Effect of Ethanol on Formation of Hippuric Acid by the Liver. (29397)

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Observation of decreased plasma clearance of sulfobromophthalein during administration of ethanol(1) suggested that the excretory or conjugative functions of the liver might be altered by this agent. In the present studies we directed our attention to the effect of ethanol on another conjugative function of the liver—the formation of hippuric acid from sodium benzoate and glycine. Ethanol is converted to acetate in the liver. The oxidative reactions, catalyzed by specific dehydrogenases, involve removal of hydrogen and its transfer to nicotinamide adenine dinucleotide (NAD), resulting in production of dihydronicotinamide adenine dinucleotide (NADH). During administration of ethanol, it follows that much of the NAD in the liver is converted to the reduced form(2). This might be expected to limit other reactions dependent on NAD or sensitive to the amount of NAD or NADH in the liver (3-5). A similar change in the NAD-NADH system is produced by sorbitol, an alternate hydrogen donor. Metabolic conversion of sorbitol to fructose also requires transfer of hydrogen, and by a process analogous to that described for ethanol results in a decrease in NAD and an increase in NADH(3,5).

This report presents the results of experiments designed to determine the effect of ethanol and of sorbitol on urinary excretion of hippuric acid by human subjects given a test dose of benzoate and glycine.

Material and methods. Non-fasting normal male volunteers were given 6 g of sodium benzoate and 3 g of glycine, divided into 2 doses given 30 minutes apart, by the oral route. Voided urine was collected for measurement of hippuric acid excretion during 2 successive 2 hour periods starting at time of the first dose. Urine specimens were acidified with acetic acid and concentrated by boiling; hippuric acid content was then determined by the gravimetric method of Quick (6). On another day, the test was repeated after administration by mouth of modest doses of whiskey or gin diluted in water. The doses were equivalent to 2-3 g of ethanol/kg body weight and were given over a period beginning 4-6 hours before administration of benzoate and glycine and ending at the 4th hour after their administration.

In a second series of studies non-fasting convalescent male patients without renal or hepatic disease were given intravenous infusions of saline solution over a 60-90 minute period. Thirty minutes before the infusion was terminated 3 g of glycine was given by mouth. At the end of the infusion period, sodium benzoate, 1.77 g in 20 ml of water, was injected intravenously. Excretion of hippuric acid during the subsequent hour was then determined. On the afternoon of the same day or on the following morning, the test was repeated with addition of ethanol as a 10-20% solution in the saline infusion. The dose of ethanol was 0.4-0.8 g/kg body weight. In one subject, ethanol was given orally in a dose of 0.9 g/kg body weight.

The effect of sorbitol was determined similarly. After control studies, 100 or 200 g of sorbitol as a 40% solution was given intravenously over a 70-110 minute period, starting 45-60 minutes before injection of benzoate.

Results. Urinary excretion of hippuric acid after administration of sodium benzoate and glycine was normal in all subjects in control tests, but was decreased after administration of ethanol (Table I). In the first 2 hour period after oral administration of precursors, the hippuric acid content of the urine averaged 6.26 g, and in the second 2 hour period 1.64 g. After ethanol by mouth, urinary excretion of hippuric acid in the first period averaged 4.71 g. The difference from the mean control value was significant at the 5% level. In the second 2 hour period after ethanol the average excretion of hippuric acid was 2.12 g, or slightly greater than control

TABLE I. Effect of Ethanol on Urinary Excretion of Hippuric Acid During 2 Consecutive 2 Hour Periods After Oral Administration of Sodium Benzoate (6 g) and Glycine (3 g).*

			Hippuric acid (g)					
			Control			After ethanol†		
Subject	Age (yr)	Wt (kg)	Period 1	Period 2	Total	Period 1	Period 2	Total
1	37	90.9	6.53	.86	7.39	4.12	1.73	5.85
			5.82	1.27	7.09	3.23	2.23	5.46
			6.05	1.49	7.54	—	—	—
2	39	64.2	6.09	2.14	8.23	5.66	2.06	7.72
			6.88	1.64	8.52	5.81	2.47	8.28
			6.16	2.45	8.61	—	—	—
		Mean	6.26	1.64	7.89	4.71	2.12	6.82

* Given in 2 divided doses 30 min apart.
† Given orally over a period beginning 4-6 hr before test and ending at 4th hr of test.

