COMPARISON OF THE FORMATION OF PROTEINS MODIFIED BY DIRECT AND INDIRECT ETHANOL METABOLITES IN THE LIVER AND BLOOD OF RATS FED THE LIEBER–DECARLI LIQUID DIET

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Abstract — It has been proposed that proteins modified by ethanol metabolites, such as acetaldehyde (AcH) or α-hydroxyethyl radicals (HER) may be an important step in the aetiology of alcoholic liver disease. Furthermore, it has also been suggested that these modified proteins may act as a marker of ethanol intake. In this study, we have measured the generation of various types of modified proteins in the liver and blood of ethanol-fed rats. Multiple types of protein modification were observed in the livers of the ethanol-fed rats. In each case, the level of modification increased over the first 6 weeks of ethanol feeding, but reached a plateau by 10 weeks. In contrast to the liver, elevated levels of proteins modified by malondialdehyde were not seen in the plasma of ethanol-fed animals, whereas elevated levels of modification due to AcH and HER were observed. In haemolysates from these animals, only modification due to AcH was seen. Further investigation of the modification of plasma proteins showed that albumin, a protein produced in the liver, carried all the types of modification investigated, whereas immunoglobulin G, a protein derived from an extra-hepatic source, only carried modifications due to acetaldehyde. This study demonstrates for the first time that modification of plasma proteins by ethanol metabolites can occur at both intra- and extra-hepatic sites.

INTRODUCTION

Ethanol metabolism leads to the production of reactive species, which may be involved in the pathogenesis of alcohol-related tissue injury. The majority of imbibed ethanol is oxidized by the liver. Therefore, it is likely that evidence of the production of these reactive compounds will be found in the liver. Indeed, the livers of ethanol-fed animals have been shown to contain proteins modified by the direct metabolites acetaldehyde (AcH) (Behrens et al., 1988; Lin et al., 1988; Worrall et al., 1991b) and α-hydroxyethyl radicals (HER) (Albano et al., 1996) as well as the indirect metabolites malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Niemela et al., 1994, 1995), using techniques such as Western blotting and ELISA (enzyme-linked immunosorbent assay). The demonstration by several researchers that such protein modification leads to the formation of neoantigens (Israel et al., 1986; Fleisher et al., 1988; Worrall et al., 1989), providing targets for immune-mediated attack, emphasizes the potential importance of protein modification in the aetiology of alcoholic liver disease. It has also been proposed that proteins modified by an ethanol metabolite, such as AcH, may be a biological marker of ethanol intake in a manner analogous to protein glycation acting as an indicator of prevailing glucose concentrations in diabetes. Furthermore, it has also been suggested by ourselves and others (Niemela et al., 1987; Hoerner et al., 1988; Worrall et al., 1991a, 1996; Koskinas et al., 1992) that measurement of antibodies directed against the modified proteins may also be useful as a marker of ethanol intake. In this study, we have investigated the generation of proteins modified by direct and indirect metabolites formed during chronic ethanol feeding in rats. Comparisons between modified proteins in the liver and in the circulation show that some types of modification are specific to the liver, whereas others are found both in liver and in blood. We have also investigated the generation of antibodies reactive with various types of modification.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (100–150 g; Central Animal Breeding House, The University of Queensland, Brisbane, Australia) were maintained under a 12 h light : 12 h dark regime at 22°C. After 3 days of acclimatization, the animals were divided into two groups. One group was fed ad libitum the Lieber–DeCarli ethanol-containing diet (Dyet Inc, Bethlehem, PA, USA), whereas the others were pair-fed the control diet in which ethanol was replaced by an isocaloric amount of maltose–dextrin. After 2, 4, 6, and 10 weeks on the diet, animals were killed by decapitation. At each time point, blood was collected in heparinized tubes, and the resulting plasma stored in small aliquots at −80°C. A haemolysate was produced from packed red cells by the addition of an equal volume of deionized water. After 20 min at 4°C, the mixture was centrifuged at 110 000 g for 20 min and the supernatant stored at −80°C. The livers were removed and some were stored at −80°C in small pieces. The remainder was homogenized with homogenizing buffer [10 ml/g liver; Tris-buffered saline (TBS), pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-tetraacetic acid and 100 µM phenylmethylsulphonyl fluoride] and also stored at −80°C. Both blood fractions and liver homogenates were assayed for adducts and/or antibodies within 2 weeks of collection.

