The microsomal ethanol oxidizing system: its role in ethanol and xenobiotic metabolism

CHARLES S. LIEBER
Alcohol Research and Treatment Center and Section of Liver Disease and Nutrition, Veterans Administration Medical Center, Bronx, New York and Mount Sinai School of Medicine (CUNY), New York, NY 10065, U.S.A.

Synopsis
After chronic ethanol consumption, the activity of the microsomal ethanol-oxidizing system (MEOS) increases and contributes to ethanol tolerance, as most conclusively shown in alcohol-dehydrogenase-negative deermice. In man and animals, there is an associated rise in microsomal cytochrome P-450, including a specific form (P-450IIIE1) with high affinity for ethanol and for the activation of some drugs (i.e. acetaminophen), carcinogens (i.e. N-nitrosodimethylamine) and hepatotoxic agents (i.e. CCI4), thereby contributing to the susceptibility of alcoholics to xenobiotics, including industrial solvents. In addition, a benzoflavone-inducible liver cytochrome P-450 isoenzyme distinct but catalytically similar to cytochrome P-450IIIE1 was purified which may play a significant role in drinkers who also are heavy smokers. Cross-induction of other microsomal enzymes is associated with enhanced metabolism of various drugs, resulting in drug tolerance. Catabolism of retinol was also found to be accelerated, in part through activation of newly discovered microsomal pathways, thereby contributing to hepatic vitamin A depletion and possibly toxicity. Thus, elucidation of the microsomal metabolism of ethanol explains a number of complications that develop in alcoholics.

Microsomal ethanol-oxidizing system: its nature and differentiation from alcohol dehydrogenase and catalase

Until recently, it was commonly believed that the primary pathway for hepatic ethanol metabolism involved cytosolic alcohol dehydrogenase (ADH), with minor contributions from catalase in the peroxisomes. Non-ADH ethanol oxidation occurring in other subcellular fractions isolated from liver was usually attributed to a hydrogen-peroxide-dependent reaction mediated by a presumed catalase contamination of these fractions. Indeed, this oxidative reaction not mediated by ADH showed substrate specificity for methanol rather than ethanol and other higher aliphatic alcohols (e.g. butanol), and was extremely sensitive to inhibitors of catalase activity, such as azide and cyanide (Orme-Johnson & Ziegler, 1965; Ziegler, 1972). The observation in rats (Iseri et al., 1964, 1966) as well as in man (Lane & Lieber, 1966) that chronic ethanol consumption was associated with proliferation of microsomal membranes prompted the suggestion that liver microsomes could be a site for a distinct and adaptive system of ethanol oxidation. Indeed, such a system was demonstrated in vitro and named the microsomal ethanol-oxidizing system (MEOS) (Lieber & DeCarli, 1968, 1970). Rates of MEOS-catalysed ethanol oxidation were 10-fold higher than those originally reported by Orme-Johnson & Ziegler and, based on various studies, it was concluded that the MEOS was distinct from ADH and catalase, and dependent on cytochrome P-450. This proposal initiated a decade of research on a newly discovered enzyme system which was finally resolved after: (a) isolation of a P-450-containing fraction from liver microsomes which, although devoid of any ADH or catalase activity, could still oxidize ethanol as well as higher aliphatic alcohols (e.g. butanol which is not a substrate for catalase) (Tescheke et al., 1972, 1974; Mezey et al., 1973) and (b) reconstitution of ethanol-oxidizing activity using NADPH-cytochrome P-450 reductase, phospholipid, and either partially or highly-purified microsomal P-450 from untreated (Ohnishi & Lieber, 1977) or phenobarbital-treated (Miwa et al., 1978) rats. That chronic ethanol consumption results in the induction of a unique P-450, was shown by Ohnishi & Lieber (1977) using a liver microsomal P-450 fraction isolated from ethanol-treated rats. An ethanol-inducible form of P-450 (LM3a), purified from rabbit liver microsomes (Koop et al., 1982; Ingelman-Sundberg & Johansson, 1984), catalysed ethanol oxidation at rates much higher than other P-450 isoenzymes, and also had an enhanced capacity to oxidize 1-butanol, 1-pentanol, and aniline (Morgan et al., 1982), acetaminophen (Morgan et al., 1983), CCl4 (Ingelman-Sundberg & Johansson, 1984), acetone (Koop & Cassaza, 1985), and N-nitrosodimethylamine (NDMA) (Yang et al., 1985). Similar results have been obtained with cytochrome P-450j, a major hepatic P-450 isoenzyme purified from ethanol- or isoniazid-treated rats (Ryan et al., 1985, 1986). Others have also provided evidence for the existence of a P-450j-like isoenzyme in humans (Wrighton et al., 1986; Song et al., 1986). Wrighton et al. (1986) employed immunoaffinity chromatography to purify from human liver a protein termed HLj; however, its catalytic activity toward ethanol was not described. Song et al. (1986) isolated DNAs complementary to human P-450j. The amino acid sequence of human P-450j, deduced by sequencing of the complementary (c) DNA inserts, was reported to be 94% homologous to the published N-termini for HLj (Wrighton et al., 1986) over the first 18 amino acid
Fig. 1. SDS/polyacrylamide gel electrophoresis of human microsomes and purified cytochromes P-450

