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Ethanol Increases Urinary and Tissue Ascorbic Acid Concentrations in Rats (41457)

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Abstract. Rats receiving a 20% ethanol drink for 24 days or 1 year were found to have an increased concentration of ascorbic acid in the urine, liver, kidney, spleen, and adrenals as compared to those receiving no ethanol or isocaloric intake of sucrose solution. This increase was not due to feed intake. *In vitro,* the enzymatic synthesis of the vitamin from glucuronolactone or gulonolactone by liver preparations of ethanol-treated rats was significantly higher than that of control and sucrose-fed animals. These results indicate that ethanol stimulates the capacity to synthesize ascorbate which, in turn, causes an increase of ascorbic acid contents in the urine and soft tissues.

The relationship between vitamin deficiency and alcoholism has been known for many years. Chronic alcoholism is frequently associated with an impairment in absorption or metabolism of thiamine (1), folate (2), vitamin B_6 (3), and vitamin B_{12} (4). In rats, chronic ethanol ingestion also resulted in an alteration of choline metabolism (5). Addition of riboflavin to the diet prevented fatty acid accumulation in the liver of ethanol-treated rats (6). Little is known in regard to the effect of ethanol ingestion on ascorbic acid metabolism. Recent studies have indicated that both acute (7) and chronic (8) ethanol administration in rats significantly increased the activity of microsomal glucose-6-phosphatase. This enzyme hydrolyzes glucose 6-phosphate to glucose, a precursor of ascorbic acid. In addition, ethanol increases the activity of liver uridine diphosphate glucuronyl-transferase (9). This enzyme has been shown to be involved in ascorbate biosynthesis. For these reasons, it would be of interest to determine the influence of ethanol ingestion on ascorbic acid metabolism. The result of such a study in rats is reported here.

Materials and Methods. *Animals and diet.* Five-week-old male rats of Sprague–Dawley strain were housed individually in stainless steel cages in a light and temperature controlled animal room. They were randomly divided into three groups of six or more rats each and fed Purina rat chow. One group of control rats had access to tap water and chow at all times. The second group of rats received 20% (v/v) ethanol as their sole beverage and was fed Purina chow *ad libitum.* The rats in the third group drank a sucrose solution which had a caloric value equivalent to the 20% ethanol solution and were pair-fed with ethanol-treated rats. At varying time intervals all the rats were fasted for 18 hr and were exsanguinated by heart puncture following ether anesthesia. Blood was taken from ethanol-fed rats at random for ethanol estimation (10). The various tissues were excised as soon as possible.

Ascorbic acid assay. Urine samples were collected in brown bottles containing 5.0 ml 10% oxalic acid as described by Hollman and Touster (11). Total ascorbic acid in the urine, liver, and other soft tissues were assayed according to the procedure of Schaffert and Kingsley (12) as modified by the 2,4-dinitrophenylhydrazine method of Roe and Kuether (13).

Biosynthesis of ascorbic acid by rat liver. This was determined by the method of Hassan and Lehninger (14). Fresh livers were washed and homogenized in 0.15 *M* KCl in a Teflon-pestle glass homogenizer for 10 sec. The crude homogenates were spun for 10 min at 3000*g* at 4° and the supernatant

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was used as the enzyme source. Usually the reaction mixture consisted of 50 μmole potassium phosphate buffer, pH 7.4, 4 μmole NAD, 4 μmole ATP, 75 μmole nicotinamide, 10 μmole magnesium chloride, 10 μmole D-glucuronolactone or 10 μmole L-gulonolactone and 0.7 ml of the supernatant in a final volume of 2.5 ml. The mixtures were incubated in conical flasks in a Dubnoff shaker at 37° in air. After 2 hr the reaction was stopped by adding 2.5 ml of 16% trichloracetic acid. Zero time control samples were obtained with the addition of trichloroacetic acid to the mixture prior to incubation. All the incubates were centrifuged and the clear supernatants were used for the determination of total ascorbic acid by the method of Schaffert and Kingsley (12). The enzyme activity was expressed in terms of micrograms of ascorbic acid formed in 2 hr per gram of liver and per milligram of protein. The protein concentration in supernatant fractions were estimated by the method of Lowry *et al.* (15).

Statistical analysis was accomplished with Student's *t* test for paired and unpaired data.

Results. Rats consuming 20% ethanol as sole beverage for 1 year excreted significantly more vitamin C than control rats (Table I). This conclusion was based upon the determinations of urinary ascorbic acid in 4 consecutive days. The daily increase ranged from 36 to 55%. Blood ethanol levels were 35 ± 8 mg%, means ± SD in ethanol-fed rats and were not detectable in control rats. Chronic ethanol intoxication also resulted in a reduced weight gain (Table I).

