtOH, and Lo-EtOH groups showed decreased total anaerobes and Bacteroides species as compared to control. Bacteroides counts, however, were not significantly correlated with numbers of tumors. By contrast, fecal aerobe counts in week 11 were correlated with numbers of right colonic tumors, but not with alcohol consumption. Thus, although ethanol affected the fecal bacterial flora, the flora itself did not appear to play a major role in the effects of alcohol consumption on colonic tumorigenesis.

Epithelial Morphometry and DNA Labeling (Table 6). Labeling index (percentage of all cells which were labeled) of the middle third of the crypt epithelium was decreased as compared to control for the right colon in three of the alcohol-consuming.
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Table 3 Summary of pertinent correlations (Spearman’s rank correlation coefficients, r)

To assess the relationships among tumor outcome, alcohol consumption, fecal bile acid and neutral sterol levels, fecal bacterial flora, and colonic epithelial DNA synthesis, the various parameters were assessed by correlation and stepwise multiple regression analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alcohol consumption</th>
<th>Fecal deoxycholic acid concentration (week 11)</th>
<th>Fecal coprostanol concentration (week 25)</th>
<th>Fecal aerobe counts (week 11)</th>
<th>Fecal Bacteroides counts (week 11)</th>
<th>Labeling index of middle 3rd of crypt</th>
<th>% of labeled cells in lower 3rd of crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>-0.350*</td>
<td>0.205</td>
<td>0.038</td>
<td>0.556*</td>
<td>0.344</td>
<td>0.024</td>
<td>0.386</td>
</tr>
<tr>
<td>Left colon</td>
<td>-0.073</td>
<td>-0.002</td>
<td>0.475*</td>
<td>0.080</td>
<td>-0.118</td>
<td>-0.083</td>
<td>0.230</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>-0.516</td>
<td>0.185</td>
<td>-0.220</td>
<td>-0.638*</td>
<td>-0.534*</td>
<td>-0.423*</td>
<td>0.011</td>
</tr>
<tr>
<td>Fecal deoxycholic acid concentration (week 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal coprostanol concentration (week 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fecal aerobe counts (week 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal Bacteroides counts (week 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labeling index of middle 3rd of crypt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of labeled cells in lower 3rd of crypt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>-0.048</td>
<td>0.152</td>
<td>0.155</td>
<td>0.260</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.001 for r.
* P < 0.01 for r.
* P < 0.05 for r.
* P < 0.02 for r.

Table 4 Mean concentrations of fecal bile acids and neutral sterols (μg/g wet weight ± SE)

Three-day fecal specimens from five rats in each experimental group were collected 1 week after the last carcinogen dose (week 11) and 1 week before sacrifice (week 25). The specimens were analyzed by chromatographic methods.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hi-Beer</td>
</tr>
<tr>
<td>Bile acids (week 11)</td>
<td></td>
</tr>
<tr>
<td>Cholic acid</td>
<td>0</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>0</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>475 ± 255</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>91 ± 32</td>
</tr>
<tr>
<td>Isolithocholic acid</td>
<td>66 ± 37</td>
</tr>
<tr>
<td>Total bile acids</td>
<td>646 ± 297</td>
</tr>
<tr>
<td>Bile acids (week 25)</td>
<td></td>
</tr>
<tr>
<td>Cholic acid</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>32 ± 32</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>241 ± 119</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>165 ± 76</td>
</tr>
<tr>
<td>Isolithocholic acid</td>
<td>155 ± 50</td>
</tr>
<tr>
<td>Total bile acids</td>
<td>516 ± 177</td>
</tr>
<tr>
<td>Neutral sterols (week 11)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2269 ± 975</td>
</tr>
<tr>
<td>Cholesstanol</td>
<td>182 ± 51</td>
</tr>
<tr>
<td>Coprostanol</td>
<td>2732 ± 780</td>
</tr>
<tr>
<td>Coprostanone</td>
<td>193 ± 90</td>
</tr>
<tr>
<td>Total neutral sterols</td>
<td>5375 ± 1533</td>
</tr>
<tr>
<td>Neutral sterols (week 25)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1395 ± 390</td>
</tr>
<tr>
<td>Cholesstanol</td>
<td>291 ± 135</td>
</tr>
<tr>
<td>Coprostanol</td>
<td>997 ± 178</td>
</tr>
<tr>
<td>Coprostanone</td>
<td>43 ± 24</td>
</tr>
<tr>
<td>Total neutral sterols</td>
<td>2727 ± 477</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. control.

