Effects of Timing and Quantity of Chronic Dietary Ethanol Consumption on Azoxymethane-induced Colonic Carcinogenesis and Azoxymethane Metabolism in Fischer 344 Rats

Stanley R. Hamilton, Ock Soon Sohn, and Emerich S. Fiala

Department of Pathology and Oncology Center, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland 21205 [S. R. H.], and Division of Molecular Biology and Pharmacology, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595 [O. S. S., E. S. F.]

ABSTRACT

Epidemiological studies have shown an association between consumption of alcoholic beverages and carcinoma of the large bowel, but studies in experimental models of colonic carcinogenesis have yielded conflicting results. We assessed the effects on azoxymethane-induced colonic carcinogenesis of both timing of chronic dietary ethanol consumption relative to carcinogen administration and quantity of ethanol consumption. Ten-week-old male Fischer 344 rats were given 11%, 22%, or 33% of calories as reagent ethanol or no ethanol by pair feeding with Lieber-DeCarli-type liquid diets providing comparable total carbohydrates, proteins, fats, and calories. Ten weekly s.c. injections of the bowel carcinogen azoxymethane (AOM), 7 mg/kg, were given to all rats in weeks 1–10. Three experimental groups were given their respective ethanol diet during acclimatization and AOM administration (preinduction and induction phases) and then were given the no-ethanol diet from week 11 until sacrifice in week 26 (postinduction phase). Three other groups received the no-ethanol diet during acclimatization and AOM administration and then were changed to their respective ethanol diet until sacrifice. The control AOM group received the no-ethanol diet throughout the study. Suppression of colonic tumorigenesis occurred in the groups with high levels of chronic dietary ethanol consumption during acclimatization and AOM administration: in the 33% and 22% diet groups, the prevalence of colonic tumors was 3% and 20% as compared with 50% in control (P < 0.001 and P < 0.02, respectively). Tumorigenesis in the left colon was more affected than in the right colon, as tumor prevalence in the left colon was decreased in both the 33% and 22% diet groups (0% in both versus 24% in control, P < 0.005), whereas prevalence in the right colon was decreased only in the 33% diet group (3% versus 38%, P < 0.001). By contrast, prevalence of colonic tumors in the 11% diet group was not significantly different from control. Chronic dietary ethanol consumption after AOM administration had no effect on tumor outcome, regardless of quantity of consumption. In an analogous study of [14C]AOM metabolism in rats fed the 33% diet during acclimatization and AOM administration, [14C]CO2 was exhaled at a slower rate than in rats fed no-ethanol diet (P = 0.05), indicating suppression of AOM metabolism. By contrast, rats changed from the 33% diet to no-ethanol diet for 12 h prior to the dose of [14C]AOM metabolized the carcinogen at a faster rate, and exhaled a larger amount as [14C]CO2 than control (P = 0.05), indicating loss of suppression with cessation of ethanol intake and enzyme induction. Our findings suggest that chronic dietary ethanol effects on experimental colonic tumorigenesis with azoxymethane are: (a) due to mechanisms affecting the preinduction and/or induction phase, including carcinogen metabolism; (b) unrelated to postinduction events such as tumor promotion and progression; and (c) dependent on ethanol dose with a threshold for inhibition of tumorigenesis which is mediated by ethanol inhibition of carcinogen metabolism.

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 in the pathogenesis of colorectal carcinoma (2–4). In particular, high dietary fat and low dietary fiber have been implicated. In addition, numerous epidemiological studies using a variety of data sources and statistical methods have found an association between consumption of alcoholic beverages and cancer of the large bowel (5–16). The relationship between rectal cancer and beer consumption has been emphasized in some studies (7, 8, 11, 13–16).

Administration to rodents of AOM or its parent compound DMH provides widely used and useful experimental models of large bowel carcinogenesis (17–20). However, studies of the effects of chronic dietary ethanol and beer consumption in these models have produced conflicting results (21–25). In one study with DMH, increased tumorigenesis occurred in the distal 5 cm of the large bowel of ethanol-fed rats (21). Our previous study with AOM showed a trend toward increased tumor formation in the left colon of rats given beer or ethanol diets but decreased tumorigenesis in the right colon (22). Another study with the direct-acting, locally applied carcinogen methyl-(acetoxyethyl)nitrosamine showed earlier occurrence of left colonic tumors in ethanol-fed rats (26). On the other hand, 2 studies in DMH-treated rats showed no effect of chronic dietary ethanol or beer consumption on large bowel carcinogenesis (23–25).