TABLE II. Effect of Ethanol on Urinary Excretion of Hippuric Acid During 1 Hour Period After Administration of Sodium Benzoate (1.77 g) Intravenously and Glycine (3 g) Orally.

				Hippuric acid (g)		
Subject	Age (yr)	Wt (kg)	Dose of ethanol (g/kg)	Control	After ethanol*	Difference
3	50	57.0	.6	1.54	.79	−.75
4	55	61.5	.4	2.13	1.38	−.75
5	21	65.5	.6	1.79	1.33	−.46
6	27	72.0	.5	2.24	1.98	−.26
7	29	57.5	.8	1.45	1.24	−.21
8	37	82.2	.9	1.79	1.69	−.10
			Mean	1.82	1.39	−.43
			p			<.02

* Given as a 10-20% solution intravenously over a 60-90 min period before injection of benzoate, except for subject 8, who received ethanol by mouth over a similar 90 min period.

value. Nevertheless, total excretion of hippuric acid in the 2 periods after ethanol was less than that in the control periods.

In each of 6 subjects, excretion of hippuric acid in 1 hour after intravenous injection of sodium benzoate was decreased after ethanol (Table II). The average urinary excretion of hippuric acid was 1.82 g in control tests and 1.39 g in tests after ethanol. Average decrease of 0.43 g was significant at the 2% level.

Sorbitol, given intravenously in doses of 100 or 200 g, caused no significant change in excretion of hippuric acid in 8 subjects (Table III). The average excretion in 1 hour was 1.87 g in control tests, and 1.79 g after sorbitol.

Discussion. Hippurate is produced by conjugation of benzoate with glycine, largely in the liver, and is excreted promptly by the kidneys. The excretion of hippuric acid after administration of precursors has been the basis for a clinical test of hepatic function since 1933(7). In the studies reported here, excretion of hippuric acid after ethanol was significantly decreased in the first 2 hours after oral administration of sodium benzoate

TABLE III. Effect of Sorbitol on Urinary Excretion of Hippuric Acid During 1 Hour Period After Administration of Sodium Benzoate (1.77 g) Intravenously and Glycine (3 g) Orally.

			Hippuric acid (g)		
Subject	Age (yr)	Wt (kg)	Control	After sorbitol*	Difference
9	54	85.4	2.06	1.75	−.31
10	41	71.3	2.05	2.31	+.26
11	53	86.4	2.05	2.06	+.01
12	19	54.5	1.92	1.32	−.60
13	22	58.8	1.90	1.36	−.54
14	41	73.6	1.86	2.14	+.28
15	27	59.5	1.69	1.93	+.24
16	47	74.0	1.44	1.47	+.03
		Mean	1.87	1.79	−.079

* Given as a 40% solution intravenously over a 70-110 min period beginning 45-60 min before test period.

and glycine and in the first hour after intravenous administration of sodium benzoate (with glycine given by mouth). A decrease occurred after infusion of as little as 0.4 g of ethanol/kg body weight. Ethanol appeared to delay the production of hippuric acid, since in the second 2 hour period after ethanol the excretion of hippuric acid was slightly greater than that in the comparable control period. A reasonable interpretation of these findings is that ethanol, even in small doses, diminishes the ability of the liver to conjugate benzoate with glycine. Premedication with glycine was considered adequate to provide more than the amount needed for conjugation, thereby eliminating the availability of this substance as a limiting factor in formation of hippuric acid(8). Alterations of gastrointestinal absorption of benzoate by ethanol was not a factor, since production of hippuric acid was decreased after both intravenous and oral administration of benzoate. Since the subjects were not fasting, lack of available glucose was unlikely to have played a role. The possibility that ethanol acted by decreasing renal clearance of hippuric acid or delaying release of hippuric acid from the liver rather than by interfering with its formation was not excluded by these studies.

Sorbitol, given intravenously in doses of 100 or 200 g, had no significant effect on excretion of hippuric acid. This dose provided a molar amount 2 or more times the molar dose of ethanol used in the preceding tests. This negative finding suggests that sorbitol does not impair conjugation of benzoate in man. Sorbitol, when given intravenously, is excreted in part in the urine and produces an osmotic diuresis. A considerable amount of sorbitol may have escaped hepatic metabolism by this route. In other studies(3), the effect of sorbitol on hepatic processes was reported to be similar to that of ethanol; however, the amount of sorbitol employed was relatively greater than that used in our experiments.