experimental groups (Hi-Beer, Hi-EtOH, and Lo-Beer). In addition, the labeling index of the middle third of the crypt epithelium in both the right and left colon was inversely correlated with alcohol consumption (Table 3). The percentage of total labeled cells in the lower third of the crypt epithelium of the left colon was correlated with alcohol consumption. Thus, alcohol consumption was associated with decreased DNA synthesis in the middle third of the crypt epithelium in both the right and left colon, and with a shift into the lower third of the epithelium in the left colon. These alcohol-associated changes in DNA synthesis at the end of the study were not correlated with tumor outcome, however. By contrast, the percentage of labeled cells in the lower third of the crypt epithelium of the right colon was correlated with the numbers of right colonic tumors, but not with alcohol consumption. Because epithelial DNA synthesis was assessed only at the end of the study, the
Table 5 Fecal bacterial flora counts (10^9 × mean colony forming units/g wet
weight ± SE)

Freshly defecated fecal specimens from six rats in each experimental group
were collected after 3 weeks of dietary acclimatization (week A3) and 1 week after
last carcinogen injection (week 11). The specimens were serially diluted in
Schaefer's broth medium and plated onto appropriate plates for total aerobic
bacteria, for aerobic/facultative gram-negative bacteria, for total anaerobic bac-
teria, and for Bacteroides.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Week A3</th>
<th>Week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobes</td>
<td>± 1</td>
<td>± 1</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>± 2</td>
<td>± 1</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>± 1</td>
<td>± 1</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>± 1</td>
<td>± 1</td>
</tr>
</tbody>
</table>

Table 6 Epithelial morphometry and DNA labeling (mean ± SE)

The rats were killed 16 weeks after last carcinogen dose (Week 26). One hour
before sacrifice, seven pair-fed groups with one rat from each experimental group
were given an i.p. injection of tritiated thymidine. Specimens from the right and
left colon were processed for autoradiography and analyzed morphometrically.

Table 7 Studies of effects of chronic dietary alcohol consumption on experimental colon carcinoma

<table>
<thead>
<tr>
<th>Author</th>
<th>Rats</th>
<th>Carcinogen administration protocol</th>
<th>Alcohol administration protocol</th>
<th>Carcinogen outcome with alcohol</th>
<th>Time and method of assessment</th>
<th>Increased tumors in distal left colon</th>
<th>No differences from control</th>
<th>No differences from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seitz 21</td>
<td>Male Sprague-Dawley</td>
<td>60 days, 12.4 weeks</td>
<td>DMH 30 mg/kg × 16 women of 4 weekly doses</td>
<td>Ethanol (36% of calories) in four alternating cycles of fluid source. All thiamin</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Nelson (22)</td>
<td>Male Sprague-Dawley (male in beer study)</td>
<td>5 weeks, 8 weeks</td>
<td>DMH 15 mg/kg weekly × 16 women of 4 weekly doses</td>
<td>Ethanol (34% of calories)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>McGarity 23-24</td>
<td>Male Sprague-Dawley</td>
<td>Not stated</td>
<td>AMMM 2 mg/kg intracolonic every 2 weeks (di)</td>
<td>Ethanol (36% of calories) in 60% liquid diet</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Garzon (25)</td>
<td>Male Fischer 344</td>
<td>10 weeks, 13 weeks</td>
<td>AO4 4 mg/kg × 10 weeks</td>
<td>Beer (alcohol providing 23% and 11% of calories)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

relationship between alcohol effects on epithelial proliferation
and colonic tumorigenesis requires further studies.

