ACUTE AND CHRONIC EFFECTS OF ETHANOL ON INTESTINAL LIPID METABOLISM

ENRIQUE BARAONA, ROMANO C. PIROLA* and CHARLES S. LIEBER

Section of Liver Disease and Nutrition, Veterans Administration Hospital, Bronx, New York and Department of Medicine, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. (U.S.A.)

(Received September 9th, 1974)

Summary

To assess the effects of ethanol on intestinal lipid metabolism, fatty acid oxidation and triacylglycerol synthesis were measured in intestinal slices incubated with ethanol. Ethanol, when used in concentrations likely to be achieved in the upper jejunum after moderate drinking, inhibited both palmitate and acetate oxidation, CO$_2$ production and triacylglycerol synthesis, whereas it enhanced the esterification of fatty acid with ethanol. The concentrations required for the inhibitory effect were much higher than those needed to saturate enzyme systems known to participate in ethanol oxidation.

In vivo administration of ethanol-containing diets produced persistent changes of the intestinal slices with respect to fatty acid oxidation and triacylglycerol synthesis. Acute intragastric administration of ethanol (3 g/kg) one hour prior to sacrifice, inhibited both processes in slices obtained from the jejunum, but not in those derived from the ileum. By contrast, chronic ethanol feeding increased the ability for fatty acid oxidation and triacylglycerol synthesis both in the jejunum and in the ileum. This stimulatory effect was associated with significant enhancement of palmitoyl-CoA synthetase activity, suggesting increased fatty acid activation.

The inhibition by ethanol in high concentrations of intestinal fatty acid oxidation and triacylglycerol synthesis probably reflects epithelial cell damage; by contrast, prolonged administration of ethanol results in a persistent enhancement of lipid metabolism which may reflect the presence of a different cell population in the intestine.

* Present address: Department of Medicine, Prince Henry Hospital, Little Bay, N.S.W. 2036 Australia.

Requests for reprints should be sent to: Dr Enrique Baraona, Section of Liver Disease and Nutrition, Veterans Administration Hospital, 130 West Kingsbridge Road, Bronx, New York 10468, U.S.A.
Introduction

Ethanol increases intestinal cholesterogenesis [1] and triacylglycerol synthesis [2] under certain experimental conditions. These effects have been attributed to the metabolic consequences of ethanol oxidation by the small intestine [3,4]. However, even after moderate alcohol drinking, the upper small intestine is exposed to concentrations of ethanol several fold higher than those in other organs [5–7]. These concentrations have direct injurious effects on epithelial cells, and indeed gross morphologic lesions of the intestinal epithelium have been found after acute ethanol administration to the rat [7,8]. Even after chronic ad libitum feeding of diets containing ethanol in low concentration, ultrastructural alterations suggesting damage [9] and changes in epithelial cell population suggesting accelerated regeneration [7] have been observed. All these effects could also influence intestinal lipid metabolism.

While changes in lipid metabolism associated with ethanol oxidation require the presence of ethanol in concentrations no greater than those needed to saturate the oxidative enzyme systems, changes secondary to cellular alterations should be enhanced by ethanol concentrations higher than those which can be readily oxidized and should persist after ethanol disappearance. In addition, the redox changes associated with ethanol oxidation in other organs increase fatty acid esterification whereas they inhibit fatty acid oxidation [10,11]. This study was undertaken to assess the effects of both the addition in vitro of various ethanol concentrations and the previous in vivo administration of ethanol, on the ability of intestinal slices to oxidize fatty acids and to synthesize triacylglycerols.

