Effect of Ethanol on Intestinal Adenosine Triphosphate (ATP) Content[1] (37201)

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Numerous studies have been conducted on the effect of the ethanol ingestion on hepatic function and structure. The intestine, however, has been less extensively studied, perhaps on the assumption that most ingested alcohol is absorbed by the stomach. However, Israel *et al.* (1) showed that significant amounts of ethanol reach the small intestine with a luminal ethanol concentration of 2% often being found in moderate drinkers.

Previous studies indicate that acute (2) and chronic (3) ethanol administration markedly affect hepatic ATP content. We now report the effects of ethanol *in vitro* and *in vivo* on small intestinal adenosine triphosphate (ATP) levels.

Methods. Female guinea pigs (weighing approx 450 g) were anesthetized with ether and sacrificed by cervical dislocation. The entire small intestine was then excised and placed in ice-cold saline. The tissue was rinsed with saline and cut into small rings as described previously (4). After gassing with 95% O_2:5% CO_2 and incubation, tissue ATP was extracted and determined as described below.

Female Sprague–Dawley rats (weighing 200 g) were killed by decapitation, the small intestine was perfused *in situ* with 50 ml of 0.1 *M* sodium phosphate (pH 7.4) containing 0.1 *M* sodium fluoride, then excised and dropped into liquid nitrogen, the entire procedure taking less than 2 min. The liver was handled in similar fashion. ATP was extracted from the frozen tissue or from the experimentally incubated tissue by homogenization in 0.6 *M* perchloric acid followed by centrifugation and subsequent neutralization with potassium phosphate (K_2HPO_4). The actual ATP content was determined as described previously (2) within 1 hr from the time of homogenization.

Acute ethanol administration was achieved by giving rats fasted 8 hr a single dose of ethanol 7.5 g/kg (39% w/v) or glucose 13.7 g/kg (60% w/v) by stomach tube. The animals were returned to their cages and fasted an additional 18 hr during which they were given free access to water. Chronic ethanol feeding was achieved by pair feeding individually caged littermates a high alcohol liquid diet (5) for 28 days. Controls received the same diet with ethanol replaced by carbohydrate. Ethanol oxidation to carbon dioxide by small intestinal tissue was measured as before (4).

Results. Incubation of guinea pig small intestinal segments with 2.6% ethanol resulted in a significant reduction of intestinal

TABLE I. Effect of Ethanol *in Vitro* on Guinea Pig Small Intestine ATP Levels.

Addition[a]	ATP concn (μmole ATP/g wet wt tissue; mean $\pm$ SD)	p[b]
0	1.34 $\pm$ 0.22	—
Glucose	1.20 $\pm$ 0.02	NS
Ethanol	0.80 $\pm$ 0.14	$<.05$
Glucose + pyrazole, 1 m*M*	1.32 $\pm$ 0.12	NS
Ethanol + pyrazole, 1 m*M*	0.82 $\pm$ 0.20	$<.05$

[a] Assay mixture consisted of 3 ml of 0.1 *M* sodium phosphate buffer (pH 7.4), 500 mg of tissue and either 570 m*M* ethanol or 240 m*M* glucose.

[b] Statistical significance of values with respect to the flask containing no additions.

[1] This research was supported in part by a grant from the National Institute of Mental Health (MH-16892).

TABLE II. Effect of Ethanol *in Vivo* on Rat Hepatic and Small Intestinal ATP Content.

Ethanol administration	ATP Concn[a]						Blood ethanol concn (Alcohol group) (mg/100 ml)[f]
	Small intestine ($N = 10$)			Liver ($N = 6$)			
	Control	Alcohol	p	Control	Alcohol	p	
Acute[b]	1.52 ± 0.69	0.90 ± 0.34	<.02	1.74 + 0.31	0.98 ± 0.18	<.001	196 ± 61
Chronic[c]	1.10 ± 0.17	0.68 ± 0.09	<.02	3.31 ± 0.14	0.87 ± 0.14	<.001	65 ± 10
Acute + pyrazole[d]	2.39 ± 0.40	1.69 ± 0.36	<.02	1.97 ± 0.19	1.43 ± 0.08	<.001	422 ± 54
Chronic + pyrazole[e]	3.52 ± 0.49	2.58 ± 0.09	<.02	2.37 ± 0.36	1.40 ± 0.48	<.001	333 ± 36

[a] Concentrations expressed as micromoles per gram of wet weight; mean ± SD.
[b] Single dose of ethanol (7.5 g/kg) or glucose (13.7 g/kg) given 18 hr prior to sacrifice.
[c] Animals fed high alcohol liquid diet (5) for 28 days. Controls received same diet with ethanol replaced by carbohydrate.
[d] Pyrazole (65 mg/kg) given intraperitoneally 2 hr before the alcohol or glucose.
[e] Animals fed high alcohol liquid diet for 28 days with pyrazole (200 mg/liter) added to both alcohol and control diets.
[f] Blood ethanol content measured by gas–liquid chromatography (14).

