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Effect of Acute Ethanol Administration on Duodenal Calcium Transport[1] (38115)

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We have recently demonstrated that chronic ethanol ingestion interferes with the ability of the rat duodenum to transport calcium by a process independent of partial starvation and of pancreatic or hepatic dysfunction (1). In view of the recently accumulated evidence that high concentrations of ethanol inhibit the intestinal transport of amino acids and carbohydrates in the rat (2–5), this investigation was undertaken to determine the effects of the acute administration of high concentrations of ethanol on calcium transport by the rat duodenum.

Methods. Albino male Sprague–Dawley rats were raised on laboratory chow and tap water in hanging cages in a windowless room with automatically controlled temperature and lighting. Food was withheld for 24 hr before experimentation.

Transport was measured by a method based on the *in vitro* everted gut sac technique of Wilson and Wiseman (6) as adapted by Martin and DeLuca (7). Sacs of 5 cm in length were filled with 0.5 ml of a medium consisting of 0.125 *M* NaCl, 0.010 *M* fructose, 0.00025 *M* $CaCl_2$, 0.03 *M* Tris–Cl, pH 7.4, and sufficient $^{45}CaCl_2$ to provide approximately 25,000 cpm/ml of medium and incubated at 37° for 90 min with continuous bubbling of 100% oxygen. After incubation, aliquots of the medium, inside and outside the sac, were counted by liquid scintillation chromatography, using automatic external standardization. Data are expressed as a final concentration ratio of the tracer inside the sac (serosal medium) over that outside the sac (mucosal medium), written S/M.

Specimens for histologic studies were fixed in 10% formalin and stained by conventional techniques with hematoxylin and eosin.

Results. As illustrated in Table I, 1 hr after the administration of a large dose of ethanol intragastrically, there was a significant decrease in the ability of duodenal gut sacs to transport calcium while no effect was apparent after the intraperitoneal administration of ethanol. Histologically, the duodenum from rats which had received intragastric ethanol demonstrated necrosis of the villous epithelium and infiltration of lymphocytes and plasma cells in the remaining crypts. No changes were seen after intragastric glucose administration or after intraperitoneal administration.

In view of this inhibition of transport, it was of interest to determine what the effect of ethanol would be when added directly to the incubation system. Concentrations of 0.1% and 1.0% ethanol did not diminish transport ratios (Table II), while a 1.5% solution significantly inhibited calcium transport by the duodenal gut sacs. An equiosmolar solution of mannitol, an inert molecule, reduced transport ratios to unity, while an initially equiosmolar solution of glucose, a metabolically active substance, resulted in no interference in transport. Histological examination of the gut sacs after incubation revealed destruction of villous architecture with sloughed and shrunken epithelial cells containing granular vaculated cytoplasm and pyknotic nuclei from the sacs incubated in the presence of mannitol and 2.6% ethanol. Similar but less striking changes were seen in the presence of 1.5% ethanol, but were not seen with glucose, as compared

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TABLE I. Effect of a Single Dose of Ethanol on Calcium Transport by Duodenal Gut Sacs.[a]

Group	Number	Route	Mean ^{45}Ca concentration ratio ± SE (serosal/mucosal)	P
Control	8	Intragastric	4.8 ± 0.6	< 0.005
Ethanol	8	Intragastric	1.2 ± 0.2	
Control	12	Intraperitoneal	3.4 ± 0.4	> 0.05
Ethanol	16	Intraperitoneal	4.0 ± 0.6	

[a] One hour before measuring transport, intragastric ethanol was administered by delivering 7.5 g/kg body weight of ethanol through a blunt needle inserted through the esophagus. Control animals received isocaloric glucose (13.7 g/kg body weight). For intraperitoneal administration, 5.0 g/kg body weight was administered and control animals received an equal volume of 0.9% NaCl. P values are calculated from nonpaired t tests.

with specimens incubated in the absence of any added substance.

Discussion. The results of this study indicate that the acute administration of ethanol also inhibits the ability of the rat duodenum to transport calcium, as measured by the *in vitro* everted gut sac technique. The fact that the depression was observed 1 hr after intragastric administration but not when ethanol was given intraperitoneally, in conjunction with the histologic damage evident on light microscopy, suggests that this is a result of a direct toxic effect on the intestinal epithelium. That this defect can be relatively rapidly reversed, however, is apparent, for when calcium transport is measured 18 hr after this single intragastric dose, transport ratios are not reduced (1).

The results of our studies, in which ethanol was added to the incubation medium bathing the everted gut sac also suggest that the inhibition is a manifestation of local toxicity to the intestine. It may be, in part, due to the hypertonic nature of the solution, since in comparing the effects of 1.5% ethanol, 5% glucose, and 4.4% mannitol (Table II) the most severe impairment of transport and histologic damage was seen with the inert molecule mannitol. Although 1.5% ethanol produced striking diminution in transport, histological changes were not as apparent and no changes in transport were seen when the metabolizable sugar, glucose, was added to the medium.

Whether the structural damage seen accompanying the inhibition of transport after acute administration of alcohol observed in this study bears any relationship to the inhibition of calcium transport seen after chronic administration cannot be determined at this time. The normal histological picture seen after chronic ethanol administration (1) would indicate that they differ but it should be noted

TABLE II. Effect of Ethanol *in Vitro* on Calcium Transport by Duodenal Gut Sacs.[a]

Addition	Percent	Osmolarity (mOsm/liter)	Number	Mean ^{45}Ca concentration ratio ± SE (serosal/mucosal)	P
None	—	290	41	3.8 ± 0.2	—
Ethanol	0.1	—	8	3.8 ± 0.4	> 0.05
Ethanol	1.0	—	9	3.6 ± 0.6	> 0.05
Ethanol	1.5	515	23	1.9 ± 0.1	< 0.01
Mannitol	4.4	520	8	1.0 ± 0.1	< 0.01
Glucose	5.0	515	13	4.7 ± 0.5	> 0.05
Ethanol	2.6	750	18	2.0 ± 0.2	< 0.01

[a] Identical concentrations of ethanol, mannitol, or glucose were added to the incubation medium bathing mucosal and serosal surfaces of everted gut sacs. P values are calculated from a Bonferoni t test for multiple comparisons.

that despite the absence of any changes on light microscopy, ultrastructural changes have been observed in the small intestine, in animals ingesting ethanol for periods of 9–12 mo (8).

Summary. To determine the effect of acute administration of high concentrations of ethanol on duodenal calcium transport, experiments were performed using an *in vitro* everted gut sac technique. One hour after the administration of ethanol intragastrically, significant decreases in transport and necrosis of epithelium were produced while intraperitoneal administration resulted in no changes. Similar changes were observed after the addition of ethanol to the incubation system. The results suggest that the inhibition of calcium transport seen after acute administration of ethanol is the result of a direct toxic effect on the intestinal epithelium.

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