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SHORT COMMUNICATIONS

Serum gamma glutamyl transferase and alkaline phosphatase as indicators of excess chronic alcohol consumption in the rat

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The practical, ethical and theoretical difficulties associated with alcohol studies involving human subjects have prompted most researchers to use some form of animal model. A number of different types of animal model exist and these have been widely reviewed [1, 2]. Perhaps the best model of chronic alcohol intake is the Isocalorific Matched Pair Feeding Technique of DeCarli and Lieber [3].

For any animal model to be an accurate representation of human alcohol abuse, consumption of ethanol must be shown to lead to pathological complications [4]. The simplest method of gauging the tissue effects of excessive alcohol consumption is the assessment of liver damage by measuring the serum levels of the hepatic enzymes which are raised as a result of alcohol abuse. The ease with which blood can be obtained from most experimental animals in sufficient quantities to allow serum enzyme analysis, means that the effects of ethanol on liver function can be readily followed throughout the course of a study. This avoids the need for repeated liver biopsy which would cause morbidity and mortality amongst the experimental animals in a long term study. The two enzymes most widely regarded as being the best indicators of alcohol abuse are gamma glutamyl transferase (GGT, EC 2.3.2.2) and alkaline phosphatase (EC 3.1.3.1). Most previous studies involving animal models of chronic alcohol abuse have measured GGT and alkaline phosphatase at a single early time point [5–7]. The aim of the present study was to measure the values of these enzymes over a longer period of alcohol intake, allowing assessment of the usefulness of each of these enzymes as markers of chronic alcohol consumption.

Materials and methods

Forty young male (6–8 weeks) Sprague–Dawley rats, were divided into two groups. The first group of 10 rats were maintained for the duration of the study on standard laboratory chow and water *ad lib*. This group formed the normal control group. The remaining thirty animals were fed a nutritionally adequate liquid diet (S.D.S. Ltd, Witham, U.K.) as their sole source of food and water. After 1 week on this liquid diet, the 30 animals were further divided into 15 closely weight matched pairs which formed the pair fed groups. One member of each pair was fed the liquid diet with ethanol substituted to provide 36% of the daily calories and the other member of the pair was fed the same liquid diet but with sucrose substituted to provide 36% of calories. By carefully monitoring the volume of diet consumed, paired animals had their nutritional and calorific intake matched exactly. All the animals were housed individually in wire bottomed cages, were weighed weekly and had 1 mL of blood removed under ether anaesthetic from the tail vein every 4 weeks from the point of pairing to the termination of the study (16 weeks). This blood was allowed to clot and was centrifuged to provide serum which was used for analysis of enzyme levels.

GGT was assayed according to the method described by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology [8]. One hundred microlitres of serum was required per assay which was carried out at 37°. Alkaline phosphatase was assayed on an LKB Rapid Reaction Rate analyser (LKB Instruments Ltd, South Croydon, U.K.) using an automated assay kit supplied by Boehringer Mannheim (BCL Ltd, East Sussex, U.K.). Twenty microlitres of serum was required per assay which was carried out at 37°.

Statistical analysis of the results was by the Mann-Whitney U-test and also, in the case of comparisons between matched pairs, by the Wilcoxon Matched Pair Signed Rank test [9]. The significance level was set at 0.05.

Small pieces of liver from each animal were obtained when killed and processed for histological analysis. Frozen sections were stained using Oil Red 'O' to highlight any hepatocyte fat deposition.

Results and discussion

As reported previously [10], all the animals gained weight throughout the study and each group appeared to adapt well to their given diet. As expected the normal control group showed a significantly higher weight gain over the course of the study (weight gain of 222.5 g) than did either of the two liquid diet groups (weight gains of 134.4 g for the alcoholic group and 155.4 g for the sucrose control group). There were no significant differences in the weight gains of the two liquid diet groups.

The results from the GGT analysis are shown in Table 1. The initial mean value for the laboratory chow group was 4.9 I.U./L and this level remained constant throughout the study. The sucrose group showed an initial mean value of 4.9 I.U./L and again this level remained constant throughout the study. The levels in the alcoholic group, on the other hand were seen to be raised within 4 weeks from the initial value of 4.9 to 7.5 I.U./L and this raised level was maintained throughout the study. Statistical analysis of the GGT values revealed that the levels in the alcoholic animals were raised, not simply with respect to the initial values but also with respect to the levels in the sucrose control animals (Wilcoxon, $P < 0.05$) and the laboratory chow animals (Mann-Whitney U-test, $P < 0.05$) at all times

Table 1. Gamma glutamyl transferase results (I.U./L)

	1	4	8	12	16
Laboratory chow	4.9 (0.98)	5.2 (1.58)	5.5 (1.28)	5.0 (1.20)	5.2 (1.17)
Sucrose	4.9 (1.93)	5.6 (1.68)	5.0 (1.36)	4.1 (0.37)	5.8 (1.71)
Alcohol	4.9 (2.48)	7.5 (2.25)	7.8 (1.88)	6.0 (1.09)	8.5 (2.29)

Animals were bled every 4 weeks from the start of the study to the termination. This blood was centrifuged to provide serum for analysis of gamma glutamyl transferase. Values quoted are means and SD (in parentheses) for each of the three experimental groups at the five bleeding times.

