The Metabolism of Ethanol to Carbon Dioxide by Stomach and Small Intestinal Slices[1] (35997)

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Recently, it has been suggested (1) that the liver is not the only organ capable of ethanol oxidation. The stomach and small intestine have been found to contain alcohol dehydrogenase (ADH) (1, 2). Furthermore, the specific activity of these preparations has been reported to be greater than that of the liver assayed under identical conditions. These observations are of importance, since it is clear that ethanol ingested orally interacts with the gastric and small intestinal mucosa before it reaches the liver. To determine if the gastrointestinal tract might contribute to the metabolism of ethanol, the production of $^{14}CO_2$ from 1-^{14}C-ethanol by slices of small intestine and stomach was measured.

Methods. Female Sprague-Dawley rats (weighing 200 g) were decapitated and the organs were excised. Liver slices were prepared with a Stadies-Riggs microtome and gastric or intestinal slices, as described previously (3). Slices weighing a total of approximately 0.5 g were placed in 3 ml of Krebs–Ringer bicarbonate buffer, gassed 10 min with 95% O_2, 5% CO_2 and incubated with 1-^{14}C-ethanol in stoppered 25 ml Erlenmeyer flasks (with center wells) for 3 hr at 37°. The reaction was stopped by injecting 2 ml of cold ethanol into the flasks, after which the flasks were cooled to 0°. One-half milliliter of 10 *N* H_2SO_4 was then injected into the incubation mixture. With the flasks still stoppered, the center well was opened and 0.5 ml of Hyamine hydroxide was added to the center well. The stoppered flasks were shaken for an additional 30 min at 4°; the Hyamine was then removed and counted in 15 ml of toluene containing 0.01% *p*-bis[2-(5-phenyloxazoyl)] benzene and 0.3% 2,5-diphenyloxazole. Alcohol dehydrogenase activity in the various organs was measured, as described previously (1). Germ-free rats were obtained from Charles River Breeding Farms, Wilmington, MA.

Results. We have confirmed previous reports that alcohol dehydrogenase (ADH) exists in the mucosa of the stomach and small intestine of male rats (Table I). Furthermore, it was found that in female as well as male rats, the ADH specific activity of the stomach was approximately three times the liver, while the small intestine was comparable to that of liver. The microsomal ethanol oxidizing system described by Lieber and DeCarli for liver (6) was not found by us in the tissues of the gastrointestinal tract.

In contrast to these observations on ADH activity, the capacity of the stomach or small intestine to convert ethanol to CO_2 was less than that of liver. Table II demonstrates that the specific activity of the stomach, middle and lower small intestine was approximately 1/5 that of liver. The upper intestine, however, demonstrated a greater capacity to convert ethanol to CO_2, the specific activity being 2/3 that of liver slices incubated under similar conditions.

Since ADH activity and ethanol metabolism might be due to gastrointestinal bacterial flora (2), studies were also carried on in germ-free rats. Thus, it was demonstrated (Table III) that effective oxidation of ^{14}C-ethanol occurred in gastrointestinal slices of germ-free rats; in fact, the oxidation to $^{14}CO_2$ was significantly greater in the upper small intestine of germ-free rats than their conventional controls.

Discussion. In the past, the liver has been

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TABLE I. Alcohol Dehydrogenase Activity in Several Rat Organs.[a]

Organ	ADH sp act	
	Male	Female
Liver	0.110	0.156
Stomach	0.480	0.465
Intestine	0.150	0.144

[a] Rate = μmoles of NADH formed/hr/mg of protein, pH 8.7, 30°.

TABLE II. Production of $^{14}CO_2$ from 1-^{14}C-Ethanol by Organ Slices.[a]

	Recovered (dpm) as $^{14}CO_2$/100 mg wet weight tissue/3 hrs	% of liver slice value[b]
Liver	12,810	—
Stomach	2270	18
Upper intestine	8690	68
Middle intestine	2820	22
Lower intestine	3330	26

[a] Assay mixture as in text. Substrate was 0.5 μl of pure ethanol (0.5 μCi). Background (Hyamine from flask with ^{14}C-ethanol but no tissue slices treated as above) was 300 dpm. Results of three animals.

[b] (Value for other organ/value for liver slices) × 100.

considered virtually the sole site of ethanol metabolism. However, recent evidence has been obtained to suggest that this concept may need to be redefined (1). The present findings emphasize that in both male and female rats, ADH is present in tissues of the gastrointestinal tract of the rat and assumes added importance in view of the apparent increase in the specific activity of this enzyme with chronic alcohol administration (1). Virtually all ingested ethanol is absorbed from the stomach and small intestine. Hence, an increase in the activity of the ADH and consequent metabolism of ethanol in these tissues could be important in explaining the increased tolerance to ethanol associated with chronic administration (5). It therefore appeared important to us to determine if the stomach or small intestine could convert ethanol to CO_2.

The present studies indicate that the small intestine and stomach are able to metabolize ethanol to CO_2, but at much lower rates than liver. This is in marked contrast to the specific activity of ADH, in which case the stomach has a greater, and the small intestine the same, specific activity as liver. It was of interest that the upper small intestine converted ethanol to CO_2 four times as rapidly as the middle or lower intestine or stomach.

Why ethanol should be metabolized at all by the gastrointestinal tract remains to be determined. Krebs and Perkins (2) recently proposed that ethanol, produced by bacteria in the gut, was absorbed into the portal vein for transport to the liver for metabolism by hepatic ADH. These workers suggested that the sole purpose of ADH might be the removal of ethanol produced by gut flora, and that intestinal ADH was, to a significant extent, of bacterial origin. In this regard, it is noteworthy that we found the same rates of ethanol conversion to CO_2 in germ-free as in conventional rats. The quantitative role of the gastrointestinal mucosa in the overall oxidation of ethanol *in vivo* is unclear. However, it would appear that the effects of ethanol

TABLE III. Production of $^{14}CO_2$ from 1-^{14}C-Ethanol by Stomach and Intestinal Slices from Normal and Germ-Free Animals.[a]

Organ	CO_2 production	
	Normal ($N = 3$) (dpm→$^{14}CO_2$/100 mg of wet wt tissue/3 hr)	Germ free ($N = 3$) (mean ± SD)
Stomach	2470 ± 304	3169 ± 233
Upper small intestine	5693 ± 1008	11,486 ± 418
Middle small intestine	979 ± 83	1159 ± 79
Lower small intestine	1482 ± 41	2099 ± 169

[a] Assay conditions as Table II.

on intestinal sugar (7), amino acid transport (8), and lipid metabolism (4) are related to the metabolism of ethanol by the mucosa.

Summary. It has been demonstrated that slices of stomach and small intestine are able to convert ^{14}C-ethanol to $^{14}CO_2$. When compared to liver slices under the same conditions, the gastrointestinal oxidation was one-fifth to two-thirds of the hepatic capacity. Furthermore, organs obtained from germ-free animals were able to convert ethanol to CO_2 at rates equal to or greater than their conventional counterparts. The significance of these observations in relation to the effects of ethanol on the intestine and to overall ethanol metabolism remains to be elucidated.

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