THE EFFECTS OF ETHANOL ON HEPATIC LIPID PEROXIDATION AND ON THE ACTIVITIES OF GLUTATHIONE REDUCTASE AND PEROXIDASE

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1. Introduction

The role of ethanol in the pathogenesis of ethanol-induced hepatic steatosis has remained a matter of some controversy. Di Luzio [1] and Comporti [2] have suggested the operation of a lipid peroxidation mechanism via free-radicle attack on the lipoprotein membrane of mitochondria. These findings are supported by an *in vitro* pro-oxidant effect of ethanol [3] and partial protection against steatosis by antioxidants [4, 5]. However, no support for this hypothesis has been received from Hashimoto and Recknagel [6].

In view of these reports we examined the effect of ethanol with time, on the structural integrity of mitochondrial and microsomal membranes. This involved the measurement of diene conjugates which are intermediates in lipid peroxidation. In addition, we report an ethanol-induced increase in the activities of the enzymes glutathione (GSH) peroxidase and (GSSG) reductase. The role of these enzymes in a protective mechanism against lipid peroxidation is discussed.

2. Methods

2.1. *Measurement of diene conjugation formation*

Four groups of four male albino Sprague-Dawley rats weighing 200–250 g were intubated daily with 5g ethanol/kg body weight as a 50% solution over 1, 2, 5 and 10 weeks respectively.

Corresponding control groups received isocalorific quantities of glucose. An additional group plus controls were left untreated at this stage and represented a 0 week group. Throughout the experiment a standard pellet diet was fed ad libitum. All groups were starved overnight prior to sacrifice. A single dose of ethanol (5g/kg) to ethanol groups and glucose to controls was administered 3 hr before being killed by stunning, and cerebral dislocation. Livers were removed, washed and homogenised in 3 vol 0.3 M sucrose:0.003 M EDTA, pH 7.4. Mitochondria and microsomes were then examined for evidence of diene conjugation by the method of Hashimoto and Recknagel [6].

2.2. *Enzyme assay*

Six groups of six male albino Sprague-Dawley rats weighing 150–200 g plus their corresponding controls were treated with the same ethanol or glucose regime as outlined above for 0, 1, 2, 3, 5 and 7 weeks respectively. Again all groups were maintained on a normal diet and starved overnight prior to sacrifice. Livers were removed, washed and homogenised in 3 vol 0.3 M sucrose. Postmicrosomal supernatants were obtained by centrifuging at 105 000 *g* for 1 hr after an initial 900 *g* spin for 10 min. This fraction was used to measure GSH peroxidase and GSSG reductase enzyme activities.

GSH peroxidase activity was measured using peroxidised mitochondria as substrate. Rat liver mitochondria sufficient to yield 0.6 g equivalents/assay were prepared and peroxidised *in vitro* by incubating them at a concentration of 0.2 g equivalents/ml in oxygen saturated 0.05 M phthalate–phosphate buffer, pH 5.8, containing 0.001 M ascorbic acid for 1 hr at 37°C. The reaction was stopped by addition of EDTA to a final concentration of 0.002 M. The peroxidised mitochondria were then recovered by centrifugation. GSH peroxidase activities were measured using the