DISCUSSION

The majority of epidemiological studies examining the rela-
tionship between large bowel cancer and alcoholic beverage
consumption have shown an association (5-16). Many of these
studies demonstrate an association between rectal cancer and
beer consumption (7, 8, 11, 13-16). Others differ, however,
in the types of alcoholic beverages incriminated or in the anatomic
sites of the associated colorectal cancers (9, 10, 12); some
epidemiological studies have not found any relationship at all
(36-43). The investigations which do show an association pro-
vide no clear evidence concerning the possible mechanism(s) by
which alcoholic beverage consumption may enhance colorectal
carcinogenesis. As a result, studies using experimental models
of colonic carcinogenesis have the potential to contribute to
understanding the conflicting epidemiological data. Unfortu-

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nately, the experimental studies to date have also produced conflicting results.

The data from available studies of dietary alcohol effects on experimental colonic carcinogenesis are summarized in Table 7. In two studies by Seitz and his colleagues in West Germany (21, 25), chronic dietary ethanol consumption appeared to enhance tumorigenesis in the left colon of the rat models. Our findings of a trend toward increased incidence and proportion of left colonic tumors tend to support these findings. By contrast, we found reduced tumorigenesis in the right colon with both beer and ethanol consumption. Other investigators have found no effect on colonic carcinogenesis with chronic dietary ethanol or beer consumption (22–24). We speculate that the conflicting data are related in part to differences in the experimental models used by various investigators (see Table 7).

Experimental colonic carcinogenesis with azoxymethane and other related systemically administered carcinogens such as dimethylhydrazine involves multiple steps which can be modulated by a variety of mechanisms (17–20). As examples, changes in hepatic metabolism of the carcinogen (44), in hepatic secretion and fecal metabolism of bile acids and neutral sterols (45), and in epithelial proliferation in the colonic mucosa (46) have been associated with modulation of experimental colonic tumorigenesis. Chronic dietary ethanol consumption can affect all of these events.

As regards effects of dietary ethanol on carcinogen metabolism in the model, we found in another study that rats with chronic ethanol consumption metabolized $[^{14}C]$azoxymethane to exhaled $^{14}CO_2$ more slowly than controls and showed reduced formation of DNA adducts in the colonic mucosa and liver. In addition, in isolated hepatic microsomes from rats fed ethanol chronically, we found that the rate of metabolism of $[^{14}C]$azoxymethane was inhibited by incubation with ethanol (47). Although other explanations are possible, these findings suggest that the ethanol dose-related decrease in right colonic tumors in the present study may be due to altered azoxymethane metabolism leading to decreased effective dose of carcinogen.

Of note, the levels of alcohol intake in the present study may be due to altered azoxymethane metabolism leading to decreased effective dose of carcinogen. Of note, the levels of alcohol intake in the present study may be due to altered azoxymethane metabolism leading to decreased effective dose of carcinogen. Of note, the levels of alcohol intake in the present study may be due to altered azoxymethane metabolism leading to decreased effective dose of carcinogen.

Although the incidences of right colonic tumors at the lower levels of intake (Lo-Beer and Lo-EtOH groups) were not statistically significantly lower than control, the left colon showed a trend toward increased tumor incidence and higher proportion of total tumors. We speculate that any lowering of the effective dose of AOM in these groups was overshadowed in the left colon by tumorigenesis-enhancing effects of alcohol. At the higher levels of alcohol consumption (Hi-Beer and Hi-EtOH groups), the inhibitory effects on tumorigenesis were apparent in the right colon. By contrast, no decrease in tumor incidence in the left colon occurred in these latter groups, possibly due to tumorigenesis-enhancing effects of chronic dietary alcohol consumption which counterbalanced, but did not overshadow, the inhibitory effects.

Bile acid metabolism is also known to be affected by dietary ethanol (48–52). Bile acids have received considerable attention as modifiers of colonic carcinogenesis (2, 45). Studies of alcohol consumption in rats have shown increased bile flow and decreased fecal excretion of bile acids (48–50) in several studies, but not all (21). In the present study in AOM-treated rats, fecal deoxycholic acid concentration was inversely correlated with alcohol consumption, supporting the previous studies (48–50).

