INTRODUCTION

Taurine (2-aminoethane sulphonic acid) is a β-amino acid, which is one of the most abundant amino acids present in tissues. It is both synthesized from dietary cysteine and methionine and ingested directly in certain foodstuffs (Huxtable, 1992). Taurine has been suggested to have a number of protective properties including protection against hepatic damage. We have recently shown that treatment with taurine reduced alcohol-induced liver dysfunction (Kerai et al., 1998, 1999). Thus, in rats in which both fatty liver and lipid peroxidation were induced by treatment with alcohol in a liquid diet for 1 month, co-administration of taurine in the liquid diet significantly decreased the fatty liver and the lipid peroxidation (Kerai et al., 1998). Furthermore, in animals in which this liver dysfunction was induced by treatment with alcohol for 1 month, administration of taurine for 2 days after alcohol treatment was terminated, caused a significant reduction in both fatty liver and lipid peroxidation (Kerai et al., 1999).

The kidney is one of the most important organs involved in taurine regulation and there is considerable evidence to demonstrate that the kidney (Chesney, 1985) regulates the whole body homeostasis of taurine. In the kidney, there is a high affinity, Na⁺-dependent, β-amino acid specific transport system, which is responsible for the reabsorption of taurine across the renal tubular brush border membrane (Goldman and Scriver, 1967; Rozen et al., 1979). In general, the transport systems for taurine are specific for β-amino acids and are both energy- and sodium-dependent. Using structural analogues of taurine, which compete for the specific β-amino acid uptake sites into cells, it is possible to deplete the body pool of taurine. β-Alanine is commonly used for this purpose in taurine research, as it utilizes the same β-amino acid uptake system in the kidney (Goldman and Scriver, 1967), and so it competitively inhibits taurine reuptake into the proximal tubular cells. The result is a loss of taurine from the body, and thus depletion of taurine in tissues such as the liver, as taurine does not accumulate in cells.

The aim of the present study was to show whether the co-administration of β-alanine with alcohol to rats (to reduce hepatic taurine levels) would alter the pathological and biochemical lesions induced by alcohol, for example, hepatic steatosis and lipid peroxidation.

MATERIALS AND METHODS

Chemicals

The following compounds were supplied by Sigma Chemical Company (Poole, Dorset, UK): taurine (synthetic), o-phthalaldehyde (OPA, HPLC grade), homoserine, (DL)-homocysteine, S-adenosyl l-methionine (SAM, iodide salt), ethanol, sodium dihydrogen phosphate, Dowex resins, adenosine triphosphate (ATP, disodium salt), GSH, firefly lantern extract (luciferase), DTNB (5,5'-dithiobis-2-nitrobenzoic acid) for measurement of total non-protein sulphydryls (TNPSH) and glutathione. Chromotropic acid, used in the determination of triglycerides, was prepared fresh from 4,5-dihydroxy-2,7-naphthalene disulphuric acid and Zeolite, activated by heating in an oven overnight at 85°C, were also obtained from Sigma Chemical Company. Water was of ultra high quality (UHQ), prepared using an Elgastat water system. The AG1-X8 resin (200–400 mesh chloride form) was purchased from BioRad and SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate) was obtained from Fluka (Gillingham, Dorset, UK). MTHF (methyltetrahydrofolate, barium salt, 50 μCi/ml) was obtained from Amersham (Little Chalfont, Bucks, UK).
**Animals**

Female Sprague–Dawley rats (125–150 g) were obtained from Charles River (UK) and acclimatized for at least 7 days after delivery. Animals were housed in communal cages, fed a rat and mouse maintenance cube diet (691 diet, Quest Nutrition Ltd., Wingham, Kent, UK) and water *ad libitum*. During experiments, animals were housed in individual metabolism cages designed to separate and collect faeces and urine (Techmate Ltd., Milton Keynes, Bucks, UK) and given a powdered diet (691 diet, Quest Nutritional Ltd., Wingham, Kent, UK) and water *ad libitum* prior to introduction of the liquid diet. Lighting was controlled to give a regular 12 h light–12 h dark cycle (08:00 on–20:00 off); room temperature was maintained at 21 ± 1°C. Urine samples (24 h) were collected over ice and diluted to 25 ml with UHQ water, centrifuged (2000 rpm, 10 min, 4°C) to remove hair and food debris and stored (–80°C) in aliquots for later analysis. The body weight and general condition of the animals were monitored twice weekly and liquid diet intake was determined daily. Animals were used under the British Home Office regulations.