Production of modified proteins

This study required the use of two modified proteins. Human plasma protein was modified using each procedure described below and used to generate antisera reactive with each type of modification. Similarly, bovine haemoglobin was also...
modified using each procedure and used as the coating protein in the ELISAs used to detect antibodies generated in vivo against each type of adduct.

**Modification by AcH.** AcH-modified proteins were produced by incubating protein (10 mg/ml) with 1 mM AcH in phosphate-buffered saline (PBS), pH 7.4. Unreduced adducts were generated by a 5 h incubation at 37°C, whereas reduced adducts were produced by a 1 h incubation at 37°C followed by the addition of 1 ml of 40% (w/v) sodium cyanoborohydride/10 ml of reaction mixture. A further 30 min incubation at 37°C was then carried out to allow the reduction of Schiff bases.

**Modification by HER.** HER-modified proteins were prepared by incubating 10 mg/ml protein with 50 mM ethanol, 100 μM ferric ammonium sulphate, 200 μM EDTA, and 100 μM hydrogen peroxide, in 50 mM sodium phosphate buffer (pH 7.4) at 37°C for 30 min as described by Moncada et al. (1994).

**Modification by MDA.** MDA-modified protein was produced by incubating 10 mg/ml protein with 1 mM MDA for 12 h at 37°C in phosphate-buffered saline, pH 7.4. MDA was produced immediately before use by the hydrolysis of the dimethylacetel with 1 M HCl for 30 min at 37°C. A portion of the acidic solution was then diluted with water and adjusted to pH 7.4 with 1 M NaOH to prepare neutral MDA solution for use in the modification reactions.

**Generation of anti-adduct antisera**

New Zealand white rabbits (8 months old) were immunized with modified human plasma protein in three sets of multi-site injections as described at Worrall et al. (1989). After the final booster injection, the rabbits were exsanguinated by cardiac puncture under nembutal anaesthesia (60 mg/kg, i.p.). Blood was collected using heparinized needles and tubes and the resulting plasma was stored at −80°C.

**Purification of anti-adduct antisera**

To decrease binding to unmodified proteins, antisera were purified as described by Worrall et al. (1989) and using the modification of Nicholls et al. (1994). Briefly, 1 ml of rabbit antiserum and 1 ml of buffer A (20 mM Tris–HCl, pH 8.0, containing 28 mM NaCl) were mixed and loaded onto a Biogel P-6 DG desalting column (Biorad). The eluate was collected as a single fraction and applied to a DEAE–Affigel blue column (Biorad) pre-equilibrated with Buffer A. The column was eluted with Buffer A, and 2-ml fractions collected. Those containing the highest protein concentrations were pooled and applied to an immunoabsorption column for 4 h at 4°C. The eluate from the immuno-absorption column was collected as a single fraction and stored at 4°C for less than a week prior to use in the ELISAs described below. The immuno-absorption column was made by reacting 2 ml of rat plasma (from a control) with 10 ml of Affigel-10 activated ester gel (Biorad) suspended in coupling buffer (40 mM HEPES, pH 7.5, containing 160 mM CaCl₂) for 4 h at room temperature. The gel was washed with 20 bed-volumes of buffer to remove unbound protein.

**Biotinylation of antibodies**

Commercially obtained rabbit anti-rat albumin or rat IgG polyclonal antibodies (Accurate Chemical & Scientific Corporation, Westbury, NY, USA) were biotinylated using a commercially available kit (Amersham plc, Amersham, UK). Briefly, the antibody concentration was adjusted to 1 mg/ml and dialysed overnight against PBS at 4°C. Biotinylation reagent was added to the solution (40 μl/mg of protein) and incubated for 2 h at 20°C. Unreacted biotinylation reagent was removed from the biotinylated antibody by chromatography on a Sephadex G-25 column. Biotinylated antibody was stored at 4°C and used within a week.

