Alcohol and absorption from the small intestine

2. Effect of ethanol on ATP and ATPase activities in guinea-pig jejunum

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SUMMARY After the exposure of isolated segments of guinea-pig jejunum to 2% ethanol for one hour, a significant reduction ($P < 0.001$) in the adenosine triphosphate content (ATP) was observed when compared with levels found in segments perfused with Krebs' solution. However, no alteration was noted in the activity of adenosine triphosphatase (ATPase). The chronic administration of 50% ethanol in a dose of 2.5 g/kg for two weeks did not affect either the ATP content or ATPase activity in jejunal mucosa.

Absorption of electrolytes from the intestinal lumen into the blood stream involves, predominantly, the process of active transport. Energy for cellular metabolism derives from the splitting of the high energy phosphate bond of adenosine triphosphate (ATP) by the enzyme adenosine triphosphatase (ATPase), (Brooks, 1973). Ethanol has been shown to affect the ATP content in several tissues and it also inhibits the active transport of Na^+ and K^+ and the (Na^+ and K^+) activated ATPase (Israel *et al.*, 1965; Israel *et al.*, 1969). It seems possible, therefore, that intestinal dysfunction in alcoholics may result, at least in part, from a direct enterotoxic effect of ethanol.

The aim of this study was thus to investigate the effect of the acute and chronic administration of ethanol on ATP levels and ATPase activities in the jejunum of guinea-pigs.

Methods

The acute effects of ethanol on the ATP content and ATPase activity in the jejunum using whole mucosal homogenates were studied in the guinea-pig. In two sets of experiments, 12 female guinea-pigs weighing approximately 225 g, were killed by cervical dislocation and the jejunum placed in ice-cold saline. The jejunum was rinsed in saline, then gassed with a

mixture of 95% O_2 and 5% CO_2 (Carter and Isselbacher, 1973). Segments of approximately 1 g were placed in hollow cylinders in a thermostatically controlled water bath at 37°C, then perfused for periods of one hour with either Krebs' solution or 2% ethanol introduced into the cylinder *via* a syringe every five minutes for the 60 minute period (Figure). Before each ethanol infusion the cylinder was flushed through with Krebs' solution to prevent a build-up in the ethanol concentration. Each animal acted as its own control since segments of jejunum from the same animal were exposed to the two solutions simultaneously. ATP was extracted and assayed using the Boehringer test kit (1973), which measures the enzymatic production of NADH after the method of Adam (Adam, 1963). ATPase activities in the whole tissue homogenate were measured by a modification of the method of Becker *et al.* (1971), in which 1 ml enzyme and 0.5 ml buffer was used instead of 0.5 ml enzyme and 1 ml buffer.

Chronic ethanol administration was achieved by gastric intubation of 12 female guinea-pigs, infusing a 50% ethanol solution in a dose of 2.5 g per kg for 14 days. A control group of 12 guinea-pigs also underwent gastric intubation and in these animals an isocaloric glucose solution was instilled for 14 days. At the end of the two-week period, ethanol was withdrawn from the test group for 24 hours to eliminate the possible acute effect of recent ethanol

Figure Apparatus for perfusion of segments of guinea-pig jejunum with 2% ethanol or Krebs' solution.

consumption. The animals were killed by cervical dislocation, jejunal segments were prepared as previously described, and ATP levels and ATPase activities measured as above.

Results

ACUTE ETHANOL ADMINISTRATION

After the acute exposure of jejunal segments to 2% ethanol (Table 1), ATP concentrations were significantly reduced when compared with levels in the corresponding segments from the same animals perfused with Krebs' solution (using paired *t* test, $P < 0.001$). However, when jejunal ATPase activities were measured, no significant difference was ap-

parent between levels found in the ethanol and Krebs' perfused segments.

CHRONIC ETHANOL ADMINISTRATION

ATP concentrations and ATPase activities were measured after two weeks in the control group of guinea-pigs receiving 50% ethanol *via* gastric intubation. Using Student's *t* test, no significant difference was found between either the mean ATP content or the mean activity of ATPase in the two groups (Table 2).

