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THE EFFECT OF ETHANOL INGESTION ON THE ERYTHROCYTE ANTIOXIDANT DEFENCE SYSTEMS OF RATS

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Abstract—The effect of ingestion of water containing 1 or 10% ethanol for a period of four weeks on serum vitamin E, hepatic glutathione peroxidase, erythrocyte-glutathione peroxidase, glutathione transferase, superoxide dismutase and selenium has been investigated in rats. The serum levels of total and free vitamin E were not significantly affected by ethanol ingestion at either level. The level of erythrocyte glutathione peroxidase was decreased to 43 or 28 per cent of the control level by intake of either 1 or 10% ethanol solution, respectively, whilst there was no alteration in glutathione transferase activity. Ethanol treatment did not alter erythrocyte selenium levels or superoxide dismutase activity. Hepatic glutathione peroxidase activity was increased to approximately 166 per cent of the control value by either 1 or 10% ethanol ingestion. The results indicate that the activity of the enzyme principally involved in preventing lipohydroperoxide-induced haemolysis is significantly reduced by ethanol ingestion.

The production of lipid hydroperoxides in the liver and their appearance in the serum is one of the toxic consequences of acute or chronic alcohol intake [1] and the hepatonecrotic action of ethanol has been assumed to be the result of the lowering of glutathione peroxidase in this organ [2]. Glutathione peroxidase is a selenium-dependent enzyme present mainly in the liver and erythrocytes which catalyses the reduction of lipid hydroperoxides into hydroxy fatty acids [3]. Vitamin E in the blood and liver partly protects against the formation of these hydroperoxides [4] and it has been reported that chronic ethanol ingestion lowers hepatic vitamin E content [4]. Free iron liberated from peroxide-haemolysed erythrocytes catalyses the formation of superoxide radicals which in turn further destructively oxidize red blood cells [5]. Superoxide dismutase reduces the superoxide radicals to hydrogen peroxide which is detoxicated by catalase and, to a lesser extent, glutathione peroxidase [5].

In this experiment the effect of chronic ethanol intake in rats was investigated by incorporating it into the drinking water at the 1 or 10% level for 28 days and determinating the levels of serum vitamin E, erythrocyte and liver glutathione peroxidase and red blood cell superoxide dismutase. To determine whether ethanol ingestion reduced glutathione availability, glutathione transferase activity was measured as an index of the status of the cofactor.

MATERIALS AND METHODS

Chemicals. [8-^{14}C]Styrene oxide, sp. act. 16.4 mCi/mmole, was purchased from Radiochemical Centre, Amersham, Bucks, U.K. All other chemicals and reagents were of the purest grade available and obtained from Sigma Chemical Co., Poole, Dorset, U.K.

Animals and treatment. Male Sprague–Dawley rats of body weight 180–190 g were purchased from Olac, Bicester, Oxon, U.K. and randomly divided into groups of six. These animals were maintained on PRM diet (Dixons, Ware, Herts., U.K.) in high density polypropylene cages. A group of 6 control animals were maintained on alcohol-free drinking water; other groups of animals received redistilled absolute ethanol in their drinking water at a level of either 1 or 10% v/v. The rats were killed after 28 days by exsanguination via cardiac puncture. The blood was divided into heparinized and non-heparinized tubes before separation into erythrocytes, plasma and serum by centrifugation. The livers were surgically excised, homogenized in 1.15% KCl and the microsome-free 100,000 *g* av. supernatant prepared by ultracentrifugation.

Assays. Erythrocyte haemoglobin content was determined using the method and kit supplied by Sigma [6]. Serum vitamin E was determined by a fluorometric technique [7]. Erythrocyte and hepatic glutathione peroxidase (E.C.1.11.1.9) was determined essentially by the Mills procedure, as modified by Hafeman *et al.* [8]. Hepatic glutathione transferase (EC.2.5.1.14) was estimated by the technique of James *et al.* [9], using [^{14}C]styrene oxide as substrate. Erythrocyte glutathione transferase was determined in essentially the same manner after the following haemolytic procedure:

Two volumes of packed erythrocytes were washed by centrifugation for 20 min at 1500 *g* av. in 5 vol. 0.9% NaCl. This process was repeated three times

before adding 1.25 vol. distilled water after the final washing. The mixture was shaken and allowed to stand for 60 min before centrifuging for a further 60 min at 10,000 *g* av. The supernatant was decanted and 100 μl portions added to the incubate in the assay procedure described. Erythocyte superoxide dismutase (EC.1.15.1.1) activity was determined by the technique of Winterbourn *et al.* [10]. Erythrocyte selenium was determined by flameless atomic absorption spectrometry on nitric acid digests using an Instrumentation Laboratory IL 157 instrument with a 555 graphite furnace.

Radiochemical analyses. The radioactive content of duplicate incubates was determined by counting for 2 cycles in a Packard 2650 liquid scintillation spectrometer.

Statistical analyses. The significance of observed differences between alcohol-treated and control groups were determined by Student's *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

Ingestion of ethanol for 28 days led to a significant increase in red blood cell volume which was reflected in the haemoglobin levels (Table 1). There was no significant difference in the free or total vitamin E levels between the control and ethanol-treated animals. Alcohol intake at either 1 or 10% approximately doubled hepatic glutathione peroxidase; conversely, erythrocyte enzyme activity was reduced to 43 per cent (at 1% v/v) or 28 per cent (at 10% v/v) of the control level (Table 2). Alcohol ingestion did not significantly affect erythrocyte selenium or superoxide dismutase activity. Consumption of alcohol at the higher dose level significantly lowered hepatic glutathione transferase, but ingestion of alcohol at either level did not significantly alter erythrocyte glutathione transferase activity (Table 3).

