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APPEARANCE OF FORMATE IN BLOOD AFTER ETHANOL INGESTION

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Summary

Human and rabbit bloods after ingestion of ethanol were analyzed for carboxylic acids, and were found to contain not only acetate but also formate. The formate level in the rabbit serum was 0.35μmole/ml at 4 hours after introduction of 10ml of 40% ethanol/kg into the stomach. Administration of [1-^{14}C] ethanol or [2-^{14}C]ethanol resulted in the presence of radioactivity in serum acetate, but not in serum formate. Pretreatment with tryptophan significantly increased serum formate, and pretreatment with folate suppressed the appearance of formate.

Introduction

Over 90% of ingested ethanol is metabolized in the liver in various animals. It has been frequently reported that the ethanol metabolizing process involves abnormalities of other metabolizing systems, such as the suppression of gluconeogenesis (1,2), the decrease of fatty acid oxidation (1), and the interference with the citric acid cycle (2). It is said that the main cause of these metabolic abnormalities is an increase in $NADH_2$ of the hepatic cells (1,2,3). But there are some phenomena which can not be explained.

During our study of the effect of ethanol on other metabolic systems by checking changes in serum carboxylic acid levels in rabbits after administration of ethanol, formate was detected. It was also found that the acid appeared in the serum of human subjects who had drunk alcoholic beverages. To our knowledge, the present paper is the first to describe such an observation.

Materials and methods

Male albino rabbits, weighing 2.5 to 3.0kg, were fasted overnight, water being allowed ad lib. Ten ml of 40% ethanol per kg of body weight was given with a stomach tube. Four hours later blood was drawn from the ear artery. Human blood samples were obtained from patients who were admitted to an emergency hospital.

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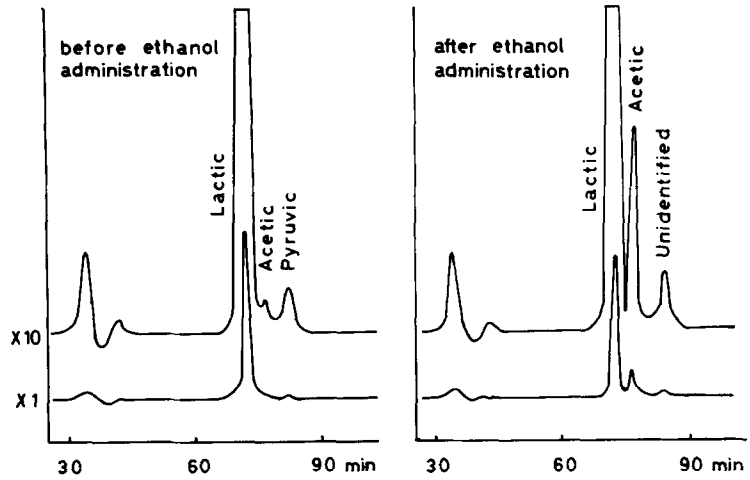

Fig. 1 Chromatograms of serum carboxylic acids before and after ethanol administration. Rabbits were given 10ml of 40% ethanol solution per kg of body weight. After 4 hours blood was collected from the ear artery. Serum was deproteinized with 10% perchloric acid, and the supernatant was used as a sample. Conditions of carboxylic acid analysis: Resin; Diaion CA-08S. Column size; 1m x 3mm. Temperature; 40°C. Eluent; 0.2N HCl. Flow rate; 0.13ml/min. Sample size; 133µl

Serum ethanol was measured with a Shimazu 6A gas-liquid chromatograph. The supernatant of serum deproteinized with 10% perchloric acid was analyzed for carboxylic acids with an auto carboxylic acid analyzer (Seishin Pharmaceutical Co.) according to the method of Y. Kasai et al (4,5,6). Formate was identified by Sawicki's method (7). [1-^{14}C]ethanol and [2-^{14}C]ethanol were purchased from New England Nuclear Co.. Radioactivity was measured using a Packard liquid scintillation spectrometer. All other reagents were obtained from commercial sources.

Results and discussion

Figure 1 is the chromatograms of carboxylic acid before and after ethanol administration. As is evident from the figure, the administration of ethanol was followed by an increase in acetate, disappearance of pyruvate, and appearance of a carboxylic acid near the point where pyruvate appeared before ingestion of ethanol.

This unidentified carboxylic acid did not react with 2,4-dinitrophenylhydrazine, a reagent which specifically combines with the carbonyl group, and was steam-distilled at pH2.8. Table 1 shows the retention times of various carboxylic acids and the unidentified carboxylic acid, eluted with 0.2N or 0.02N HCl for analysis with the auto carboxylic acid analyzer. On elution

Table 1

Retention times of various carboxylic acids and the unidentified carboxylic acid.