The data in Table II demonstrate that ethanol ingestion for 24 days also enhanced the urinary excretion of ascorbic acid in immature rats. This increase was approximately 100 and 33% over the sucrose-drinking and control rats, respectively. Table II again indicates that ethanol inhibited the rate of growth in young animals.

Adrenal weight expressed as milligrams per unit of body weight was significantly increased by ethanol or sucrose drinking. The weights of liver, kidney, and spleen did not show any significant alteration in ethanol-treated rats (Table III).

The results appearing in Table IV show that ascorbic acid concentrations in the liver, kidney, spleen, and adrenals were found to be significantly increased in ethanol-intoxicated rats as compared to control rats and the rats drinking sucrose solution. With the exception of kidney, the concentrations of ascorbic acid in other tissues did not show any differences between sucrose-treated rats and control animals.

As shown in Table V, the liver preparations from ethanol-treated rats had an augmented capacity in the utilization of glucuronolactone for the synthesis of ascorbate. The average increases were 30 and 37% when the results were expressed per gram of liver and per milligram of protein,

TABLE I. THE EFFECT OF ETHANOL ON URINARY EXCRETION OF ASCORBIC ACID IN ADULT RATS[a]

Treatment	Body weight (g)	Daily feed intake (g)	Ascorbic acid (μg/24 hr)	Percentage change
None	367 ± 15[b]	9.3 ± 1.16	510 ± 79.2	
Ethanol	325 ± 10[c]	9.8 ± 0.34	718 ± 118.4[c]	+40.7
None	—	13.2 ± 2.56	572 ± 106.4	
Ethanol	—	12.1 ± 0.74	838 ± 115.0[c]	+46.5
None	—	13.3 ± 1.47	518 ± 55.6	
Ethanol	—	10.1 ± 1.73	804 ± 168.8[c]	+55.2
None	371 ± 18	11.5 ± 0.69	690 ± 56.1	
Ethanol	326 ± 13[c]	10.8 ± 1.40	936 ± 119.4[c]	+35.5

[a] Weanling rats were kept on experiment for 1 year. The 24-hr urine specimens were collected daily for the last 4 consecutive days of the experiment. Total ascorbic acid in the 24-hr urine was expressed as μg/24 hr.
[b] Mean of six rats ± SD.
[c] $P < 0.01$.

TABLE II. URINARY EXCRETION OF ASCORBIC ACID IN YOUNG ADULT RATS[a]

Treatment	Final body wt in 24 days (g)	Daily feed intake (g)	Ascorbic acid (μg/24 hr)
None	219 ± 5[b]	19.8 ± 1.1	1079 ± 159
Ethanol	184 ± 14[c]	12.2 ± 0.7[d]	1433 ± 313[c]
Sucrose	212 ± 11	12.2 ± 0.3[d]	698 ± 294[e]

[a] Weanling rats were kept on experiment for 24 days. The 24-hr urine specimens were collected daily in the last 4 consecutive days of the experiment. Values in ascorbic acid were the means of four 24-hr urinary excretion specimens.
[b] Mean of six rats ± SD.
[c] Ethanol vs none, $P < 0.05$.
[d] Ethanol or sucrose vs none, $P < 0.01$.
[e] Sucrose vs ethanol or none, $P < 0.05$.

TABLE III. EFFECT OF ETHANOL ON GROWTH AND ORGAN WEIGHTS[a]

Treatment	No. of rats	Initial body weight (g)	Final body weight in 28 days (g)	Liver (mg%)	Kidney (mg%)	Spleen (mg%)	Adrenal (mg%)
None	6	87 ± 6[b]	263 ± 8	3747 ± 173	765 ± 23	233 ± 26	14 ± 1.8
Ethanol	8	86 ± 5	205 ± 7[c]	3913 ± 142	805 ± 35	248 ± 25	17 ± 1.1[c]
Sucrose	8	87 ± 4	229 ± 4[d]	4272 ± 277[d]	759 ± 38	237 ± 13	16 ± 1.3[d]

[a] Young rats were kept on experiment for 28 days, after which they were killed and their liver, kidney, spleen, and adrenal were weighed and used for determination of ascorbic acid concentration (see Table IV).
[b] Mean ± SD.
[c] Ethanol vs none, $P < 0.01$.
[d] Sucrose vs none, $P < 0.05$.