The previous studies in experimental models of colonic carcinogenesis varied in the timing of ethanol diet administration relative to carcinogen administration and in the quantity of ethanol consumed. We, therefore, systematically evaluated these 2 factors in the AOM-treated rat model. We also studied the in vivo metabolism of AOM and compared the results to tumor outcome.

MATERIALS AND METHODS

Animals and Housing. Guidelines for 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 210–260 g were obtained from Harlan Sprague-Dawley, Indianapolis, IN. The rats were housed singly in suspended wire-bottomed metabolic cages in animal quarters with controlled temperature (21–22°C), humidity (30–50%), and light (12-h cycles).

Diets, Experimental Groups, and Pair-feeding Technique. Four iso-caloric Lieber-DeCarli-type liquid diets were formulated by BioServ, Frenchtown, NJ, to provide 11%, 22%, or 33% of total calories as reagent ethanol and a no-ethanol control diet. The diets had equivalent...
percentages of calories from proteins, carbohydrates, and fats to allow assessment of ethanol effects using the method of concomitant variation (27). The dietary compositions are shown in Table 1.

The experimental groups are shown in Table 2. Three groups were given their respective ethanol diet during the 3 weeks of acclimatization (preinduction phase, weeks A1–A3) and 10 weeks of AOM injection (induction phase, weeks 1–10) and then were given the no-ethanol diet until sacrifice (postinduction phase, weeks 11–26). Three other groups were given the no-ethanol diet during acclimatization and AOM administration and then received their respective ethanol diet until sacrifice. The control AOM group was given the no-ethanol diet throughout the study.

Pair feeding with graduated feeding tubes (BioServ) was used to control diet consumption as in our previous study (22). The diets were changed daily in the mid-afternoon. Ethanol-fed rats were tapered onto their diets over a 4-day period (1:3 mixture of the ethanol diet and no-ethanol diet on the first day, 1:1 on second, 3:1 on third, and undiluted ethanol diet on fourth). Diets were begun 3 weeks before first carcinogen administration.

Carcinogen and Administration Procedures. All carcinogen-handling procedures were carried out in accordance with regulations promulgated by the Office of Safety and Environmental Health of The Johns Hopkins Medical Institutions. Azoxymethane (CAS:25843-45-2; Ash Stevens, Detroit, MI) was prepared and administered as in our previous study (22). After 3 weeks of dietary acclimatization, all rats were given 10 weekly s.c. injections of AOM at a dosage of 7 mg/kg. Injections were given in the mid-morning.

Necropsy Procedures. The rats were killed 16 weeks after the last injection of azoxymethane (week 26). Necropsy procedures were those of our previous study (22).

Studies of Azoxymethane Metabolism. The procedures were analogous to those for the first carcinogen dose in the studies of tumor outcome in rats given the 33% diet during acclimatization and AOM administration (Table 2, group C). Ten-week-old male Fischer 344 rats (Charles River, Kingston, NY) were pair fed the liquid ethanol-containing diet (33% of total calories) or isocaloric control diet. One group was fed the ethanol diet continuously for 21 days; a second group was fed the ethanol diet for 20.5 days and then switched to the no-ethanol diet for 0.5 day; the control group was fed the no-ethanol diet continuously for 21 days. On the morning of the 22nd day of feeding, 4 rats from each group received a s.c. injection of [14C]AOM (dimethyl-14C, specific activity, 3.99 mcI/mmol; New England Nuclear, Boston, MA), 7 mg/kg body weight (106 x 44.353 x 103 x 54.978 dpm). The rats were immediately placed in Delmar-Roth-type metabolism cages for the determination of exhaled 14CO2, by methods described previously (28). Exhaled air was collected continuously, and samples for analysis of 14CO2 were obtained hourly for the first 7 h and at 24 h. At the end of 24 h, the rats were killed. Throughout the experimental period, the rats had their respective liquid diets available ad libitum.