Materials and Methods

Materials

The composition of the synthetic liquid diets used in this study has been previously reported [12]. They contain 18% of the total calories as protein, 35% as fat, 11% as carbohydrate and 36% either as ethanol or additional carbohydrate (control diet). Sodium [1-14C]acetate (57.3 Ci/mol), [1-14C]-ethanol (2.7 Ci/mol) and [1-14C]palmitic acid (10 Ci/mol), of a purity greater than 98% as determined by radiochromatography, were purchased from New England Nuclear Corp. (Boston, Mass.). [1-14C]Palmitic acid was included in a lipid emulsion containing 6 mM palmitic acid (Sigma Chemical Co., St. Louis, Mo.), 3 mM glyceryl monooleate (Calbiochem, Los Angeles, Calif.) and 24 mM sodium taurocholate (Calbiochem, Los Angeles, Calif.), dissolved in Krebs-Ringer phosphate buffer (devoid of calcium and magnesium), pH 6.3. The emulsion was prepared according to the procedure of Johnston and Borgström [13] and its modifications by Thompson et al. [14] and passed through a Millipore filter (0.22 ± 0.02 μm pore diameter).

Ethanol feeding procedures

Weanling CD® male rat littermates were purchased from Charles River Breeding Laboratories (Wilmington, Mass.) and when they reached a weight of 130–150 g, they were pair-fed the liquid diets for 3–4 weeks. All animals were
tested after administration by gastric tube of a dietary load in a dose of 6 ml per 100 g body weight. In the case of the alcohol-containing diet, this load is equivalent to a dose of 3 g of ethanol per kg body weight and represents approximately one fifth of the daily ethanol consumption in rats. To ensure an equal rate of food intake during the last 24 h, oral pair-feeding was replaced by simultaneous gastric intubations, the last of which (3 ml of diet per 100 g body wt) was given 3 h prior to the tests.

To compare the effects of acute and chronic ethanol administration, rats were fed the liquid diets in groups of three: ethanol-containing diet was fed ad libitum to one rat (group I) in each trio, and the other two littermates were limited to the ingestion of similar amounts of diet containing isocaloric carbohydrate instead of ethanol. The rat fed alcohol chronically and one of the pair-fed controls (group II) were given intragastrically the ethanol-containing diet (chronic and acute ethanol administrations, respectively); the other pair-fed control received the control diet (group III).

The effects of chronic alcohol feeding in the absence of ethanol at the time of sacrifice, were studied in a second series of pair-fed rats. In this series the alcohol-fed rats (group IV), as well as their pair-fed controls, were tested after an intragastric load of control diet. To allow for ethanol disappearance, the last intubation with alcohol diet was given 20 h before the tests, and replaced thereafter by administration of control diets.

Animals were sacrificed by decapitation and the small intestine, from the angle of Treitz to the ileo-cecal junction was stripped from the mesentery. Intestinal contents were flushed out with ice-cold saline. Segments of intestine from the proximal 20 cm of jejunum and from the ileum (between 30 and 10 cm proximately to the cecum) either were sliced in rings 1 mm wide by means of a McIlwain tissue chopper (Brinkmann Instruments, Westbury, New York) for the metabolic studies in vitro or were used for the enzyme assays described below.

**In vitro metabolic studies**

The intestinal rings were washed in 10 vols of ice-cold saline, 150–200 mg of tissue were incubated at 37°C, under O₂ in 3 ml of Krebs-Ringer phosphate buffer (devoid of calcium and magnesium), pH 6.3, containing 10 mM glucose, and the micellar solution of palmitate described above. CO₂ was trapped in the center well containing 0.4 ml of 0.1 M barium hydroxide. In the radioactive studies, either [1-¹⁴C]palmitic acid (0.166 Ci/mol) was included in the micellar solution or 2 mM sodium [1-¹⁴C]acetate (0.625 Ci/mol) was added to the medium and the radioactive CO₂ was trapped in the center wells containing 0.5 M Hyamine® (p-diisobutylcresoxyethoxyethyl)-dimethylbenzylammonium hydroxide). The reaction was stopped by the addition of 0.2 ml of 5 M sulfuric acid to the medium. The production of CO₂ was determined by titration of the remaining barium hydroxide in the center wells, according to the method of Conway [5] or by liquid scintillation counting of the Hyamine®-trapped radioactivity. After completion of the incubations, the radioactivity remaining in the media was counted in Bray's scintillation fluid [16] and the obtained values were subtracted from the initial radioactivity to assess the rate of uptake. Tissue slices were then digested in 1 M NaOH for protein determina-
tion [17] or washed in 24 mM taurocholate and homogenized in chloroform-methanol 2:1 for lipid extraction [18]. Triacylglycerols were isolated from the lipid extracts by thin-layer chromatography in a solvent system containing n-hexane, diethyl ether and acetic acid (83:16:1), eluted with diethyl ether, and counted by liquid scintillation. All the results were compared to those obtained with boiled intestinal slices.