**Liquid diet technique of ethanol administration**

Due to the tendency of animals given alcohol to reduce their solid food consumption, animals were given a liquid diet. Chronic ethanol feeding was achieved by incorporation of ethanol in a nutritionally adequate totally liquid diet obtained from Dyets Inc. (Pennsylvania, USA). The liquid diet provided 1 kcal/ml of which 35% of total calories were derived from fat, 47% from carbohydrates and 18% from protein. Ethanol-treated animals were given diet, where maltose dextrin was isocolarically replaced by ethanol. The alcohol provided 36% of the calories (Lieber and DeCarli, 1989). Animals were started on the diet at a body weight of 125–150 g and ethanol was introduced progressively with 30 g/l of the liquid diet for 2 days, 40 g/l for the subsequent 2 days followed by the final formula containing 50 g/l (Lieber and DeCarli 1989; Kerai et al., 1998, 1999).

**Preparation of diet.** As vitamin A degrades when mixed in with other dry ingredients, vitamins and minerals were incorporated into the diet at the time of preparation of the liquid diet. The liquid diet was prepared in cold tap water using a kitchen blender. The diet was kept refrigerated in the dark and used within 1 week of preparation.

**Pair-feeding.** The alcohol-fed animals were allowed liquid diet consumption *ad libitum* and their daily intake was monitored. The control animals were then given the same amount of liquid control diet during the following 24-h feeding period. This pair-feeding process was repeated every 24 h. The technique of daily pair-feeding was adopted to assure a strict caloric intake in both ethanol-treated animals and their individual pair-fed controls (Lieber and DeCarli, 1989).

**Study design**

Rats were either provided with 3% β-alanine in the drinking water for 2 days (*n* = 12) or given tap water (*n* = 12). Rats (*n* = 6) were then treated with alcohol which was administered in the liquid diet for 28 days. Pair-fed control rats (*n* = 6) were also provided with the same liquid diet but without alcohol. A second group of animals (*n* = 6) received alcohol administered in the liquid diet which also contained 3% β-alanine. Pair-fed control rats (*n* = 6) were given the same liquid diet containing 3% β-alanine only. After 28 days of treatment, animals were killed and blood and tissue removed for analysis and microsomes prepared from the liver.

**Post-mortem procedure**

Animals were exsanguinated from the abdominal aorta under anaesthesia (Hypnorm: Hypnovel: water, 1:1:2, 3.33 ml/kg, i.p.) and blood samples were collected into Microtainers (Becton Dickinson & Co., Rutherford, NJ, USA) for the separation of serum. After standing at room temperature for at least 45 min, the Microtainers were centrifuged (13,000 rpm, 45 s, MSE minifuge) and stored at –80°C. Serum was analysed for enzymes and biochemical parameters using appropriate kits (Boehringer Mannheim GmbH Diagnostica, Germany) with a centrifugal IL Monarch 2000 (Instrumentation Laboratory, UK, Ltd). The liver was removed, weighed, and approximately 200 mg taken from the right lobe and immediately homogenized in trichloroacetic acid (TCA, 10% w/v, 4 ml, 4°C), frozen in liquid nitrogen and stored at ~80°C for subsequent analysis of ATP. Approximately 200 mg of liver were also taken from the right lobe and immediately homogenized in sulphasalicylic acid (0.2 M, 2 ml, 4°C), frozen in liquid nitrogen and stored at ~80°C for subsequent analysis of taurine, TNPSH, and oxidized glutathione (GSSG).