Some of these values are reprinted [10] with permission from Pergamon Press.

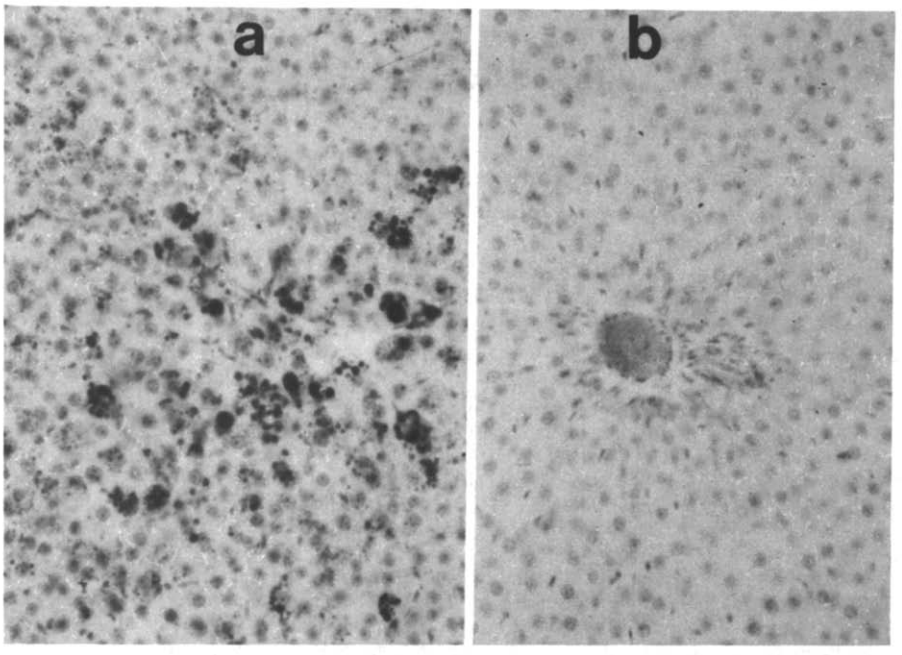

Fig. 1. Results from the histological analysis of the alcoholic and control liver sections. At the termination of the study, the animals were killed and small pieces of liver were obtained from each animal. This tissue was processed for histological analysis and frozen sections were stained using Oil Red 'O' to highlight any hepatocyte fat deposition. The results demonstrate substantially greater fatty deposition in the alcoholic liver (a) than in the control liver (b). The control liver shown here is from a sucrose control animal.

subsequent to the start of the study. There were no significant differences in the enzyme levels of the sucrose control and laboratory chow groups, suggesting that the serum GGT levels were unaffected by the liquid diet. This was particularly important given the previous evidence of dietary effects on serum GGT levels [11].

The results from the alkaline phosphatase assays are shown in Table 2. The initial mean value for the laboratory chow group was 490 I.U./L and this value decreased significantly over the course of the study to a final value of 200 I.U./L (Mann-Whitney U-test, $P < 0.002$). The sucrose group had an initial mean value of 340 I.U./L and again, this value decreased to a final value of 130 I.U./L (Mann-Whitney U-test, $P < 0.002$). The initial mean value for the alcoholic group was 320 I.U./L. This value subsequently decreased significantly (Mann-Whitney U-test, $P < 0.002$) unitl at the termination of the study, it was 160 I.U./L. Statistical analysis revealed that the alkaline phosphatase values from the laboratory chow group were significantly higher (Mann-Whitney U-test, $P < 0.02$) than those from the two liquid diet groups at the beginning of the study and also at weeks 8 for the alcoholic animals and 8 and 16 for the sucrose control group. This implies a very rapid effect of the liquid diet on the alkaline phosphatase levels in the two pair fed groups being evident after only 1 week on the liquid diet. The mean alkaline phosphatase values from the alcoholic animals were higher than those from the sucrose controls at all points subsequent to the start of the study. This difference reached levels of significance at weeks 4 (Wilcoxon, $P < 0.019$), 12 (Wilcoxon, $P < 0.028$) and 16 (Wilcoxon, $P < 0.05$). This suggests that alkaline phosphatase is also a useful marker of alcohol consumption in long term studies. The enzyme does not however appear to be as sensitive or consistent in this respect as GGT and the apparent effect of the liquid diet on the enzyme levels suggests that it may be unsuitable for use in liquid diet experiments. The raised levels observed at weeks 12 and 16 suggest that alkaline phosphatase may become a more consistent indicator of excess alcohol consumption following prolonged exposure. This has already been shown to be the case for the human serum alkaline phosphatase response to alcohol consumption [12]. A significant temporal reduction in the alkaline phosphatase levels was noticeable in all three experimental groups. Unfortunately, data in the literature (which tends to give a single time value for activity) is unable to substantiate this although it appears [13] that such an 'ageing' effect is normal.