**Detection of modified proteins and rat plasma, haemolsyate and liver homogenate**

The presence of modified proteins in liver homogenate, plasma or haemolsyate was detected by indirect ELISA. Briefly, samples were diluted to 100 μg protein/ml in PBS, and 100 μl were added to each well. After 2 h at 4°C, the plates were washed with Tris-buffered saline (TBS), pH 7.4. Non-specific binding sites were blocked by incubation with 200 μl of saturated casein solution [20% (w/v), pH 7.5–8.0] for 1 h at 4°C. Purified rabbit anti-adduct antiserum (100 μl) was then added to each well and incubated for 4 h at 4°C. The plate was washed with TBS–casein (TBS containing 0.5% casein) and 100 μl of biotinylated anti-rabbit immunoglobulin antibody was added. After 2 h at 4°C, the plates were washed again with TBS–casein and 100 μl of streptavidin–alkaline phosphatase complex added. After 1 h at 37°C, the plates were again washed with TBS. The final step was to add 100 μl of p-nitrophenyl phosphate (1 mg/ml) in diethanolamine buffer (10 mM diethanolamine, 0.5 mM MgCl₂, pH 9.5) and incubate at 37°C. The absorbance of each well at 405 nm was measured using a Titerrek multiscan plate reader after a 30 min–3 h incubation, depending on the type of adduct and whether liver, plasma or haemolsyate was assayed.

Modification of plasma albumin and IgG was detected by using a sandwich ELISA. Microtitre plates were coated with 100 μl of purified rabbit anti-modified human plasma protein diluted to 20 μg/ml in TBS, pH 7.4. After 4 h at 4°C, the plates were washed with TBS and the samples added (50 μl plasma or liver homogenate mixed with 150 μl TBS) and incubated for 2 h at 4°C. The plate was then washed with TBS and 100 μl of biotinylated anti-rat albumin or IgG diluted 1:100 with TBS containing 0.1% (w/v) casein was added to each well. After 2 h at 4°C, the plate was washed with TBS, and streptavidin–alkaline phosphatase complex added. The rest of the assay was as described above. The absorbance of each well at 405 nm was measured using a Titertek multiscan plate reader after a 1–3 h incubation, depending on the protein to be measured.

**Detection of anti-adduct antibodies in rat plasma**

The method used to determine plasma immunoreactivity with modified protein was essentially the same ELISA as that described in Worrall et al. (1991a). However, for these assays, the microtitre plates were coated with 100 μl of a solution containing 50 μl/ml unmodified or modified bovine...
haemoglobin in PBS, pH 7.4. After blocking with casein solution, 100 µl of a 1:10 dilution of plasma in TBS containing 0.5% (w/v) bovine casein (TBS–casein) were added to each well and incubated for 1 h at 37°C. The wells were then washed with TBS–casein and incubated with biotinylated antibodies for total rat Ig, or class-specific ones for IgA, IgG or IgM (1:1000 dilution in TBS–casein buffer). From this point, the assay was carried out as described previously.

**Definitions and statistical evaluation of results**

Antibody binding to modified epitopes was defined as the difference in absorbance between wells coated with unmodified and AcH-modified bovine haemoglobin and was termed adduct-specific reactivity (ASR). Statistical analyses were performed on an IBM-compatible PC using SigmaStat™ for Windows 95 Version 2.03 (Jandel Scientific Software, San Rafael, CA, USA).

**RESULTS**

**Nutritional parameters**

No significant difference in weight gain was seen between animals fed the ethanol-containing and control forms of the Lieber–DeCarli liquid diet (data not shown). Ethanol consumption of rats fed the ethanol-containing diet was 11.3 ± 1.5 g/kg/day, with a range of 8.3 to 14.2 g/kg/day. Plasma ethanol concentrations were determined after 6 weeks on the diet. Approximately 1 h after consuming the diet, plasma ethanol concentration varied from 85 to 138 mg/dl, with an average of 111.8 ± 15.6 mg/dl. Ethanol could not be detected in the plasma of animals fed the control diet. These results are consistent with those previously reported by Lieber and De Carli (1980).

**Modified proteins in liver**

Figure 1 shows that multiple types of modification occur in the livers of rats fed ethanol for up to 10 weeks. In each case, the amount of modification increased gradually over the first 6 weeks of feeding, but was relatively steady after that time. In the control animals, the levels of modification were stable throughout the experiment and were always lower than those of the ethanol-fed group.

**Modified proteins in plasma**

A different pattern of modification was seen when plasma proteins were analysed. Figure 2 shows that the overall level of modification was lower in plasma and that the appearance of modifications was generally later, compared to that in the liver. Thus, elevated levels of modification were only seen in the ethanol-fed animals after 4–10 weeks of feeding, but not earlier.