Jejunal rings from chow-fed rats, fasted overnight, were used to study the effects of ethanol added to the incubation medium. Ethanol was used in final concentrations ranging from 0.125 to 5 g per 100 ml. The effects of 2.5 g of ethanol per 100 ml were compared with those of an equal volume of distilled water and those of isosmotic concentrations of urea (2.95 g per 100 ml) or mannitol (9.11 g per 100 ml). Ethanol was measured [19] in the medium to assess its disappearance. In 6 experiments, [1-14C]ethanol (2.5 Ci/mol) was added to the medium and its incorporation into tissue lipids was assessed.

**Enzyme assays**

Intestinal mucosa was scraped from jejunal and ileal segments and was homogenized in 0.154 M KCl in 0.01 M phosphate buffer pH 7.4. Palmitoyl-CoA synthethase activity was measured in the homogenates by the hydroxamate-trapping method of Kornberg and Pricer [20], as modified by Rodgers et al. [21].

**Statistics**

Student's t-test was applied to the mean of the individual differences between pair-fed animals or between incubations with and without ethanol (paired comparisons) [22].

**Results**

**Effects of in vitro addition of ethanol on lipid metabolism by intestinal slices**

The addition of ethanol to the incubation media in concentration of 0.125 g/100 ml did not change significantly the incorporation of [1-14C]-palmitate into CO2 or into the intestinal triacylglycerols. At higher concentrations, ethanol inhibited both fatty acid oxidation and triacylglycerol formation (Table I). These reactions proceeded in a nearly linear fashion for up to 30 min. During this period, palmitate disappeared from the media at a rate of 150 ± 10 nmol per min per g of intestine. This process was unaffected by ethanol in various concentrations (Table I).

The incorporation of the palmitate label into 14CO2 was inhibited by ethanol in concentrations of 2.5 g/100 ml or greater, but it was unaffected by concentrations of 0.5 and 1.0 g/100 ml (Table I). This inhibition was associated with a reduction in the total production of CO2 by the intestinal slices: with 2.5 g/100 ml ethanol, the slices produced 3.09 ± 0.29 µl per mg of tissue protein in 30 min, compared to 3.97 ± 0.39 µl without ethanol (P < 0.05). Urea and mannitol, in concentrations isosmotic to 2.5 g of ethanol per 100 ml, also inhibited 14CO2 production by 40% (P < 0.02) and by 73% (P < 0.01), respectively. Ethanol at a concentration of 2.5 g/100 ml (but not at a concentration of 0.5 g/100) also inhibited the incorporation of the acetate label into
TABLE I

EFFECTS OF ETHANOL ON PALMITATE OXIDATION AND ESTERIFICATION BY INTESTINAL SLICES

Jejunal slices obtained from 7 chow-fed rats (fasted overnight), were incubated in Krebs-Ringer phosphate medium containing a micellar solution of $[^1\text{C}]$ palmitate, monooleate and taurocholate for 30 min, with and without ethanol. Each value represents the mean ± S.E.