ATP content (Table I). This reduction was not observed when an amount of glucose isocaloric to the amount of ethanol added was incubated with the tissue under the same conditions. It was noteworthy that the effect of ethanol on intestinal ATP content could not be blocked by additions of pryazole (6) which reduced ethanol oxidation to CO_2 by 70%.

Likewise, the small intestines of rats treated with ethanol *in vivo*, either acutely or chronically, exhibited decreased ATP levels compared to isocalorically fed controls (Table II). This effect of ethanol also could not be blocked by pretreatment of the animals with pyrazole (6) or by the inclusion of this inhibitor in the diet. However, pyrazole, by decreasing ethanol metabolism, produced the expected increase in blood ethanol levels (Table II).

In normal rats the small intestinal ATP content was found to be 1.41 ± 0.21 μmoles/g wet weight. This value was not significantly different from that of animals given a single dose of glucose or fed the liquid control diet for 28 days. However, animals given a single injection of pyrazole or fed a pyrazole containing diet for 28 days exhibited increased small intestinal ATP content. There was 70% more ATP in the small intestine of animals given glucose and pyrazole ($p < 0.01$) and 135% more in the small intestine of animals fed pyrazole in the diet for 28 days ($p < 0.001$) than the normal animals. Animals given alcohol plus pyrazole also showed increased intestinal ATP levels (compared to controls) but these values were lower than the animals given pyrazole alone (Table II). No alterations in hepatic ATP were observed in pyrazole treated rats.

Consistent with earlier reports (2, 3), treatment of animals with ethanol *in vivo*, either acutely or chronically, depressed hepatic ATP levels (Table II). However, this effect of ethanol upon hepatic ATP content could also not be prevented by pretreating the animals with pyrazole or by including this inhibitor in the diet.

Discussion. Over the past several years a number of studies have shown that ethanol has a number of effects on the function and structure of the small intestine. Ethanol in-

hibits the active transport of Na^+ and K^+ and the (Na + K) activated ATPase in several tissues and species (7). A number of investigators have demonstrated that both *in vitro* and *in vivo* ethanol may interfere with the uptake and transport of amino acids and glucose (1, 8, 9, 10). Israel *et al.* (1) found in the human small intestine that at a luminal ethanol concentration of 2%, a concentration found in the intestinal lumen of moderate drinkers, the intestinal adsorption of L-methionine was inhibited by 50%. Thomson, Baker and Levy (11) found that 25% of patients given 1.5 g/kg of ethanol prior to thiamine-^{35}S would have a 40–60% reduction in absorption of this vitamin. As discussed in a recent review (12) chronic alcoholic patients have malabsorption of many substances including fat, xylose, folic acid, thiamine, vitamin B_{12}, fat-soluble vitamins, and methionine. Rubin *et al.* (13) have found that chronic ethanol administration to human volunteers led to abnormalities in mitochondria, dilatation of the endoplasmic reticulum and the cisternae of the Golgi apparatus.

The present findings demonstrate that ethanol also affects intestinal ATP content. The results suggest that ethanol, either fed to animals *in vivo* acutely or chronically or added *in vitro* to normal small intestinal tissue at concentrations equivalent to luminal ethanol concentrations found in moderate social drinkers, lowered intestinal ATP content. However, this effect was not blocked when ethanol metabolism was inhibited by pyrazole either *in vitro* or *in vivo*. Hence, it appears that ethanol exerts an effect upon intestinal ATP content independent of its metabolism to acetaldehyde and CO_2.

It is possible that this action of ethanol on intestinal ATP content may explain, at least in part, the inhibitory effects of ethanol on the intestinal mucosal transport.

Summary. Ethanol given *in vivo* either acutely or chronically or added *in vitro* in amounts comparable to those found in the small intestine of moderate social drinkers, markedly lowered the ATP content of the small intestine. This effect could not be prevented when ethanol metabolism was inhibited by pyrazole. Thus, it appears that ethanol exerts a direct effect on the ATP content of the small intestine. This action may explain, in part, the inhibition of various intestinal mucosal transport processes by ethanol.

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Received Dec. 13, 1972. P.S.E.B.M., 1973, Vol. 142.