Furthermore, elevated levels of MDA-modified proteins were not observed in the ethanol-fed group. Analysis of the types of modifications carried by a liver-derived protein, albumin, and one produced outside the liver, immunoglobulin G
(IgG) was performed. Albumin carried all the types of modifications investigated, whereas IgG only carried those modifications derived from AcH (data not shown).

**Modified proteins in haemolysate**

Unlike the liver, where multiple types of modification were detected, only a single type of modification was found in haemolysate. Figure 3 shows the development of unreduced AcH adducts in haemolysate from these animals. In the early stages of ethanol feeding, there was no elevation in modification of the haemolysate when compared to controls. However, by 6 weeks, there was a significantly higher level of modification in the ethanol-fed animals which was further increased by 10 weeks.

**Antibodies reactive with modified proteins**

Figure 4 shows the detection of circulating antibodies reactive with various types of modification. The antibody profiles were similar for all the types of modification studied. In control animals, the antibody levels remained low and virtually constant over 10 weeks, whereas antibody levels rose steadily in the ethanol-fed animals, in most cases reaching a plateau by 6 weeks. The only exception was the reactivity against reduced AcH adducts which was only beginning to reach a plateau at 10 weeks. Figure 5 shows the antibody classes involved in the immune responses against the various types of adduct after 10 weeks of ethanol feeding. Ethanol-fed rats had higher IgM reactivity than controls for each type of modification. However, this was not true with IgG and IgA. IgG was only elevated against unreduced and reduced AcH adducts, HER-derived adducts and MAA adducts in the ethanol-fed animals whereas IgA was only elevated against unreduced and reduced AcH adducts.

**DISCUSSION**

The production of modified proteins during chronic ethanol oxidation, together with the associated immune responses, has been widely studied over the past 10–15 years. This extensive research effort has focused on modified proteins as potential targets for immune attack leading to tissue damage, and also as biological markers of hazardous ethanol intake. Many reactive metabolites have been shown to be generated during ethanol oxidation. These reactive species can occur directly as a result of the action of ethanol metabolizing enzymes (e.g. AcH and HER) or can be generated indirectly by prevailing conditions within the liver (e.g. lipid peroxidation leading to MDA). In the present study, we have investigated the formation of six types of modification within the liver and blood of ethanol-fed rats. However, due to the use of different antisera to detect the various types of modification, we have not attempted to compare the relative levels of the adducts. To do this would require either chemical analysis to measure absolute amounts of the modified amino acids or the ability to construct standard curves for the ELISAs using known amounts of pure adducts. At present both of these approaches are not available. Thus, we have limited comparisons in the amount of adducts to within single adduct types measured using the same reagents. For example, we can demonstrate that there was more of an adduct after 10 weeks of feeding than there was of the same adduct after 4 weeks.

The liver is the main site of ethanol oxidation and it would be expected that this tissue would have the highest levels of modification and the widest variety of adduct types. The liver oxidizes >90% of imbibed ethanol to produce AcH by the action of alcohol dehydrogenase and the microsomal ethanol-oxidizing system (MEOS), an important component of which

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**Fig. 3.** Detection by ELISA of unreduced AcH adducts in red blood cell haemolysates.

Haemolysates were prepared from rats fed the control (□) and ethanol-containing (●) forms of the Lieber–DeCarli liquid diet for 10 weeks (*P < 0.05 ethanol vs control).
is cytochrome P-450 IIE1 (Lieber, 1999). During chronic ethanol oxidation, appreciable levels of AcH can accumulate due to the inability of aldehyde dehydrogenase to oxidize all AcH to acetate. Studies using freshly isolated rat hepatocytes suggest that a free AcH concentration of about 1 mM can be obtained at an ambient ethanol concentration of 25 mM (Irving et al., 1985). The MEOS system can also produce highly reactive HER as a secondary reaction to the production of AcH (Lieber, 1999). An indirect effect of ethanol metabolism is to decrease the hepatic concentration of glutathione, making cellular membranes more prone to peroxidation and leading to the increased production of lipid peroxide breakdown products, such as MDA and 4-HNE. Thus, ethanol oxidation leads to the production of two well-characterized groups of reactive species, namely aldehydes (AcH and MDA) and free radicals (HER). Each of these species can react individually with macromolecules, such as proteins, to produce covalent modifications. In addition, it has recently been shown that AcH and MDA can act in concert to produce an adduct which contains two molecules of MDA and one molecule of AcH, and another which contains one molecule of each. These adducts have been designated MAA (malondialdehyde–acetaldehyde adducts) by Tuma et al. (1996).