**Biochemical determinations**

**Taurine.** A high-performance liquid chromatographic method with fluorimetric detection was used for the determination of taurine in urine, serum, and liver tissues essentially by the method of Waterfield (1994). Taurine was derivatized with o-phthalaldehyde/2-mercaptoethanol prior to injection onto a C18 column. Isocratic elution of the adduct was carried out using NaH2PO4 (0.05 M, pH 5.4) in methanol and water (43:57 v/v). Homoserine was used as an internal standard to facilitate the standardization and quantification of samples. Analysis was completed in 6 min with homoserine and taurine eluting after 3 and 4 min respectively.

**Triglycerides.** The hepatic content of triglyceride was determined by a modified method of Butler et al. (1962). Briefly, phospholipids were separated from triglycerides by adsorption on a synthetic Zeolite. The triglycerides were then extracted into chloroform, hydrolysed, and measured as esterified glycerol with non-esterified samples used as individual blanks.

**Lipid peroxidation.** Lipid peroxidation, measured as malondialdehyde production in liver samples, was determined by the method of Sawicki et al. (1963) employing malondialdehyde as standard.

**ATP.** ATP content of liver samples was determined by luciferase-linked bioluminescence in TCA extracts of liver samples using a firefly lantern extract (Jenner and Timbrell, 1994).

**TNPSH.** Liver TNPSH were determined by the method of Ellman (1959) as a measure of reduced liver glutathione, which constitutes most (>95%) of the liver TNPSH (De Master and Redfern, 1987).

**GSSG.** GSSG was determined by the method of Griffith (1980) using 2-vinylpyridine to mask GSH.

**Microsomal analysis.** Microsomes were prepared from livers, as described by Lake (1987). Total cytochrome P-450...
content of liver samples was determined by the method of Omura and Sato (1964). 4-Nitrophenol hydroxylase activity was determined by the modified method of Prough et al. (1978). 4-Nitrophenol is a substrate for the ethanol-inducible CYP2E1. The method relies on the formation of p-nitrocatechol, which can be detected spectrophotometrically after total ionization under alkaline conditions. The protein content of microsomes was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Homocysteine and cysteine. A high-performance liquid chromatographic method with fluorimetric detection was used for determination of total homocysteine and cysteine, (oxidized and reduced) in urine and serum by the procedure of Fortin and Genest (1995). Homocysteine and cysteine were reduced by 10% tri-n-butylphosphine in dimethylformamide, then derivatized with SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate) at 60°C for 1 h (stable for 1 week at 4°C), prior to injection onto a C18 column. Isocratic elution of the adduct was carried out using sodium acetate (0.1 M), acetic acid (0.1 M) and 2% methanol, pH 4.0. N-Acetylcysteine was used as an internal standard to facilitate the standardization and quantification of samples. Analysis was completed in 14 min with cysteine, homocysteine, and N-acetylcysteine eluting after 2.5, 3.5, and 6 min, respectively.

Methionine synthase. Methionine synthase was measured in the liver cytosol as described by Nicolaou et al. (1997). Assay mixtures (total volume 300 µl) contained 50 mM potassium phosphate buffer pH 7.2, 400 µM (D,L)-homocysteine, 35 µM SAM, 236 µM MTHF (2658 dpm/nmol), 60 µM hydroxycobalamin, 25 mM DTT (D,L-dithiorthreitol) and the enzyme source. Incubations were performed in light-protected stopped serum vials under nitrogen. Reaction mixtures were preincubated for 5 min (37°C), prior to the initiation of the reaction by the addition of homocysteine through a syringe. Incubations were performed for 45 min at 37°C. The enzyme reaction was terminated by the addition of ice-cold water (400 µl) and solutions immediately passed through a 0.5 × 5.0 cm column of BioRad AG1-X8 resin. [14C]Methionine was eluted with 2 ml of UHQ water, collected and quantified by scintillation spectrometry. Protein concentrations were determined with the BioRad protein assay based on the method of Bradford (1976) with BSA as standard.

Urinary protein. Urinary protein was measured by the Coomassie Plus Protein assay kit supplied by Pierce and Warriner (Chester, UK).