Statistical Analysis. In the study of tumor outcome, 4 rats dying before week 16 of causes unrelated to AOM-induced tumors were excluded from analysis, while 2 rats dying in week 16 or 18 as a result of tumors were included. Incidences of tumors in the various segments of the gastrointestinal tract were compared by the Fisher exact test. The cecum, ascending, and transverse colon were considered together as right colon. Differences between means were assessed by calculation of 2-tailed t tests for paired and unpaired data and Mann-Whitney U tests. Correlation was assessed by calculation of Pearson's product moment and Spearman's rank (nonparametric) correlation coefficients. Stepwise multiple regression analysis was used to assess multiple correlations with tumor outcome, and only those parameters which remained statistically significant by F test were considered acceptable.

RESULTS
Effects on Tumorigenesis of Chronic Dietary Ethanol Consumption during Acclimatization and AOM Administration (Preinduction and Induction Phases) (Table 2, Groups A, B, and C). Mean caloric intake (kcal/kg body weight/week) is shown in Fig. 1 (top). Intake was high during the first acclimatization week (week A1) due to the gradual introduction of ethanol diets. Rats dying before week 16 of causes unrelated to AOM-induced tumors were excluded from analysis, while 2 rats dying in week 16 or 18 as a result of tumors were included. Incidences of tumors in the various segments of the gastrointestinal tract were compared by the Fisher exact test. The cecum, ascending, and transverse colon were considered together as right colon. Differences between means were assessed by calculation of 2-tailed t tests for paired and unpaired data and Mann-Whitney U tests. Correlation was assessed by calculation of Pearson's product moment and Spearman's rank (nonparametric) correlation coefficients. Stepwise multiple regression analysis was used to assess multiple correlations with tumor outcome, and only those parameters which remained statistically significant by F test were considered acceptable.

Table 1 Compositions of reconstituted liquid diets (Kcal/l)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>11%</th>
<th>22%</th>
<th>33%</th>
<th>No-ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>177</td>
<td>177</td>
<td>177</td>
<td>177</td>
</tr>
<tr>
<td>t-cystine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>dL-methionine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto-dextrins</td>
<td>357</td>
<td>244</td>
<td>131</td>
<td>470</td>
</tr>
<tr>
<td>Reagent ethanol (95%)</td>
<td>113</td>
<td>226</td>
<td>339</td>
<td>0</td>
</tr>
<tr>
<td>Fats</td>
<td>350</td>
<td>350</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Total calories</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Proteins</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Fats</td>
<td>350</td>
<td>350</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Carbohydrates including ethanol</td>
<td>470</td>
<td>470</td>
<td>470</td>
<td>470</td>
</tr>
<tr>
<td>Ethanol contribution to total carbohydrates</td>
<td>113</td>
<td>226</td>
<td>339</td>
<td>0</td>
</tr>
<tr>
<td>% Total calories from ethanol</td>
<td>11.3</td>
<td>22.6</td>
<td>33.9</td>
<td>0</td>
</tr>
</tbody>
</table>

* All diets included vitamin mix 711 and salt mix 711 (BioServ) as in our previous study (22).

Table 2 Summary of experimental groups

Ten-week-old male Fischer 344 rats were used in the study. The isocaloric Lieber-DeCarli-type liquid diets (see Table 1) were fed with pair-feeding technique. After 3 weeks of dietary acclimatization, 10 weekly s.c. injections of azoxymethane were given at a dose of 7 mg/kg. The rats were killed 16 weeks after the last dose of azoxymethane.
ETHANOL AND EXPERIMENTAL COLONIC CARCINOGENESIS

Fig. 1. Caloric intake in groups given ethanol diet during the preinduction and induction phases (top) and postinduction phase (bottom). Caloric intake/kg body weight/week was similar to control as a result of pair feeding isocaloric liquid diets, except for the higher values in the 22% and 33% diet groups during the induction phase (top) due to lower body weights in these groups (see Fig. 3).

Fig. 2. Ethanol consumption (g/kg body weight/week) in groups given ethanol diet during the preinduction and induction phases (left) and postinduction phase (right). Ethanol consumption of each group reflected the intake of its respective liquid diet and the ethanol content (see Table I).

attributed in previous studies to energy wastage associated with ethanol metabolism (29).