<table>
<thead>
<tr>
<th>Ethanol concentration (g/100 ml)</th>
<th>$[^1\text{C}]$ palmitate disappearance (dpm/mg tissue)</th>
<th>$[^1\text{C}]$ CO$_2$ production (dpm/mg tissue)</th>
<th>$[^1\text{C}]$ triacylglycerol production (dpm/mg tissue)</th>
<th>$[^1\text{C}]$ ethylpalmitate production* (dpm/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1679 ± 98</td>
<td>246 ± 17</td>
<td>343 ± 46</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1543 ± 50</td>
<td>202 ± 15</td>
<td>263 ± 34</td>
<td>91 ± 81</td>
</tr>
<tr>
<td>1.0</td>
<td>1466 ± 100</td>
<td>220 ± 20</td>
<td>98 ± 10**</td>
<td>15 ± 81</td>
</tr>
<tr>
<td>2.5</td>
<td>1500 ± 128</td>
<td>88 ± 10**</td>
<td>82 ± 14**</td>
<td>433 ± 116**</td>
</tr>
<tr>
<td>5.0</td>
<td>1808 ± 101</td>
<td>34 ± 4**</td>
<td>44 ± 9**</td>
<td>551 ± 120**</td>
</tr>
</tbody>
</table>

* Identification based on chromatographic characteristics and labeling with $[^1\text{C}]$ ethanol (see text).

** P < 0.01, paired comparisons with slices incubated without ethanol.

$[^1\text{C}]$ CO$_2$: 354 ± 21 dpm/mg of tissue were incorporated into CO$_2$ by the slices incubated with ethanol for 30 min, compared to 531 ± 62 dpm without ethanol (P < 0.01). The latter inhibition was associated with a reduction in acetate disappearance from the media: 1367 ± 36 dpm/mg of tissue disappeared in 30 min from the ethanol containing media, whereas 1560 ± 61 dpm disappeared from the media without ethanol (P < 0.01). After correction for the difference in acetate uptake, the oxidation of acetate to CO$_2$ was still significantly (P < 0.01) inhibited by ethanol in concentration of 2.5 g/100 ml.

The incorporation of $[^1\text{C}]$ palmitate into intestinal triacylglycerols was unaffected by 0.5 g of ethanol per 100 ml, but it was significantly (P < 0.01) inhibited by ethanol in concentrations of 1 g/100 ml or greater (Table I). By contrast, increasing concentrations of ethanol in the media resulted in enhanced incorporation of the fatty acid label into a fraction of tissue lipids which migrates slightly faster than triacylglycerols in our chromatographic system. From the data in Table I, it can be estimated that 50.3 nmol of palmitate were incorporated into this fraction per min per g of slices incubated with ethanol (5 g/100 ml). This lipid fraction had migratory characteristics identical to those of ethyl- and methylpalmitate. Eluates of this fraction were further analyzed in a gas chromatograph (Hewlett-Packard, model 402) using a 4 ft column of 3.8% SE 30 on 80–100 mesh chromosorb, at 225°C: a single major peak with a retention time identical to that of ethylpalmitate (3.15 min) and different from that of methylpalmitate (2.41 min) was identified. Ethylpalmitate was not present in tissue incubated without ethanol. Furthermore, after incubation of the slices with $[^1\text{C}]$ ethanol (5 g/100 ml), 60% of the label recovered in tissue lipid extracts was incorporated into this fraction. This is equivalent to 46 nmol of ethanol/min/g of tissue. Thus, the molar ratio of ethanol to palmitate incorporated into this fraction was 0.9–1. By contrast, only 2% of the ethanol radioactivity in the lipid extracts was recovered in triacylglycerol and 38% in the remainder lipid fractions. 83% of the $[^1\text{C}]$ ethanol label incorporated into
TABLE II

EFFECTS OF ACUTE AND CHRONIC ETHANOL PRETREATMENT ON INTESTINAL OXIDATION OF PALMITATE AND TRIACYLGlycerol SYNTHESIS IN VITRO