DISCUSSION

From these reults it was concluded that ingestion of water containing either 1 or 10% ethanol for 28 days enhances hepatic glutathione peroxidase activity but significantly decreases erythrocyte glutathione peroxidase levels. Acute ethanol intoxication increased lipid hydroperoxide products in the liver, whereas chronic intake of an order similar to the present experiment reportedly lowers hepatic vitamin E levels without increasing lipid peroxidation [4]. No evidence of any vitamin E deficiency was observed in this study, however. Intake of larger doses of ethanol results in fatty infiltration of the liver and generation of lipid hydroperoxides which are released into the serum [1]. It has been suggested that the increased serum lipoperoxide in cirrhotic patients may be due to reduced hepatic glutathione peroxidase [2]. In a previous experiment, it was demonstrated that prolonged intake of ethanol in the manner employed in this experiment progressively increased hepatic microsomal protein [11]. It is possible that in the present experiment there was a similar 'induction' of glutathione peroxidase activity. The presence of a non-selenium-dependent form of glutathione peroxidase in the liver has been

Table 1. The effect of chronic ethanol intake on erythrocyte volume, haemoglobin and serum vitamin E level*

Treatment level	PCV (%)	Haemoglobin (g/100 ml)	Serum vitamin E	
			Free (μg/ml)	Total (μg/ml)
1%	53.8 ± 3.5	13.2 ± 1.1	5.4 ± 2.1	8.3 ± 1.9
10%	58.8 ± 3.5	16.6 ± 0.5	3.6 ± 2.4	9.1 ± 1.8
0 (Controls)	50.0 ± 3.2	14.7 ± 0.8	5.1 ± 1.0	7.4 ± 0.9

* Results represent the means ± S. D. for duplicate assays on samples derived from six animals.

Table 2. The effect of chronic ethanol intake on hepatic and erythrocyte glutathione peroxidase levels and erythrocyte selenium levels*

Treatment level	Hepatic glutathione peroxide (units/mg liver)	Erythrocyte glutathione peroxidase (units/mg haemoglobin)	Erythrocyte selenium (μg/ml RBC)
0 (Control)	11.7 ± 2.9	63.5 ± 11.6	7.6 ± 0.6
1%	19.7 ± 4.5	27.3 ± 7.9	6.9 ± 1.4
10%	20.0 ± 3.8	17.8 ± 1.0	7.5 ± 1.5

* Results represent the means ± S.D. of duplicate assays on samples derived from six animals.

Table 3. The effect of chronic ethanol intake on erythrocyte superoxide dismutase and hepatic and erythrocyte glutathione transferase activity*

Treatment level	Erythrocyte superoxide dismutase (units/mg haemoglobin)	Glutathione transferase	
		Hepatic† (nmoles/mg protein/ml)	Erythrocyte‡ (nmoles/min/ml erythrocytes)
1%	3.7 ± 0.69	74 ± 13.2	42 ± 8.0
10%	3.6 ± 0.71	72 ± 5.0	46 ± 4.1
0 (Control)	3.9 ± 0.29	79 ± 3.5	43 ± 3.9

* Results represent the means ± S.D. of duplicate samples derived from six animals.

reported [12], and in the absence of this essential element, this enzyme might be selectively induced. Whereas the intake of ethanol at the low levels employed in this study could result in the proliferation of the hepatic smooth endoplasmic reticulum [13] and the induction of protein synthesis [11], no corresponding increase in the selenium-dependent erythrocyte enzyme levels would be possible.

Erythrocyte glutathione status as estimated by glutathione transferase activity has been postulated as an index of ageing of the whole organism [14]. Using this enzyme as a monitor of the status of the cofactor indicated that diminished glutathione was not responsible for the decreased peroxidase activity in the present experiment.

Alcohol abuse is normally associated with decreased serum zinc and magnesium, and diminished serum selenium levels in alcoholics is not usually observed [15], although decreased serum selenium levels in alcoholic cirrhosis patients has recently been reported [2]. Alcohol ingestion in the manner of the present experiment produced no decrease in selenium levels. A large amount of selenium in vegetable sources occurs as selenomethionine [16]. It is believed that this essential trace element is absorbed from the gut largely in the form of selenomethionine [16]. Vitamin B_6 has been demonstrated to be essential for the activity of the enzymes which metabolize selenomethionine to the form essential for glutathione peroxidase activity [17]. Diminished B vitamin levels are characteristic of alcohol abuse [18] and thus it is possible that although adequate selenium is present, it cannot be utilized by the enzyme.

A major problem in studies investigating the source of alcohol toxicity is finding animal models suitable for comparison with man [1]. The mode of administration of alcohol in this experiment has been criticised [1, 13]. It has been calculated that this method could provide a calorific equivalent of only 10–25 per cent of the daily requirement and produces no significant fatty infiltration of the liver [1]. The present experiment emphasizes, however, the toxicity of low doses of alcohol taken as a dietary supplement as in the nonalcoholic human situation. Furthermore, the decreased erythrocyte glutathione peroxidase resulting from low ethanol intake suggests that this deficiency could have a role in the causation of the elevated serum lipohydroperoxides characteristic of cirrhosis, rather than being a consequence of their toxicity.

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