The anatomic sites of tumors are shown in Fig. 4 (top), and tumor incidence by anatomic site is illustrated in Fig. 5 (solid bars). Colonic tumor incidence was reduced in both the 22% and 33% diet groups as compared to control AOM (Fig. 5, open bars). The occurrence of tumors in the left colon was particularly affected: both the 22% and 33% groups showed reduced incidence of tumors in that site whereas only the 33% group showed reduced incidence in the right and transverse colon. Numbers of colonic tumors were inversely correlated with ethanol consumption during the preinduction and induction phases (e.g., for week 1, \( r = -0.349, P < 0.001 \)).

Effects on Tumorigenesis of Chronic Dietary Ethanol Consumption after AOM Administration (Postinduction Phase) (Table 2, Groups D, E, and F). Mean caloric intake (kcal/kg body weight/week) is shown in Fig. 1 (bottom). The curves are similar to those in the groups fed the ethanol diets in the preinduction phases.

Mean ethanol consumption (g/kg body weight/week) is shown in Fig. 2 (right). The low ethanol consumption in the first week of administration (week 11) reflects the gradual introduction of ethanol diet.

Mean body weight is shown in Fig. 3 (bottom). The weights were similar when all groups were receiving no-ethanol diet, but some divergence occurred during the period of ethanol diet consumption.

Fig. 3. Body weights in groups given ethanol diet during the preinduction and induction phases (top) and postinduction phase (bottom). Top, body weights of the 22% and 33% diet groups lagged behind the control and 11% groups despite pair feeding, possibly due to energy wastage by ethanol metabolism (29). Bottom, weights were similar when all groups were receiving no-ethanol diet, but some divergence occurred during the period of ethanol diet consumption.

Fig. 4. The anatomic sites of tumors are shown in Fig. 4 (bottom), and tumor incidence by anatomic site is illustrated in Fig. 5 (hatched bars). There were no statistically significant differences from the no-ethanol control AOM group (Fig. 3, open bars). Numbers of colonic tumors showed no statistically significant correlation with ethanol consumption after AOM administration was completed.

4307

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was exhaled as \( {^{14}C}\text{O}_2 \) is shown in Fig. 6. In the control rats, 6-h consumptions represented the majority of dietary intake consumptions in the two ethanol-fed groups probably resulted from the ethanol diet to no-ethanol diet 12 h before \([^{14}\text{C}]\text{AOM}\) administration, ethanol diet group, 17 ± 1 g for the group switched from metabolism cages, mean liquid diet consumption was 32 ± 4 g for the ethanol fed rats. By contrast, rats fed the ethanol diet continuously metabolized the carcinogen at a faster rate for the second through the sixth h (P = 0.05 versus control by Mann-Whitney U test). The rise in mean exhaled \( {^{14}\text{C}\text{O}_2} \) in the sixth and seventh h was attributable to a large increase in the 1 rat which consumed little ethanol diet in the metabolic cage. The rats switched from ethanol diet to no-ethanol diet 12 h before \([^{14}\text{C}]\text{AOM}\) administration metabolized the carcinogen at a faster rate than control; metabolism of \([^{14}\text{C}]\text{AOM}\) was essentially complete by 3 h. In addition, these rats exhaled a larger quantity of the \([^{14}\text{C}]\text{AOM}\) dose as \( {^{14}\text{C}\text{O}_2} \) than the no-ethanol control (P = 0.05 by Mann-Whitney U test). Taken together, our findings suggest that metabolism of the carcinogen was inhibited by the presence of consumed ethanol; but the enzymes involved in AOM metabolism were induced by chronic ethanol consumption, as became apparent after ethanol consumption ceased.

**DISCUSSION**

The azoxymethane-treated rat model of colonic carcinogenesis has multiple steps which occur between administration of carcinogen and tumorigenesis (30). Modulation of many of these steps in this and related models has the potential to modulate tumorigenesis. The AOM model does not have classic initiation and promotion phases in most studies because multiple doses of carcinogen are given to avoid excessively long latent periods to tumorigenesis. For discussion of ethanol effects in modulating tumorigenesis, we separated the model into preinduction, induction, and postinduction phases, as shown in Table 3.