Intestinal slices were obtained from 7 groups of 3 rats one hour after intragastric administration of a single dose of diet containing either ethanol (3 g/kg of body wt) or isocaloric carbohydrate. The slices were incubated in Krebs-Ringer phosphate medium containing a micellar solution of [14C]palmitate, monoooleate and taurocholate for 30 min. One rat of each trio was previously fed ethanol-containing diet for 3–4 weeks, and 2 were pair-fed control diet. Each value represents the mean ± S.E. of 7 determinations. N.S., not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chronic treatment</th>
<th>Acute treatment</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>14CO2</td>
<td>14CO2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>production</td>
<td>production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(dpm/mg of tissue)</td>
<td>(dpm/mg of tissue)</td>
</tr>
<tr>
<td>I.</td>
<td>Alcohol-fed rats</td>
<td>Ethanol diet</td>
<td>403 ± 64</td>
<td>1649 ± 243</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P*</td>
<td>0.01</td>
<td>1.01</td>
</tr>
<tr>
<td>II.</td>
<td>Pair-fed controls</td>
<td>Ethanol diet</td>
<td>216 ± 27</td>
<td>815 ± 146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P*</td>
<td>0.01</td>
<td>1.01</td>
</tr>
<tr>
<td>III.</td>
<td>Pair-fed controls</td>
<td>Control diet</td>
<td>409 ± 17</td>
<td>1775 ± 319</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

* The significance values are for paired comparisons between the results above and below the P value.

the presumed ethylpalmitate, was split off by saponification in KOH (17 g per 100 ml of diethyleneglycol) for one hour.

The disappearance of ethanol from the media was too small to be measured accurately.

Effects of in vivo ethanol pretreatment on intestinal slices incubated without ethanol

Acute intragastric administration of a diet containing ethanol (in a dose of 3 g per kg body weight and in a concentration of 5 g/100 ml) one hour prior to sacrifice, decreased the ability of the jejunal slices to oxidize palmitate and to synthesize triacylglycerols (Table II). No such changes were found in slices obtained from the ileum.

By contrast, chronic ethanol feeding had stimulatory effects both in the jejunum and in the ileum. One hour after the intragastric administration of the alcohol containing diet, the intestine from rats chronically fed ethanol showed significantly greater capacity to oxidize palmitate and to synthesize triacylglycerol than that from pair-fed controls given an equal acute load of ethanol. The enhancement of 14CO2 production was associated with an increased production in total CO2 (5.72 ± 0.27 μl/mg of jejunal protein in 30 min, compared to 4.49 ± 0.18 μl in the pair-fed control rats given an equal acute load of alcohol; P < 0.05).

The stimulatory effects of chronic ethanol feeding were also observed when the ethanol pretreated rats were tested after administration of diet without ethanol for 20 h prior to sacrifice (group IV). The jejunal slices from rats chronically fed alcohol showed again a greater capacity for palmitate oxidation (620 ± 50 dpm/mg of tissue; P < 0.01) and esterification to triacylglycerols.
TABLE III
EFFECTS OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION ON SMALL INTESTINAL ACYL CoA SYNTHETASE ACTIVITY

Mucosal homogenates were obtained from 7 groups of 3 rats one hour after intragastric administration of a single dose of diet containing either ethanol (3 g/kg of body wt) or isocaloric carbohydrate and assessed for acyl-CoA synthetase activity [20, 21]. One rat of each trio was previously fed ethanol-containing diet for 3–4 weeks, and 2 were pair-fed control diet. Each value represents the mean ± S.E. of 7 determinations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chronic treatment</th>
<th>Acute treatment</th>
<th>Activity (nmol of acyl-CoA formed/min/mg of mucosa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jejunum</td>
</tr>
<tr>
<td>I.</td>
<td>Alcohol-fed rats</td>
<td>Ethanol diet</td>
<td>2.44 ± 0.18*</td>
</tr>
<tr>
<td>II.</td>
<td>Pair-fed controls</td>
<td>Ethanol diet</td>
<td>1.69 ± 0.19*</td>
</tr>
<tr>
<td>III.</td>
<td>Pair-fed controls</td>
<td>Control diet</td>
<td>1.95 ± 0.20</td>
</tr>
</tbody>
</table>

* P < 0.01 for paired comparisons between these results.