TABLE IV. ASCORBIC ACID CONTENTS IN TISSUES

Treatment	No. of rats	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)	Adrenal (μg/100 mg)
None	6	311 ± 13[a]	166 ± 16	683 ± 33	410 ± 36
Ethanol	8	361 ± 3[b,d]	244 ± 19[b,d]	744 ± 28[c,e]	473 ± 25[c,e]
Sucrose	7	319 ± 16	207 ± 36[f]	691 ± 35	391 ± 35

[a] Mean ± SD.
[b] Ethanol vs none, $P < 0.01$.
[c] Ethanol vs none, $P < 0.05$.
[d] Ethanol vs sucrose, $P < 0.01$.
[e] Ethanol vs sucrose, $P < 0.05$.
[f] Sucrose vs none, $P < 0.05$.

TABLE V. ACTIVITY OF ENZYME SYSTEMS FORMING ASCORBIC ACID FROM GLUCURONOLACTONE AND GULONOLACTONE[a]

Treatment	Substrate used	Ascorbic acid synthesized in 2 hours	
		Per g liver	Per mg protein
Sucrose, pair-fed	D-glucuronolactone	285 ± 17[b]	1.95 ± 0.08
Ethanol	D-glucuronolactone	370 ± 25[c]	2.68 ± 0.04[c]
Sucrose, pair-fed	L-gulonolactone	271 ± 18	1.73 ± 0.07
Ethanol	L-gulonolactone	320 ± 19	2.25 ± 0.08[c]

[a] Weanling rats were kept on experiment for 28 days, after which their livers were isolated for enzyme studies.
[b] Mean of six rats ± SD.
[c] $P < 0.05$.

respectively. Likewise, ethanol significantly increased the synthesis of ascorbic acid from gulonolactone.

Discussion. Vitamin C deficiency diseases have been shown to be associated with alcoholism (15). Taverna (16) found significantly low vitamin C levels in the blood of alcoholic patients. Lester *et al.* (17) demonstrated that the percentage of urinary excretion of vitamin C after oral test dose was significantly lower for alcoholic patients than nonalcoholic subjects. Recently Fazio *et al.* (18) reported that the ingestion of ethanol reduced plasma ascorbic acid concentrations for at least 24 hr. The later finding was probably due to an impairment in absorption of ascorbic acid by ethanol rather than an increased in the excretion, catabolism, or utilization of this vitamin.

In the present study the evidence indicates that ethanol consumption markedly influences urinary excretion and tissue distribution of ascorbic acid in young and adult male rats. Since the increased excretion after ethanol administration is not accompanied by any decrease in tissue levels but, on the contrary, by an increase, it is justifiable to infer that ethanol intake favorably influences the biosynthesis of this vitamin. This could be accomplished by increasing the liver enzymes involved in vitamine C biogenesis or by increased provision of essential precursors. The former appears plausible in view of the findings that incubation of D-glucuronolactone or L-gulonolactone *in vitro* formed more ascorbic acid in the liver extract of ethanol-treated rats than those of control animals. However, further work is still required to clarify *in vivo* the stimulating effects of ethanol on biosynthetic pathways of ascorbic acid.

The clinical significance of these findings is not known since human beings, unlike rats, lack the activity of liver microsomal enzyme L-gulonolactone oxidase for the synthesis of ascorbic acid. Forbes and Duncan (19) have demonstrated the occurrence of an increased ascorbic content in the liver of guinea pigs 48 hr after alcohol intoxication. Because the guinea pig is unable to synthesize vitamin C, these authors suggested that this high level of vitamin C in the animals injected with alcohol probably represented a migration of vitamin from other areas of the body into the liver. Whether these changes apply to humans is not known.

Another consideration which may explain the present findings is that ascorbic acid, a strong reducing agent, can function as an electron donor similar to NAD in ethanol oxidation. This adaptation spares the NAD/NADH and accelerates the conversion of ethanol to its metabolites. The earlier work of Krasner *et al.* (20) in man showing a direct correlation between leukocytes ascorbic acid level, hepatic alcohol dehydrogenase activity, and the clearance of ethanol from the blood appears to support this possibility. This is further substantiated by recent work of Yunice and Lindeman (21) indicating that the rats pretreated with ascorbic acid had an increased survival and decreased blood ethanol level after a single ethanol load test.

Others have observed that chronic and acute ethanol intoxication in rats resulted in a marked decrease of liver glutathione level (22, 23). The present work discloses that ethanol had a stimulating effect on ascorbic acid synthesis which, in turn, resulted in high content of vitamin C in the liver and other tissues. These diametrically opposed changes are of interest because of their common biological roles as reducing agents and detoxifying compounds. However, it is not clear whether the decrease in glutathione resulting from ethanol intake is in any way associated with the increase of ascorbic acid levels.

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