Statistical analysis
Statistical evaluation of data was performed by Duncan’s multiple range test to make comparisons between groups. Values quoted are means ± SEM of six animals. The level of significance (P) was set at <0.05.

RESULTS

Liquid diet consumption, ethanol intake, and body weights
Initially, animals receiving alcohol plus β-alanine drank significantly less than animals given alcohol alone. There was a gradual decrease in liquid diet consumption in the alcohol-treated animals, as alcohol was progressively increased into the liquid diet (Fig. 1a). The liquid diet intake remained relatively constant throughout the treatment period up to day 25, at which point, alcohol-treated animals consumed more liquid diet than alcohol plus β-alanine-treated animals. An ethanol intake of 10.5 g/kg (1.7 g/rat) by the alcohol-treated animals and 10.2 g/kg (1.6 g/rat) by alcohol plus β-alanine treated animals (data not shown) was achieved. Figure 1b shows the gain in body weight in all animals during the 28 days of liquid diet consumption.

Fig. 1. Liquid diet consumption (a) and body weight (b) of animals during 28 days of ethanol administration.

Results are expressed as means ± SEM of six animals. Asterisks indicate values significantly different between treated and respective pair-fed controls (*, P < 0.05; **, P < 0.01). Symbols indicate significant differences between the treated groups and between the pair-fed control groups (●, P < 0.05; □, P < 0.01; ⬤, P < 0.001).
Urine volumes and urinary protein

After initial administration of the liquid diet, the urine volume of all animals was increased (data not shown). However, the urine volumes were not changed during 28 days of ethanol administration. However, the urine volume in the alcohol plus β-alanine-treated and β-alanine-only-treated animals was significantly less on days 1, 2, 4, and 6, compared to non-β-alanine-treated animals. There was no effect of alcohol on urinary protein excretion, although animals given alcohol plus β-alanine and β-alanine alone, excreted significantly greater urinary protein compared to non-β-alanine-treated animals, throughout the treatment period (data not shown).

Post mortem

Both total and relative liver weights (Fig. 2a) and total kidney weights (Fig. 2b) were significantly raised in the alcohol- and alcohol plus β-alanine-treated animals, compared to the pair-fed controls. There was no significant difference in liver and kidney weights between the alcohol- and alcohol-plus β-alanine-treated animals.

Serum analysis

Serum alkaline phosphatase (ALP a marker of bile duct damage) was significantly raised by alcohol treatment, with a greater increase following alcohol plus β-alanine treatment (Fig. 3). Serum ALP was significantly greater in the alcohol plus β-alanine-treated animals compared to alcohol only-treated animals, and in the β-alanine-treated animals compared to the untreated control group. Serum bile acid levels (indicating early cholestasis) were raised by alcohol and alcohol plus β-alanine treatment (Fig. 3). Serum bile acid levels were significantly greater in alcohol plus β-alanine-treated animals, compared to alcohol only-treated animals, and in the β-alanine-treated animals, compared to the untreated control group.

Urinary, hepatic, and serum taurine

There was no effect of alcohol on urinary taurine excretion, other than after 21 days, when the levels were reduced (Fig. 4a). However, animals consuming the β-alanine- and alcohol plus β-alanine-containing liquid diet excreted significantly more taurine compared to animals receiving the β-alanine-free liquid diet (Fig. 4a). There was no effect of alcohol on serum taurine levels, but hepatic taurine was significantly reduced (Fig. 4b). In the β-alanine-treated animals, there were significantly lower levels of liver taurine and serum taurine, compared to non-β-alanine-treated animals. Furthermore, liver taurine levels were markedly lowered in the alcohol plus β-alanine-treated animals, compared to β-alanine only-treated animals.

Hepatic and serum triglyceride and lipid peroxidation

Alcohol and alcohol plus β-alanine treatment significantly raised hepatic triglyceride levels (Fig. 5a) which were significantly but only slightly lower in alcohol plus β-alanine-treated animals, compared to those treated with alcohol alone. Serum triglyceride levels were significantly raised by alcohol and alcohol plus β-alanine treatment (Fig. 5a). There was
no significant difference in serum triglyceride levels in the alcohol-treated animals, compared to alcohol plus β-alanine-treated animals. Alcohol and alcohol plus β-alanine treatment caused a significant increase in malondialdehyde levels (Fig. 5b), but there was no significant difference between the alcohol and alcohol plus β-alanine-treated animals.