(2938 ± 478 dpm/mg of tissue; P < 0.02) than those from control rats (399 ± 14 and 1688 ± 289 dpm/mg of tissue, respectively).

The enhanced fatty acid oxidation and triacylglycerol synthesis observed in slices from ethanol pretreated rats was associated with 44% increase in palmitoyl-CoA synthetase activity in the jejunum (Table III). A similar tendency was observed in the ileum, but the changes in this activity did not reach a level of statistical significance.

Neither acute nor chronic ethanol administration affected the protein concentration of the intestine significantly (102 ± 4 mg/g of tissue in control rats; 114 ± 6 and 110 ± 5 after acute and chronic ethanol administration, respectively.)

Discussion

In vitro, ethanol inhibited both the incorporation of [1-14C]palmitate into triacylglycerol and its oxidation to 14CO2 by small intestinal slices (Table I). The concentrations of ethanol required to inhibit triacylglycerol synthesis were 1 g/100 ml and those to inhibit oxidation, 2.5 g/100 ml. These concentrations have been found in the upper small intestine after ethanol ingestion both in man [6] and in rats [5,7].

The small intestine has some alcohol dehydrogenase activity [3] and it is able to convert [1-14C]ethanol to 14CO2 [4]. However, the observed alterations in lipid metabolism are probably not linked to ethanol oxidation. The redox changes expected from the oxidation of ethanol through the alcohol dehydrogenase pathway would inhibit palmitate oxidation [10] and stimulate triacylglycerol synthesis [11]. In addition, an ethanol concentration of 0.5 g/100 ml should be sufficient to saturate not only alcohol dehydrogenase activity, but also that of the microsomal ethanol oxidizing system [23]. However, ethanol in this concentration did not produce significant changes in intestinal lipid metabolism. The possibility that the inhibition of [14C]palmitate oxidation and incorporation into triacylglycerols could merely reflect dilution
of the acetyl-CoA pool by the acetate resulting from the oxidation of ethanol also appears unlikely because of the concomitant decrease in the production of total CO₂. Previous observations indicate that, at these high ethanol concentrations, the intestinal oxygen consumption is also inhibited [24] and the structural integrity is affected [25]. Thus, the inhibition of fatty acid metabolism is probably a reflection of diffuse tissue damage, rather than a direct consequence of ethanol oxidation.

The injurious effect of ethanol could be due in part to hyperosmolarity, as suggested by the similarity of the effects observed with isosmotic controls in this and previous studies [7]. Also, at concentrations greater than those optimal for oxidation, ethanol participates in a variety of chemical reactions; for instance, alcoholysis of biologically important phosphate esters with formation of ethylphosphate is known to occur in vivo [26]. Ethanol can also be esterified with fatty acids in vivo [27]. The discrepancy between our results and the reported 30% increase in triacylglycerols synthesis by ethanol in vitro [2] may be due to contamination of the triacylglycerols fraction with esters of fatty acids and ethanol. These esters were readily apparent in our experimental conditions, probably because of the solvent system used [28] and the much higher concentrations of fatty acids used in our incubation media. Most of the ethanol label present in the tissue lipid extracts was recovered in this fraction, suggesting the formation of ethylpalmitate. Furthermore, the incorporation of palmitate into these esters was enhanced by increasing the concentration of ethanol in the media. Such an increase did not occur when the slices were killed by boiling, suggesting that the ethylpalmitate formation requires some enzymatic system either in the intestinal cells or in the contaminating luminal contents [29]. Contamination with labeled ethylpalmitate could account for up to a 50% increase in apparent triacylglycerol synthesis.