The tissue samples analysed in the study were from male Wistar rats maintained on the Lieber–DeCarli liquid diet. Those animals fed the ethanol-containing form of the diet consumed 8–14 g ethanol/kg/day, whereas the control animals were pair-fed the control form of the diet in which the ethanol was isocalorically replaced by maltose–dextrin. After 6 weeks on the liquid diet, blood-ethanol concentrations ranged from 85–138 mg/dl 1 h after consumption in the ethanol-fed animals, whereas ethanol was not detectable in the blood of control animals. Thus, comparison between tissues from the ethanol-fed and control animals will show whether the quantity or type of adduct is related to ethanol consumption.

Previous studies have shown that proteins modified by AcH (Barry et al., 1987; Behrens et al., 1988; Lin et al., 1988; Worrall et al., 1991b), MDA (Niemela et al., 1994) and HER (Albano et al., 1993; Clot et al., 1996) are formed in the liver of ethanol-fed animals. However, no simultaneous measurement of the generation of multiple adduct types has been previously carried out. In this study, ethanol-fed animals exhibited higher levels of each type of adduct after 10 weeks. Furthermore, as expected, the amount of each type of adduct increased after the initiation of ethanol feeding until a plateau was reached, in most cases at about 6 weeks. However, the time taken to reach significantly elevated levels of each adduct varied. Reduced AcH adducts (thought to be largely N-ethylated amino groups) and MAA adducts were elevated after 2 weeks, whereas unreduced AcH adducts (chemical nature unknown, but probably includes imidazolidinone and thiazolidine derivatives), HER-derived modifications, and N-ethylated lysine residues (recognized by the monoclonal antibody RT1.1) were only elevated after 4 weeks. The slow increase in HER-modified proteins is probably related to the induction of MEOS activity, which takes about 2 weeks of chronic ethanol feeding to become maximal (Lieber, 1999). However, MDA adducts took 10 weeks to become significantly elevated in the ethanol-fed animals. The discrepancy between the generation of reduced AcH adducts and the N-ethylated amino groups detected by RT1.1 is interesting given that N-ethylated amino groups were thought to be the main type of reduced adduct formed. The reduced AcH adducts are significantly elevated in the ethanol-fed animals 2 weeks prior to the N-ethylated residues, suggesting that other types of adduct are initially formed in vivo that are recognized by the antisera raised against proteins incubated with AcH and then reduced with sodium cyanoborohydride, but are not recognized by RT1.1. Further characterization of the adducts formed by this modification protocol will help to identify the nature of the other adducts. A comparison of the generation of MDA-modified protein and MAA adducts is also of interest, as both types of modification require MDA. It appears that, at least in the initial stages, MDA produced reacts together with AcH to produce MAA adducts, such that they are significantly elevated after 2 weeks, whereas MDA adducts are not elevated until the concentration of MAA adducts becomes maximal.

Plasma proteins from ethanol-fed animals were also found to carry elevated amounts of each adduct type after 10 weeks of feeding, when compared to control animals. Again in the same way as hepatic modification, the degree of modification of plasma protein was found to increase over time, and to then plateau. However, with the exception of the N-ethylated residues, each type of adduct took longer to be significantly elevated in plasma than it did within the liver. A potential explanation for these data is that ethanol metabolites initially react with internal components of hepatocytes and only react with secretory proteins such as albumin during the secretory process once the initial sites have been modified. However,
this raises the question of whether plasma proteins are modified prior to secretion by the hepatocyte or are modified in the circulation by reactive metabolites which have leaked out of the liver. In an attempt to answer this question, we compared the types of modification carried by albumin, the major plasma protein which is secreted by hepatocytes, and IgG, a protein secreted by circulating plasma cells (i.e. non-hepatic origin). Albumin was found to carry all of the types of modification studied. However, IgG carried only unReduced and reduced AcH adducts. These data suggest that at least some types of modification carried by albumin probably occur prior to its release into the circulation. For example, we have shown that plasma albumin carries HER-derived modifications. However, due to the highly reactive nature of these free radicals, it is unlikely that they are able to diffuse out of the liver cells to react with extra-hepatic albumin. Furthermore, the production of HER outside the liver is likely to be low. This is further supported by the fact that circulating IgG does not carry this type of modification, presumably because it is secreted by cells which do not contain the MEOS activity required to make HER and do not encounter the radical in the circulation. Similar arguments can be made for MDA-derived modification and for MAA adducts, both of which do not appear to form on circulating IgG.