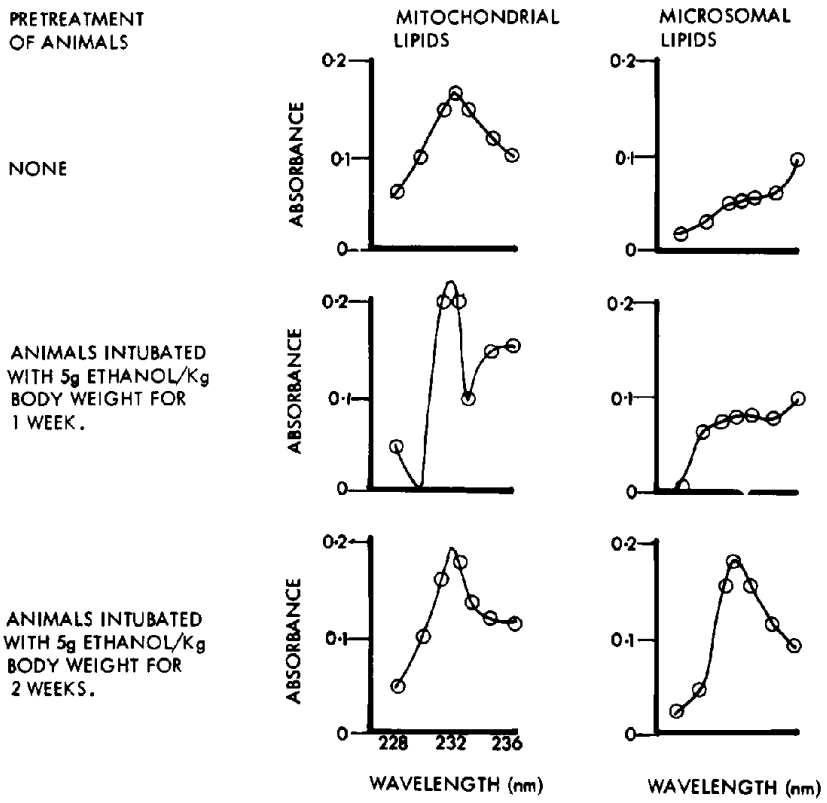

Fig. 1. Difference spectra showing diene conjugation in rat liver mitochondrial and microsomal lipids, 3 hr after receiving 5 g ethanol/kg body weight by stomach intubation.

following assay system; 0.6 g equivalents peroxidised mitochondria, 0.13 mM GSH, 0.1 ml enzyme supernatant and 0.3 M Tris–HCl buffer, pH 7.6 in a total volume of 1 ml. Duplicated controls in which buffer replaced substrate were also carried out and incubated for 20 min at 37°C. The reaction was stopped by addition of 4 ml 6.3% TCA and the GSH remaining measured by the method of Beutler et al. [7]. Activity was expressed as nmoles GSH oxidised/min/mg protein.

GSSG reductase was measured by the method of Bergmeyer [8] and protein by the method of Lowry [9].

3. Results and discussion

3.1. *Evidence of lipid peroxidation*

The measurement of diene conjugation is regarded as a direct and sensitive method for detecting lipid peroxidation. Fig. 1 shows the effects of acute ethanol administration on hepatic mitochondrial and microsomal lipids of rats which had received varying degrees of ethanol pretreatment. These graphs represent difference spectra between ethanol groups and their equivalent glucose controls. The occurrence of diene conjugation is demonstrated by an absorption peak at around 233 nm.

Only two out of the four animals in group 0 showed signs of peroxidation. This was confined to mitochondrial lipids. None displayed any evidence of microsomal peroxidation. Three animals from group 1 also showed only mitochondrial peroxidation. In group 2, however, peroxidation was found in both cellular compartments. This result, although not illustrated, was repeated in groups 5 and 10.

The fact that diene conjugation could only be established in half of the group 0 animals confirms the relatively high incidence of failure in detecting lipid per-

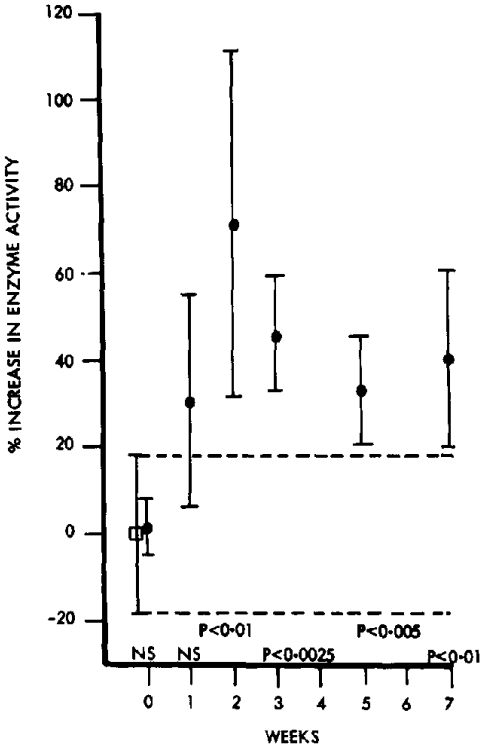

Fig. 2. Effect of ethanol on the activity of rat liver GSH peroxidase: □ Control groups; • Ethanol treated groups.