We found that the inhibitory effect on tumorigenesis of chronic dietary ethanol consumption during the preinduction and induction phases stood in marked contrast to the absence of ethanol effect during the postinduction phase. Other modulators of colonic carcinogenesis have been tested similarly to gain insight into their mechanisms of action. For example, enhancement of tumorigenesis by barbiturate (31), increased dietary fat of some types (32, 33), and chronic neomycin administration (34) occurred during the postinduction phase. Other types of dietary fat produced enhancement when given in the preinduction and induction phases (33). Inhibitors of tumorigenesis can also exert their effects in different phases. Some produce inhibition during the induction phase, e.g., disulfiram and pyrazole (35,36). Of note, these compounds inhibit hepatic metabolism of AOM (28, 37). Other inhibitors are effective only in the postinduction phase, e.g., butylated hydroxytoluene (38) and difluoromethylornithine (39, 40).

Ethanol has wide-ranging biochemical effects with the potential to affect carcinogenesis (41). In the present study, chronic dietary ethanol consumption affected AOM-induced colonic tumorigenesis only when given during the preinduction and induction phases (Table 2, groups A, B, and C). Thus, ethanol does not appear to affect tumorigenesis in this model via mechanisms operant during the postinduction phase, i.e., promotion and/or progression of established tumors.

Our finding that ethanol produces dose-dependent inhibition of colonic tumorigenesis contrasts with most previous studies in this and related models which have shown either enhancement or no effect (21, 23–25). Our previous study did show an ethanol dose-dependent decrease in tumorigenesis in the right colon, but there was also a trend toward increased tumor formation in the left colon (22). The occurrence of dose dependency with a threshold for ethanol effects has not been investigated by other authors who have used 1 high level of ethanol consumption. In contrast to our previous study, the left colon did not show evidence of increased tumorigenesis in the present study but rather decreased tumor formation. The explanation for the differences between our 2 studies is unknown.

We found that left colonic tumorigenesis was inhibited at
Fig. 5. Colonic tumor incidences. Left, all sites; middle, right and transverse colon; right, left colon. Tumor incidence was reduced in the groups given the 22 and 33% diets during the preinduction and induction phases (R, * P < 0.02; *** P < 0.001). Although only the 33% diet group showed reduced incidence in the right colon, the occurrence of tumors in the left colon was particularly affected, as both the 22 and 33% diet groups showed reduced incidence of tumors in that site (** P < 0.005). A threshold for suppression of tumorigenesis by ethanol was evident, as the 11% group was similar to control. In the groups given ethanol diets during the postinduction phase (C), there were no significant differences from the no-ethanol AOM control group.

Fig. 6. 14C-AOM metabolism study. The procedures were analogous to those for the first carcinogen dose in the studies of tumor outcome in rats given the 33% diet during acclimatization and AOM administration (Table 2, Group C). In the no-ethanol control rats, AOM metabolism was essentially complete by 5 h. By contrast, rats fed the 33% diet throughout the study metabolized the carcinogen at a faster rate, as metabolism was essentially complete by 3 h, and exhaled a larger amount as 14CO2 than the control group. These latter findings suggest that cessation of ethanol consumption resulted in loss of suppression of carcinogen metabolism, permitting enzyme induction to become evident.

**Table 3 Summary of phases of model**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Preinduction</th>
<th>Induction</th>
<th>Postinduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks of study</td>
<td>A1-A3</td>
<td>1-10</td>
<td>11-26</td>
</tr>
<tr>
<td>Procedures</td>
<td>Acclimatization to first diet</td>
<td>Weekly AOM injections, continuation of first diet</td>
<td>Acclimatization to second diet, continuation of second diet until sacrifice</td>
</tr>
<tr>
<td>Representative events</td>
<td>Induction of hepatic microsomal enzymes by ethanol</td>
<td>AOM metabolism, DNA adduct formation, altered epithelial proliferation</td>
<td>Altered epithelial proliferation, development of dysplasia, appearance and growth of tumors</td>
</tr>
</tbody>
</table>