**Hepatic ATP, GSH, and GSSG and microsomal analysis**

Alcohol had no effect on hepatic levels of ATP, but β-alanine alone significantly raised ATP levels compared to alcohol plus β-alanine treatment and no alcohol treatment (Fig. 6a). Hepatic GSSG was significantly reduced by alcohol and alcohol plus β-alanine treatment (Fig. 6b). There was no significant difference in hepatic GSSG levels between alcohol- and alcohol plus β-alanine-treated animals. However, alcohol and alcohol plus β-alanine treatment significantly raised levels of hepatic GSH compared to pair-fed controls (Fig. 6b). There was no significant difference in hepatic GSH levels between alcohol-treated and alcohol plus β-alanine-treated animals. Although there was no change in 4-nitrophenol hydroxylase activity, total cytochrome P-450 levels were significantly raised by alcohol treatment and alcohol plus β-alanine treatment (Fig. 7). There was no statistical difference in total cytochrome P-450 levels between alcohol- and alcohol plus β-alanine-treated animals.

**Serum and urinary homocysteine and cysteine and hepatic methionine synthase**

Urinary homocysteine (Fig. 8a) and cysteine (Fig. 8b) levels were raised significantly by β-alanine treatment and significantly more in the alcohol plus β-alanine-treated animals, compared to controls. The higher levels of urinary homocysteine and cysteine were maintained in these animals throughout 28 days of alcohol treatment. Although there was no effect of β-alanine on levels of serum homocysteine or cysteine,
alcohol and alcohol plus β-alanine treatment significantly raised levels of serum homocysteine, and alcohol alone significantly decreased levels of serum cysteine (Fig. 8c). Serum homocysteine levels were also significantly higher in the alcohol plus β-alanine-treated animals, when compared to alcohol only-treated animals. Animals treated with alcohol and alcohol plus β-alanine had significantly inhibited hepatic methionine synthase activities, compared to pair-fed controls (Fig. 8d). There was no significant difference in hepatic methionine synthase activity between alcohol- and alcohol plus β-alanine-treated animals.

Histological analysis
Histological examination of liver tissue (Fig. 9) showed that animals treated with alcohol (Fig. 9b) had developed a marked steatosis, compared to pair-fed controls (Fig. 9a). The fat accumulation in animals given alcohol plus β-alanine (Fig. 9d) was also marked, compared to pair-fed controls (Fig. 9c), but was more than that observed in animals treated with alcohol alone. No method of scoring fat accumulation was used, as triglycerides were also measured biochemically.

DISCUSSION
Previous studies have shown the protective properties of taurine in reducing alcohol-induced hepatic steatosis and lipid peroxidation both when given with and after alcohol (Kerai et al., 1998, 1999). The present study aimed to evaluate the effect of β-alanine, a structural analogue of taurine, which will deplete levels of hepatic taurine, on alcohol-related damage (e.g. hepatic steatosis and lipid peroxidation). The liquid diet containing alcohol resulted in the accumulation of liver triglycerides and lipid peroxidation and thus provided a model for alcoholic liver dysfunction, as has been previously reported (Kerai et al., 1998, 1999). The administration of alcohol to rats for 28 days caused a significant increase in total and relative liver and kidney weight. The increase in liver weights could not be accounted for by triglyceride accumulation, as this was less than 2% of the total increase in liver weights, measured in animals treated with alcohol alone. The increase in kidney weights may be indicative of kidney dysfunction and the raised levels of urinary protein are consistent with this. Despite the fatty liver, urinary taurine was unchanged by alcohol treatment, as found in previous studies (Kerai et al., 1998, 1999). Although other compounds causing fatty liver, such as hydrazine and ethionine, have previously been shown to raise urinary taurine, inhibition of protein synthesis is believed to be the underlying mechanism (Waterfield et al., 1993a,b). Both β-alanine- and alcohol plus β-alanine-treated animals excreted significantly greater amounts of taurine in urine than non-β-alanine-treated animals as a result of the competition between β-alanine and taurine for reuptake in the kidney. Therefore β-alanine treatment depleted liver and serum taurine levels, but liver taurine levels became depleted significantly more in the alcohol plus