As shown in this study, when the intestinal slices were incubated without ethanol in the medium, their ability to oxidize fatty acids and to synthesize triacylglycerols was also affected by previous ethanol treatment. The effects of acute and chronic ethanol administration prior to sacrifice were compared in an experimental model designed to minimize differences in the previous treatment of the animals. The importance of these requirements is illustrated by the differences in the capacity of the intestinal slices for triacylglycerol synthesis between the chow-fed rats used for the in vitro experiment after an overnight fast (Table I) and the rats pair-fed control liquid diets and tested in the fed state (Table II). This difference probably reflects also the more extended area of intestine chosen for the in vitro studies and most likely, the previous feeding with liquid diet whose fat content is much greater than that of chow diet. This adaptation of the intestinal re-esterifying capacity to high fat diets has been previously reported by Singh et al. [30].

Acute administration of a single dose of ethanol-containing diet by gastric tube, one hour prior to sacrifice, inhibited fatty acid oxidation and triacylglycerol synthesis in the jejunum but not in the ileum. The administered dose is equivalent to one fifth of the daily ad libitum intake of ethanol-containing diet. This administration produced intraluminal concentrations in the upper small intestine which are maximal within 10 min and they are similar in magnitude to the inhibitory concentrations observed in vitro [7].
Thus, the inhibition after acute administration of ethanol in vivo is most likely due to the high local concentrations achieved in the upper small intestine and is consistent with other evidence of tissue damage after acute ethanol administration in vivo [7].

By contrast, chronic ethanol feeding increased the ability of the intestinal slices to oxidize palmitate and to synthesize triacylglycerols. This was associated with increased production of total CO₂ and oxygen consumption [7]. The latter findings render unlikely the possibility that the changes in fatty acid-label incorporation could be due to changes in the fatty acid pool, but rather suggest a metabolically more active tissue. These activities were further enhanced when the intestine from alcohol-pretreated rats was tested in the absence of acute ethanol effects, indicating a permanent metabolic change of the intestinal cell. In the small intestine, an organ with a rapid cell turnover, the changes can occur very rapidly. Already 16 h after acute administration of a high ethanol dose (which is known to produce gross hemorrhagic erosions of the mucosa), there is enhanced capacity for triacylglycerol [2] and cholesterol [1] synthesis.

The mechanism for this stimulatory effect of chronic ethanol feeding is unknown. Increased triacylglycerol synthesis has been attributed to the metabolic disturbances resulting from ADH-dependent ethanol oxidation because the increase was inhibited by pyrazole [2], an ADH inhibitor which also exerts a number of toxic effects [31]. In the present study a similar enhancement of triacylglycerol formation was observed in the absence of significant ethanol oxidation. Furthermore, unlike the consequences of redox changes linked to ethanol oxidation, both fatty acid esterification and oxidation were enhanced. This association is probably due to enhanced fatty acid activation, a common step for both processes, as suggested by the increased activity of palmitoyl-CoA synthetase (Table III). This enzyme activity is present both in mitochondria and in microsomes, two organelles of the intestinal epithelial cells which have been shown to be altered by chronic ethanol feeding in man and in rat [9]. The possibility that these alterations might reflect changes in the epithelial cell population secondary to the damaging effects of ethanol should also be considered.

Thus, the high concentrations of ethanol to which the small intestine is exposed during drinking inhibit fatty acid oxidation and esterification to triacylglycerols apparently as a consequence of a direct damaging effect of alcohol. By contrast, prolonged ethanol administration promotes functional and structural changes which result in an increased capacity for fatty acid oxidation and triacylglycerol synthesis. It must be pointed out that these changes are not necessarily relevant to the effects of ethanol on fat absorption since there is no significant transport of fat in the absence of lymphatic circulation and the processes studied here might not be rate-limiting steps for the transport of fat through the small intestine. Actually, the transport of dietary fat into the lymph was shown to be enhanced by a comparable single ethanol dose, whereas chronic ethanol feeding prevented these acute effects and exerted a mild inhibition of fat absorption [32]. Recent evidence indicates that these effects on fat absorption depend, at least in part, on the lymphagogue effect of ethanol [33].
Acknowledgements

This investigation was supported by USPHS Grants AM 12511, AA 00224, the Veterans Administration and Research Scientist Development Award AA 70409. The expert assistance of Miss Frances Finkelman is gratefully acknowledged.

References