At this time it is not possible to specify at what step in the formation of hippurate ethanol may have an effect. The first step in synthesis is activation of benzoate by coenzyme-A. The activated benzoyl group is then transferred to glycine to form hippurate. The latter reaction is catalyzed by the enzyme glycine N-acylase(9). Energy for the reaction is provided by hydrolysis of the high-energy phosphate bond of adenosine triphosphate. During oxidation of ethanol, impairment of formation of hippurate might result from inhibition of glycine N-acylase or reduced availability of adenosine triphosphate. An example of inhibition of enzymatic activity during oxidation of ethanol was described by Isselbacher and Krane, who noted that during ethanol oxidation rise of NADH inhibited uridine diphosphate galactose 4-epimerase(3). The possibility of reduced availability of adenosine triphosphate is supported by the fact that ethanol appears to trap NAD in the reduced form. Such trapping effect might limit the supply of adenosine triphosphate by impairment of oxidative metabolism and/or by limitation of glycolysis, both of which processes depend on the availability of NAD(10). Alternatively, ethanol might have a direct effect on cellular respiration and thereby affect oxidation of NADH. If this were the case, it would explain the difference between the effects of ethanol and sorbitol.

Summary. The effect of ethanol on the formation of hippuric acid was determined in studies on non-fasting human males without renal or hepatic disease after oral or intravenous administration of sodium benzoate and premedication with glycine by mouth. Ethanol, given orally or intravenously, resulted in significant decreases in urinary excretion of hippuric acid in all subjects tested. Each subject served as his own control. In similar studies, intravenous administration of sorbitol produced no significant changes in urinary excretion of hippuric acid. The results suggest that the hepatic processes that conjugate benzoate with glycine to form hippurate are impaired during oxidation of ethanol.

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Creatine Synthesis in Perfused Liver of Vitamin E-Deficient Rats.* (29398)

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An intensive creatinuria and a decrease in the amount of creatine in skeletal muscle are among the manifestations of metabolic disorder observed in vit. E deficiency(1,2). Results of previous experiments(3) have suggested that a lowered uptake of creatine by skeletal muscle without accompanying changes in rate of creatine synthesis by the liver is responsible for the disturbance in creatine metabolism in vit. E-deficient rats. The view that creatine synthesis is unchanged in vit. E-deficient animals is supported by the observations of van Pilsum *et al*(4) on the changes in excretion and body content of creatine in vit. E-deficient rabbits. However, others(2,5,6) have suggested that creatinuria in vit. E deficiency results from an increased synthesis of creatine by the liver and an increased release of creatine by skeletal muscle. The present investigation tests the hypothesis that creatine synthesis is not altered in vit. E deficiency by comparing the rate of creatine synthesis in isolated, perfused livers of rats fed a vit. E-depleted diet with that of rats fed a normal stock diet and, also, of rats fed a vit. E-depleted diet supplemented with α-tocopheryl acetate.

Methods. Six male rats of the Wistar strain (obtained from Carworth Co.) after weaning were placed on a diet deficient in vit. E(7) for a period of 4 to 14 months. At the time of hepatectomy, the deficient rats had developed intensive creatinuria and creatinemia. Three rats of the same strain maintained on the same high fat diet for 16-35 months but supplemented with vit. E (50 mg α-tocopheryl acetate/week) served as controls. Observations made on male rats used in another study(10) which were fed a stock Purina fox chow diet, are included in this study as a second control.

The liver was removed from each rat and perfused with whole heparinized rat blood diluted with one-third of its volume of Ringer's solution containing 250 mg of glucose and 4 μc (0.185 mg) of glycocyamine-2-C^{14} (purchased from New England Nuclear Corp.). Blood donors were normal adult Sprague-Dawley rats, fasted 18 to 20 hours before bleeding. Details of the perfusion technique and apparatus have been described(8, 9). During the first hour of the experiment (initial phase), the synthesis of creatine was determined at glycocyamine concentrations approximating the levels ordinarily found in the blood. After one hour, 20 mg of nonradioactive glycocyamine and 35 mg of L-methionine were added to the perfusate and the perfusion continued for 5 hours (final phase of experiment). Thus, in the latter phase, synthesis of creatine was determined at high precursor concentrations normally not found in the blood. At regular intervals

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