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**Fig. 6.** Changes in hepatic levels of adenosine triphosphate (ATP) (a), reduced glutathione (GSH), and oxidized glutathione (GSSG) (b). Results are expressed as means ± SEM of six animals. Asterisks indicate values significantly different between treated and respective pair-fed controls (*, \( P < 0.05; **, \( P < 0.01; ***, \( P < 0.001). Symbol indicates value significantly different between β-alanine- and no alcohol-treated animals (+, \( P < 0.01).**

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**Fig. 7.** The effect of ethanol on hepatic 4-nitrophenol hydroxylase (4-NPOH) activity and total cytochrome P-450 levels. Results are expressed as means ± SEM of six animals. Asterisks indicate values significantly different between treated and respective pair-fed controls (**, \( P < 0.01; ***, \( P < 0.001).**
β-alanine-treated animals. Serum ALP activity was significantly raised by alcohol treatment, and was significantly higher with the alcohol plus β-alanine treatment. Another indication of cholestasis was the raised levels of bile acids following alcohol treatment, which were significantly greater after alcohol plus β-alanine treatment.

Although hepatic triglyceride levels were found to be slightly but significantly lower in the alcohol plus β-alanine-treated animals, compared to alcohol treatment alone, the hepatic steatosis seemed greater in the alcohol plus β-alanine-treated animals when assessed histologically. Ethanol decreases triglyceride release into the blood (Dajani and Konyoumjian, 1967; Koga and Hira yama, 1968; Madsen, 1969), which may be one of the mechanisms underlying ethanol-induced hepatic steatosis. Although alcohol and alcohol plus β-alanine treatment raised serum triglyceride levels, the difference between the two groups was not significant. Thus, unlike taurine (Kerai et al., 1999), β-alanine does not affect triglyceride transport from the liver.

Chronic ethanol feeding results in proliferation of the membranes of the smooth endoplasmic reticulum. As many of the enzymes involved in the synthesis of triglycerides and phospholipids are bound to the membranes of the endoplasmic reticulum, the lipid-synthesizing capacity of the liver could be enhanced. As ethanol is also metabolized by the microsomal enzyme system, it has the potential to interfere with lipid metabolism. Microsomal ethanol oxidation may interfere with lipid metabolism by generating oxygen radicals such as O2•− and HO•, which initiate a cascade of lipid peroxidation and damage cell membranes (Polavarapu et al., 1998). In this study, both alcohol and alcohol plus β-alanine treatment significantly increased hepatic lipid peroxidation, but there was no significant difference between the treatment groups, suggesting that taurine depletion did not affect lipid peroxidation. Previous studies, however, have shown that taurine treatment decreases the extent of lipid peroxidation (Kerai et al., 1998, 1999).

Alcohol and alcohol plus β-alanine treatment raised hepatic GSH levels and reduced GSSG levels after 28 days of ethanol administration. Fernández-Checa et al. (1993) have shown that GSH depletion precedes steatosis and lipid peroxidation, and have suggested that the depletion of GSH could be a contributing factor in the development of alcoholic liver disease. It is possible that GSH depletion occurred earlier in this study and that by 28 days, GSH levels were raised as a result of: (1) rebound synthesis in GSH; (2) conversion of homocysteine to GSH; or (3) mild cholestasis (Dahm et al., 1991; Seabra and Timbrell, 1997). As with previous studies, there was no effect
of alcohol on hepatic ATP levels. However, β-alanine treatment alone raised ATP levels, but the reason for this is unclear.