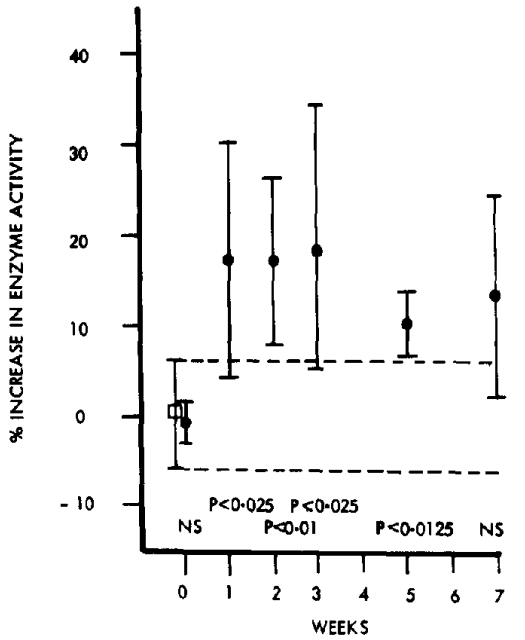

Fig. 3. Effect of ethanol on the activity of rat liver GSSG reductase: □ Control groups; • Ethanol treated groups.

oxidation following a single administration of ethanol [2, 6]. The reasons for this are not clear. It is possible that some livers may be more susceptible than others to acute ethanol intoxication. Increased susceptibility is also suggested in the results observed with the other groups, where the frequency of detecting mitochondrial peroxidation and the establishment and potentiation of microsomal peroxidation was increased with increased duration of ethanol pretreatment. Whether this might be attributed to nutritional imbalance relative to the calorific contribution of ethanol or to an accumulative toxic action of ethanol 'per se' remains unsolved.

In view of these findings we feel justified in agreeing with the observations of Di Luzio [1] for the operation of an ethanol-induced lipid peroxidation mechanism. Initially only mitochondrial lipids appear to be affected, but following chronic ethanol exposure this effect becomes more generalised.

3.2. *An enzymatic protective mechanism*

Lipid peroxides are extremely toxic [10–13]. Animals, however, have the ability to metabolise lipid peroxides [14] and to develop tolerance following sublethal exposure [15]. This, together with the limited effectiveness shown by antioxidants in protecting against lipid peroxidation, has led to the belief that other protective mechanisms may also be operative. Little and O'Brien [16] have demonstrated the ability of intracellular GSH peroxidase to utilize a variety of lipid peroxide substrates. This enzyme is specific for thiols, especially GSH, as hydrogen donors. Christophersen [17] has identified the lipid products formed by such a decomposition as the corresponding monohydroxy polyenoic fatty acids.

In the present experiment we examined the activities of hepatic GSH peroxidase and GSSG reductase in the rat following varying lengths of chronic ethanol administration. As indicated in figs. 2 and 3, results showed that the activities of these enzymes were increased by approximately 45% and 15% respectively. Statistically these results were highly significant.

Elevated enzyme activities of this nature are consistent with an induction process. The role of GSH peroxidase may, therefore, be one of an adaptable enzyme in a protective mechanism against lipid peroxide toxicity. This would involve the reduction of lipid peroxides to the corresponding monohydroxy fatty acids as previously suggested [17], using GSH as hydrogen donor. The accompanying increase in GSSG reductase is probably a reflection of the increased demand for GSH.

It is significant that these increases are a consequence of ethanol administration and, therefore, further implicates the involvement of ethanol in lipid peroxidation. At this stage it is not possible to evaluate the importance of these results in terms of the total protective mechanisms operating in the cell. They may, however, help to explain the development of increased tolerance and catabolism of lipid peroxides.

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