Our in vivo study showed that dietary ethanol consumption inhibited metabolism of [14C]AOM to [14CO2]. In an analogous study using AOM at a higher dose (14 mg/kg as contrasted with 7 mg/kg in the present study), we found that formation of DNA adducts in the colonic mucosa was decreased in rats fed the ethanol diet. Acute administration of 4-iodopyrazole, a potent inhibitor of alcohol dehydrogenase, also inhibited lower levels of ethanol consumption than right colonic tumorigenesis. The differential susceptibility of left colonic tumorigenesis to modulation by ethanol has been demonstrated previously, as Seitz et al. (21) found that DMH-induced tumorigenesis in the distal left colon was selectively increased in rats consuming an ethanol diet. In addition, ethanol produced selective stimulation of colonic crypt cell production in the left colon (42). The anatomy, histology, and epithelial proliferation of the right and left colon in the normal rat are strikingly different (43, 44), but the mechanism(s) of differential ethanol effects remains to be determined.

The inhibition of tumorigenesis that we observed with chronic dietary ethanol consumption during the preinduction and induction phases appears to be due to suppression of AOM metabolism by ethanol. According to current views, AOM is metabolically activated by a series of reactions (28, 45). First, AOM is hydroxylated in the liver to MAM by the mixed-function oxidase of the endoplasmic reticulum. This reaction does not appear to occur in the colonic mucosa due to the lower levels of or lack of necessary enzymes. MAM may be further metabolized by alcohol dehydrogenase (46) in the liver and colonic mucosa or by microsomal oxidase in the liver to the highly unstable methyldiazonium ion and formic acid (45). The methyldiazonium ion can methylate cellular nucleophiles, including DNA, to form adducts or react with water to form methanol. DNA adduct formation is considered to be the determining step in initiation events which may ultimately result in tumorigenesis (47, 48). Of particular note, the enzymes involved in metabolism of AOM (microsomal oxidase in the liver and alcohol dehydrogenase in the liver and colonic mucosa) are also responsible for the metabolism of ethanol to acetaldehyde. Thus, the level of DNA adduct formation, and ultimately tumorigenesis itself in the model, appear to depend mainly on the activities of these enzymes and the competition between AOM and ethanol for the active sites.
ETHANOL AND EXPERIMENTAL COLONIC CARCINOGENESIS

MAM-induced colonic mucosal and hepatic DNA adduct formation (45). We have obtained evidence in vitro which supports our interpretation of our in vivo findings: metabolism of [14C]-AOM and [14C]MAM by isolated rat liver microsomes was inhibited by the presence of ethanol as well as by pyrazole (49). Inhibition by ethanol of various mixed-function oxidation reactions is well known (50–53) and may involve indirect mechanisms (50, 51) as well as direct competition for active sites. Taken together, our findings suggest that the inhibition of tumorigenesis by dietary ethanol consumption during the preinduction and induction phases is in large part due to the reduced effective dose of carcinogen resulting from ethanol suppression of AOM activation.

Our in vivo study also showed that in rats with chronic dietary ethanol consumption which ceased 12 h before the [14C]AOM dose, the carcinogen was metabolized to CO2 more rapidly than in controls. Thus, loss of ethanol suppression of [14C]-AOM metabolism occurred with cessation of ethanol intake. The more rapid metabolism of AOM than in controls is attributable to ethanol induction of enzyme activity. Ethanol is well known to induce microsomal oxidase activity (52–54). Hepatic microsomes isolated from rats fed as in the present study with cessation of ethanol diet 12 h before AOM were 2 to 3 times more active in AOM metabolism than microsomes from control rats (49). Induction of microsomal oxidase activity was even greater in microsomes from rats fed the ethanol diet continuously (12–18 times control). Thus, a rapid decline in microsomal activity followed cessation of ethanol consumption. In the rats fed ethanol continuously, the enzyme induction was overshadowed in vivo by the presence of ethanol acting as an inhibitor of AOM metabolism, manifested by slower metabolism of [14C]AOM to CO2. After ethanol intake ceased, the enzyme induction became evident, manifested by the more rapid metabolism of [14C]AOM to CO2. Interactions between acute and chronic effects of ethanol on hepatic metabolism of other substances have been reported previously (52, 53).

The relationship of our findings to colorectal carcinogenesis in human beings is uncertain. Clearly, our results do not provide experimental support for the epidemiological association between consumption of alcoholic beverages and occurrence of large bowel cancer. On the other hand, the phases of the experimental model which are relevant to large bowel carcinogenesis appear warranted for comparison with epidemiological findings.

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