Changes in methionine metabolism or methylation in the liver may have an important role in alcohol toxicity (Fig. 10). Methionine is converted to SAM, which is important in maintaining the integrity of the liver. SAM is important in the conversion of phosphatidylethanolamine to phosphatidylcholine. Phosphatidylcholine has been shown to be an important constituent of lipoproteins that are involved in the transport of fat from the liver. This would prevent accumulation of fat in the liver and subsequent liver injury (Gigliozzi et al., 1998). The conversion of homocysteine to methionine has been considered to be an essential reaction for conserving methionine, detoxifying homocysteine and for the production of SAM (Lucock et al., 1996). The initial decrease in urinary homocysteine in the control animals and β-alanine-treated groups can be explained by the change from the powdered diet (4.7 g/l methionine), compared to the liquid diet (0.3 g/l methionine). Previous studies have shown that taurine does not protect against the alcohol-induced increase in urinary homocysteine levels and the inhibition of hepatic methionine synthase activity (Kerai et al., 1998, 1999). Although ethanol itself does not inhibit methionine synthase, acetaldehyde is known to inhibit highly purified methionine synthase in vitro (Kenyon et al., 1998). However, in the present study, β-alanine- and especially alcohol plus β-alanine-treated animals excreted significantly greater amounts of homocysteine and cysteine into the urine.

Our previous studies suggested that the protective effect of taurine might be related to the likely increased conjugation of bile acids with taurine (Kerai et al., 1998). Taurine conjugated bile acids are known to inhibit CYP2E1 (Chen and Farrell, 1996) and therefore the metabolism of alcohol by this enzyme, and the subsequent increase in lipid peroxidation and oxidative stress would be reduced. However, in this study, CYP2E1 was not increased and the activity was not decreased by β-alanine and alcohol treatment.

Consistent with this is the lack of effect of β-alanine on hepatic lipid peroxidation. The data on the effect of β-alanine on fatty liver and triglyceride accumulation is difficult to interpret and reconcile. Biochemically, there was a decrease in triglycerides in the liver of alcohol plus β-alanine-treated animals, whereas histologically there seemed to be an increase. This could reflect the nature of the lipids which accumulate and are detected by Oil Red O staining, in contrast to the triglycerides measured biochemically. Thus, there could be an overall increase in liver fats but a decrease in triglycerides caused by β-alanine. Clearly, however, unlike taurine, β-alanine

Fig. 9. Liver histology in rats treated with alcohol or alcohol plus β-alanine.
Frozen sections of liver (×40) taken from (A) control, (B) alcohol-, (C) β-alanine- and (D) alcohol plus β-alanine-treated animals. Frozen sections were stained with Oil Red O and counterstained with Mayer’s haematoxylin. Lipid droplets were stained red.
treatment did not decrease cytochrome P-450 levels or hepatic lipid peroxidation. Also in the present study, serum bile acids and ALP were raised in alcohol-treated animals and significantly more so in the alcohol plus β-alanine-treated animals, suggesting that cholestasis may have occurred. However, with lower levels of taurine, less taurocholate may be formed. Thus, the accumulation of toxic bile acids in the alcohol plus β-alanine-treated animals could result in greater hepatotoxicity in these animals.

The data indicate that treatment with β-alanine has a number of effects. These may be due to the depletion of taurine or to the direct effects of β-alanine itself, or to a combination of these factors. This study does not distinguish between these effects. As β-alanine is an analogue of taurine, it is quite possible that some effects may be common. For example the decrease in the level of triglycerides and the rise in the level of bile acids and ALP seen in the serum were also observed in rats treated with alcohol and taurine. Thus, in this study the depletion of hepatic and serum taurine levels by treatment with β-alanine significantly increased the hepatotoxicity of ethanol as determined histologically and by some biochemical measurements. The present results support previous findings (Kerai et al., 1998, 1999) that taurine has a protective role in the liver against ethanol-induced hepatic steatosis. However it should be noted that the rat is a good synthesizer of taurine, unlike humans, who rely more heavily on dietary sources. Therefore changes brought about by the depletion of taurine with β-alanine treatment would have more impact in humans than in the rat.

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