Several previous studies have shown that AcH reacts with haemoglobin in vivo to produce covalent adducts (Niemela et al., 1990; Sillanaukee et al., 1991; Lin et al., 1993). In this study, we also found significantly elevated immunoreactivity with AcH-derived adducts in haemolysates from ethanol-fed rats, compared to control animals. However, the adducts were only of the unReduced type, with no reduced adducts being detected. This is again an interesting finding, since it demonstrates that adducts formed in erythrocytes are different from those formed in the plasma. This is not surprising, given that mature erythrocytes do not contain endoplasmic reticulum and therefore do not have MEOS activity. Thus, they are unlikely to generate large amounts of HER and would not therefore contain HER-modified proteins. The AcH adducts formed may be derived from AcH produced inside the erythrocyte or from AcH that has leaked out of the liver. Under most conditions, erythrocytes are well protected against free radical damage, being amply supplied with reduced glutathione and a glutathione regenerating system (al-Turk et al., 1987). This probably explains the absence of MDA and MAA adducts.

Many researchers have shown that adduct formation leads to the generation of neoantigens and to the production of antibodies reactive with the modification (hapten) and the carrier protein. In this study, we were able to detect antibodies reactive with each type of modification. While previous studies have shown that antibodies reactive with a single type of modification are generated in vivo (Israel et al., 1986; Hoerner et al., 1988; Worrall et al., 1989; Clot et al., 1995; van de Vijver et al., 1996), this is the first study to compare the generation of antibodies against multiple types of adduct in the same animal. Initially, we analysed the total immunoglobulin reactivity (IgG, IgA, and IgM together) against each type of modification. After 6–10 weeks on the diet, the ethanol-fed animals had elevated reactivity with each type of modification. Indeed, the immune responses were similar for most adducts with a maximal response being obtained after about 6 weeks of ethanol feeding. However, the immunoreactivity with reduced AcH and MAA adducts was still increasing after 10 weeks of feeding. This was especially true of the reactivity against MAA adducts, where a similar response was seen in each ethanol-fed animal tested (data not shown). We then dissected the antibody responses by looking at the classes of immunoglobulin involved in the response to each type of adduct. An elevated IgM response against all of the adduct types was observed after 10 weeks on the diet. An elevated IgG response of each adduct type other than MDA adducts was also observed in the ethanol-fed animals. However, only ethanol-fed animals exhibited a significantly elevated IgA response against unReduced and reduced AcH adducts. We have previously demonstrated a relationship between elevated IgA reactivity with AcH-modified proteins and ethanol abuse in humans (Worrall et al., 1996). The causal relationship for the elevated IgA reactivity has not yet been delineated, but must lie in the close relationship between IgA and the liver (Brown and Kloppel, 1989).

The Lieber–DeCarli liquid diet is the most commonly used paradigm to study the pathological effect of ethanol. However, it does not cause significant liver damage, such as hepatitis or cirrhosis unless an additional stimulus such as lipopolysaccharide (Pennington et al., 1997) is used. However, we have previously shown that rats fed the ethanol-containing form of the diet and injected with AcH-modified proteins, leading to the generation of a high titre of anti-AcH adduct antibodies, exhibited panlobular piecemeal necrosis, whereas control-fed animals did not (Worrall et al., 1992). These data suggest that the Lieber–DeCarli liquid diet is a good starting point for the generation of advanced liver disease. Comparison between the adducts formed by feeding the Lieber–DeCarli liquid diet and the Tsukamoto–French paradigm, in which ethanol feeding does not require additional factors to produce hepatitis and fibrosis (Nanji et al., 1989), may help to identify which adducts are important in the aetiology of significant liver damage. In this study, we have demonstrated that multiple types of protein modification are generated as a result of chronic ethanol feeding of rats. We have also shown that different types of modification occur in the liver when compared to plasma and erythrocytes. These data show that it is likely that modification of plasma proteins occurs at two sites. Plasma proteins may be modified during their synthesis and secretion by hepatocytes, but the data generated by analysis of IgG (not synthesized and secreted by the liver) suggest that modification can also occur in the plasma. Whether this is as a result of ethanol metabolism in the circulation or due to ethanol metabolites leaking out of the liver remains to be determined. We are now completing a similar study using human tissue.

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REFERENCES