Acid	Retention time (min.)	
	0.2N HCl	0.02N HCl
Glutamic	40	40
Lactic	70	70
Acetic	76	70
Pyruvic	81	—
Formic	85	95
Malic	90	117
Unidentified	85	95

Carboxylic acids dissolved in 0.2N or 0.02N HCl (4μmoles/ml) were applied. Analytical conditions were the same as in the footnote of Fig. 1 except for the use of both 0.2N and 0.02N HCl.

with either eluent, the retention times of carboxylic acid were the same as that for formate. The solution containing the carboxylic acid was collected by fractionation on the column of the analyzer. The concentrate of this solution strongly reacted with 1-methylquinalidinium toluene-p-sulfonate, a reagent to detect formate which was prepared by the method of Sawicki (7). These results suggested that the carboxylic acid was formate, and was different from both pyruvate and malate, the retention times of which closely resemble that of formate on carboxylate analysis.

At 4 hours after ethanol administration the serum level of formate was 0.354±0.029μmole/ml, of ethanol 70.4±0.21μmoles/ml, and of acetate 2.443±0.087 μmoles/ml. The appearance of formate was not a phenomenon peculiar to rabbits. Figure 2 shows a negative correlation between ethanol and formate levels in human samples. Formate was invariably detected from the sera containing ethanol. That no relationship existed between the levels of ethanol and formate was presumably due to the fact that the test samples were taken at varying times after ingestion of different quantities of alcohol.

It is well known that methanol is oxidized to formaldehyde and subsequently to formate. It is clear, however, that the formate detected in this study was not derived from methanol, because it was confirmed by gas-liquid chromatography that the ethanol used was not contaminated by methanol. Table 2

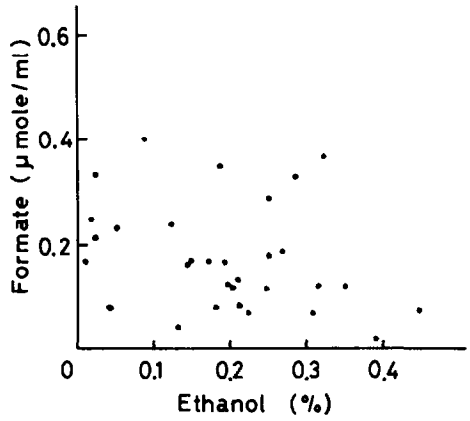

Fig. 2 Negative correlation between ethanol and formate levels of human sera after drinking.

Table 2

Radioactivity of serum acetate and formate after administration of labeled-ethanol.

	Acetate	Formate
[1-^{14}C]ethanol	426	0
[2-^{14}C]ethanol	466	0

(c.p.m./μmole)

Rabbits were given 10ml/kg of 40% ethanol solution containing 50μCi of [1-^{14}C]ethanol or [2-^{14}C]ethanol. Serum acetate and formate were fractionated by chromatography on a column of the carboxylic acid analyzer. The fractions containing acetate or formate were adjusted to pH9.0 with potassium hydroxide, and the radioactivity was measured with a scintillation spectrometer as cocktails of toluene-triton X-100 system.

shows the incorporation of orally administered [1-^{14}C]ethanol or [2-^{14}C]ethanol into serum acetate but not into serum formate, meaning that the ethanol ingested is not a precursor of the formate.

In vivo, formate is produced on the pathway of tryptophan metabolism(8,9). In our experiments prior administration of tryptophan significantly increased serum formate ($p < 0.001$) in comparison with administration of ethanol or tryptophan alone (Table 3). Badawy and Evans demonstrated that tryptophan pyrrolase activity was acceralated by ethanol administration(10). Thus, it seems likely that the appearance of formate following ethanol administration is due to acceralation of tryptophan metabolism.

Table 3

The effect of pretreatment with tryptophan or folate on serum ethanol and formate levels.

Treatment	Number	Ethanol (μmole/ml)	Formate (μmole/ml)
Ethanol	13	70.4±0.21	0.354±0.029
Tryptophan	4	—	0.091±0.035
Tryptophan + Ethanol	5	69.8±0.43	0.638±0.055*
Folate + Ethanol	5	67.4±0.28	0.241±0.024**

Values are means ± standard errors.
* $p<0.001$ ** $p<0.05$

Ethanol was given orally and blood sample was obtained as described in Materials and Methods. Rabbits were given 1g of tryptophan/kg suspended in water (about 4%) and force fed by a stomach tube 30 minutes before ethanol administration. Folate was injected intraperitoneally in 0.5% aqueous suspension at a dose level of 5mg/kg every day for a week.

On the other hand formate, in vivo, is consumed on the pathway of folate (11). As shown in table 3, the increase of formate following administration of ethanol was significantly suppressed by prior administration of folate ($p<0.05$). Bertino and his co-workers reported that a survey of enzymes catalyzing the transfer of one-carbon units in liver revealed that only tetrahydrofolate formylase (the formate-activating enzyme) was inhibited by ethanol at a comparable concentration (12). Admitting that this is also the case in vivo, the appearance of formate is partially prevented by prior administration of folate.

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