Table 2. Alkaline phosphatase results (I.U./L)

	1	4	8	12	16
Laboratory chow	490 (61.6)	280 (108)	330 (83.7)	210 (50.9)	200 (57.4)
Sucrose	340 (88.9)	225 (24.2)	150 (15.2)	180 (49.7)	130 (23.1)
Alcohol	320 (67.1)	310 (108)	190 (55.4)	240 (59.3)	160 (49.4)

Animals were bled every 4 weeks from the start of the study to the termination. This blood was centrifuged to provide serum for analysis of alkaline phosphatase. Values are quoted as means and SD (in parentheses) for each of the three experimental groups at the five bleeding times.

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Results from the histological analysis of the liver sections (Fig. 1) revealed substantially greater centrilobular fatty deposits in the alcoholic animals than in either the sucrose or the laboratory chow control groups. Additionally a mild mixed inflammatory response was noted in the alcoholic animals. No evidence of liver cell necrosis or increased collagen deposition was noted. It is interesting to note that

in none of the alcoholic animals was the liver disease seen to develop beyond the stage of fatty liver which is in agreement with the findings of Lieber [14] in this respect.

In summary, the lack of consistency in the raised alkaline phosphatase levels, the apparent effect of the liquid diet on the enzyme levels and the presence of an 'ageing' effect would indicate that, of the two enzymes investigated in the present study, GGT provides the most useful and sensitive index of alcohol consumption for use in a long term alcohol study. The fact that the GGT appears to be completely unaffected by the composition of the liquid diet also argues in favour of its use in such studies.

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Effect of cumene hydroperoxide or hypoxia-reoxygenation on glutathione status in guinea-pig heart

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Hypoxia-reoxygenation induces oxidative damage [1]. To examine the mechanism by which oxidative injury occurs, cumene-hydroperoxide (Cum-OOH) was used in the guinea-pig heart. Guinea-pig heart was selected because while it is devoid of selenium dependent glutathione peroxidase activity [2] it possesses selenium independent activity (GPD), known as glutathione transferase of class alpha [3].

Materials and methods

The hearts were excised and mounted on Langendorff apparatus as described elsewhere [4]. The perfusion medium was a modified Krebs–Henseleit bicarbonate solution at 37° and pH 7.4 gassed with 97% O_2:3% CO_2 (pO_2 650 mm Hg) and containing 5 mM glucose (normal perfusion). The experimental time course consisted of 45 min of aortic perfusion (about 6 mL/min) followed by 60 min of normal perfusion or by 30 min of perfusion with different concentrations of Cum-OOH plus 30 min of perfusion with Cum-OOH-free buffer (washout); or 30 min of perfusion with a glucose-free medium gassed with 97% N_2:3% CO_2 ($pO_2 < 50$ mm Hg) (hypoxia); then hearts were returned to the normal perfusion for 30 min (reoxygenation).

Developed and resting tension, coronary pressure and surface electrocardiogram (ECG) were measured. The lactate dehydrogenase activity (LDH) was measured in the effluent as previously described in Ref. 5.

After hypoxia-reoxygenation and Cum-OOH perfusion and washout or 60 min of normal perfusion, malondialdehyde (MDA) was measured in frozen tissue as previously described in Ref. 6.

Glutathione transferase (GST), thioltransferase (TT), GPD and glutathione reductase activities (GSSG red) were assayed in 14,000 *g* clear supernatant [7–10], respectively, with minor modification. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured in the supernatant obtained after trichloroacetic extraction of samples [11, 12]. Proteins were determined by the method of Bradford [13].

Data (mean ± SE) were subjected to variance analysis and Bonferroni multiple comparison test. Ventricular arrhythmias were compared by chi square test. A P value of 0.05 was regarded as significant.

Results and discussion

Figure 1 shows that Cum-OOH and washout decreased developed tension and increased resting tension as does hypoxia followed by reoxygenation. The effect on devel-