Discussion

An ethanol concentration of 2% has been shown to

ATP		ATPase	
2% Ethanol	Krebs' solution	2% Ethanol	Krebs' solution
0.98	1.22	2.42	1.31
0.94	1.33	3.01	2.53
0.61	1.75	1.68	1.91
1.04	1.49	3.26	3.66
0.85	1.46	2.61	2.69
0.19	1.50	2.88	4.11
0.53	1.36	1.83	2.61
0.74	1.46	2.06	2.63
1.08	1.20	2.59	1.75
1.28	1.48	—	2.84
1.30	1.70	2.11	2.88
1.20	2.03	2.04	3.05
Mean $\pm$ SEM		2.41 $\pm$ 0.15	
0.98 $\pm$ 0.07	1.49 $\pm$ 0.07	2.66 $\pm$ 0.22	NS
$P < 0.001$			

Table 1 ATP levels ($\mu\text{mol g wet weight}^{-1}$) and ATPase activities ($\mu\text{mol Po}_4$ released. g wet weight $^{-1}$. h $^{-1}$) in jejunal segments of guinea-pigs after acute exposure to 2% ethanol or Krebs' solution for one hour

ATP		ATPase	
50% Ethanol	Glucose solution	50% Ethanol	Glucose solution
1.48	1.26	7.85	5.83
1.26	1.28	8.15	7.68
1.03	1.65	4.13	5.81
1.02	1.08	4.21	4.27
1.5	0.82	4.11	5.05
1.44	1.4	6.06	2.63
1.61	0.9	1.22	1.19
1.48	1.44	3.01	2.42
1.7	1.51	2.59	2.46
1.27	1.27	1.45	4.0
1.23	1.51	1.85	1.79
1.69	1.33	1.73	2.14
Mean $\pm$ SEM		3.86 $\pm$ 0.69	
1.39 $\pm$ 0.07	1.29 $\pm$ 0.07	3.77 $\pm$ 0.58	NS
NS			

Table 2 ATP levels ($\mu\text{mol g wet weight}^{-1}$) and ATPase activities ($\mu\text{mol Po}_4$ released. g wet weight $^{-1}$. h $^{-1}$) in jejunal segments of guinea-pigs after chronic administration of 50% ethanol (2.5 g/kg) for 14 days, and in control animals

be present frequently in the small intestine in moderate drinkers (Israel *et al.*, 1969). The acute administration of ethanol at this concentration produced a reduction in the ATP content in isolated guinea-pig jejunal segments. These findings confirm the results of Carter and Isselbacher (1973). No effect, however, was demonstrated on activities of ATPase after similar exposure to ethanol. Chronic ethanol administration failed to produce significant alterations in either ATP levels or ATPase concentrations. Interference with the active transport of sodium and chloride probably results from recent ethanol ingestion as shown in the human perfusion study in alcoholics (Krasner *et al.*, 1976). This disturbance of intestinal function in the alcoholic patients is probably a direct effect of recent ethanol ingestion and may provide one mechanism leading to the development of malabsorption, particularly of water-soluble nutrients, in chronic alcoholics.

We would like to acknowledge the technical assistance which we received in this study from Mr P. Higgins, Mr K. McKirdy, Mrs A. Donald, Mr D. Sellar, and Miss J. Toms. We wish to extend our thanks to Professor A. Goldberg for his advice and

encouragement in the preparation of this and the previous paper.

References

- Adam, J. (1963). Adenosine-5-triphosphate: determination with phosphoglycerate kinase. In *Methods of Enzymatic Analysis*, p. 539. Edited by H-U. Bergmeyer. Verlag Chemie: Weinheim.
- Becker, T., Viljoen, D., and Kramer, S. (1971). The inhibition of red cell and brain ATPase by δ -aminolaevulinic acid. *Biochimica et Biophysica Acta*, **225**, 26-34.
- Boehringer Mannheim, G.m.B.H. (1973). *Biochemica Test Combination. Enzyme Determination of the Concentration of Adenosine-5-Triphosphate in Blood*, pp. 1-5.
- Brooks, F. P. (1973). Gastrointestinal absorption. (*Clinician*) (Searle). *Gastroenterology in Medical Practice*, pp. 15-22.
- Carter, E. A., and Isselbacher, K. J. (1973). Effect of ethanol on intestinal adenosine triphosphate (ATP) content. *Proceedings of the Society for Experimental Biology and Medicine*, **142**, 1171-1173.
- Israel, Y., Kalant, H., and Laufer, I. (1965). Effects of ethanol on Na, K, Mg-stimulated microsomal ATPase activity. *Biochemical Pharmacology*, **14**, 1803-1814.
- Israel, Y., Valenzuela, J. E., Salazar, I., and Ugarte, G. (1969). Alcohol and amino acid transport in the human small intestine. *Journal of Nutrition*, **98**, 222-224.
- Krasner, N., Cochran, K. M., Russell, R. I., Carmichael, H. A., and Thompson, G. G. (1976). Alcohol and absorption from the small intestine. I. Impairment of absorption from the small intestine in alcoholics. *Gut*, **17**, 245-248.