The fecal levels of bile acids were not correlated with tumor outcome, however. The effects of ethanol consumption on fecal neutral sterol excretion have not been studied extensively. In our study, fecal coprostanol concentration at the end of the experiment was correlated with numbers of left colonic tumors. There was no correlation earlier in the experiment (week 11), however, and the fecal levels were not correlated with ethanol consumption. As a result, it appears that this fecal sterol finding may be a consequence of the presence of tumors. Similarly, the fecal *Bacteroides* counts which were inversely correlated with ethanol consumption were not correlated with tumor outcome, and the fecal aerobe counts which were correlated with tumor outcome were not correlated with ethanol consumption. Thus, we found no evidence that fecal bile acids and neutral sterols, and the bacterial flora important in their metabolism, had a major pathogenic role in alcohol effects on tumorigenesis in our study.

Ethanol produces alterations in colonic epithelial proliferation (53), and abnormal proliferation has been extensively studied as regards its role in colonic carcinogenesis (46). Simanowski et al. (53) found that crypt cell production rate was increased in the distal left colon of rats fed ethanol chronically. In the present study, we found an inverse relationship between alcohol consumption and DNA synthesis in the middle third of the colonic crypt epithelium of AOM-treated rats. These alcohol-associated alterations in epithelial proliferation, however, were not correlated with tumor outcome. In another study, we found increased activity of ODC in the mucosa of both the right and left colon of rats fed ethanol-containing diet. ODC is the first and rate-limiting enzyme in the biosynthesis of polyamines which appear to play an important role in regulation of cell proliferation (54). Increased mucosal ODC activity is a marker for susceptibility to colonic carcinogenesis in the rat model (55, 56) and in human beings (57). Thus, ethanol effects on colonic epithelial proliferation may play a role in modulating experimental colonic carcinogenesis.

In addition to the examples discussed above, ethanol has been shown to cause a wide variety of other effects which have the potential to alter steps in colonic carcinogenesis (58–60). These effects include alteration of colonic microsomal enzyme induction and carcinogen metabolism, cytotoxic effects of alcohol and its metabolites, interference with DNA repair, dietary and nutritional disturbances, endocrinological effects, and immuno-suppressive effects. Thus, complex interactions among the potential mechanisms for alcohol effects in the models of colonic carcinogenesis are possible. The results of our study provide evidence that such complex interactions do occur.

Many epidemiological studies have found an association between beer consumption and rectal cancer. About a decade ago, the occurrence of nitrosamines in beer was considered as a possible factor in the association with beer alone (61), but nitrosamines have been eliminated by modifications of the manufacturing process. Asbestos fibers in beer were also a concern in the past (62–64). The relationship between the findings in the experimental models and in human beings remains to be determined with certainty. In the two studies evaluating the effects of beer on experimental colonic carcinogenesis [the present study and the study of Nelson and Samelson (22)], the colonic tumor outcome in the two different models was not significantly different with beer and reagent ethanol. Thus, there is no experimental evidence that beer produces effects other than those due to its alcohol content.

As an additional consideration in comparing the results in
experimental models and epidemiological studies, the relevance of the animal models to rectal cancer in human beings is uncertain. Seitz et al. (21) and Garzon et al. (25) found enhancement by chronic dietary ethanol of left colonic tumorigenesis with both a systemically administered carcinogen (DMH) and a locally-administered, direct-acting carcinogen (acetoxyethylmethyl nitrosamine). Our results tend to support these findings. These workers have also reported a selective stimulation of cell proliferation in the distal left colon by ethanol feeding (53). The right and left colon of the rat, however, show striking anatomic and histopathological differences, in contrast to the relatively subtle differences in human beings (65). As a further consideration, studies by McGarrity et al. (23, 24) in a model similar to that of Seitz et al. (21) produced disparate results, as there was no enhancement of tumor formation in the distal left colon. Thus, additional studies in appropriate experimental models are needed to determine the mechanisms that may be involved in the epidemiological association between beer consumption and rectal cancer.

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REFERENCES

Effects of Chronic Dietary Beer and Ethanol Consumption on Experimental Colonic Carcinogenesis by Azoxymethane in Rats


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