Samples were analysed on a slab gel 0.75 mm thick containing 7.5% (w/v) acrylamide using a discontinuous buffer system. Migration proceeded from top to bottom. Lanes 2 and 7, microsomes (10 μg); lanes 3, 4 and 5, cytochrome P-450-B, P-450-ALC (P-450IE1) and P-450-C, respectively (0.5 μg); lane 6, mix of all three P-450s (0.25 μg each); lanes 1 and 8, protein standards with molecular masses of 98 000, 68 000, 58 000, 53 000, 43 000 and 29 000 (0.5 μg each). (From Lasker et al., 1987a.)

residues. We now have succeeded in obtaining the purified human protein (Fig. 1) in a catalytically active form, with a high turnover rate for ethanol and other specific substrates (Lasker et al., 1987a). The N-terminal sequences of cytochrome P-450-ALC, P-450-B and P-450-C were determined as shown in Fig. 2. The N-terminal sequences of HLj (Wrighton et al., 1986) and human P-450 (Song et al., 1986) are given for comparison, and those residues identical to P-450-ALC are boxed. In a new nomenclature for cytochromes P-450, it was proposed that the ethanol-inducible form be designated as P-450IE1 (Nebert et al., 1987). The designation P-450IE1 should be reserved for this specific P-450 alcohol oxygenase. However, other microsomal cytochrome P-450 isoenzymes can also contribute to ethanol oxidation (Lasker et al., 1987b). Thus, the term MEOS should be maintained when one refers to the overall capacity of the microsomes to oxidize ethanol rather than to that fraction of the activity which is specifically catalysed by P-450IE1. Since P-450IE1 may play a key role in the toxicity of various agents, particularly after chronic alcohol consumption (see below), the molecular mechanism(s) by which other P-450 isoenzymes by compounds such as phenobarbital, 3-methylcholanthrene or pregnenolone-16α-carbonitrile is mediated by an increase in their corresponding mRNAs (Phillips et al., 1981; Pickett et al., 1983; Hardwick et al., 1983a,b; Kawajiri et al., 1984), resulting primarily from transcriptional activation of P-450 structural genes. In contrast, other reports have concluded that P-450IE1 protein induction by certain 'ethanol-like' agents may be regulated by a post-translational event rather than via increased P-450IE1 gene transcription (Song et al., 1986; Khani et al., 1987). Neither study found, using P-450IE1 cDNA probes, an accumulation of mRNA transcripts.

More recently, Kubota et al. (1988) showed an increase in translatable hepatic P-450IE1 mRNA in hamsters following treatment with either ethanol or pyrazole. This increase in

<table>
<thead>
<tr>
<th>Cytochrome</th>
<th>Amino acid residue</th>
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<tr>
<td>P-450 MP-1,2</td>
<td>M D S L V V L V X</td>
</tr>
<tr>
<td>P-450-C</td>
<td>M* X S* L* V* V* L* L* S* L* X</td>
</tr>
<tr>
<td>P-450-B</td>
<td>M E P F V L V L X L S S M L</td>
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<tr>
<td>P-450-ALC</td>
<td>M A L G V T V A L L V W A A F L L L V</td>
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<tr>
<td>HLj</td>
<td>A A L G V T V A L L V W A A F L L L V</td>
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<tr>
<td>Human P-450j (M)</td>
<td>F A A L G V T V A L L V W A A F L L L V</td>
</tr>
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Fig. 2. N-Terminal amino acid sequences of human P-450-ALC, P-450-B, P-450-C and related proteins

The N-terminal sequences of cytochrome P-450-ALC (P-450IE1), P-450-B and P-450-C were determined as described in the text. The N-terminal sequences of HLj (Wrighton et al., 1986) and human P-450 (Song et al., 1986) are given for comparison, and those residues identical to P-450-ALC are boxed. Whether the N-terminal Met of human P-450j is present in the native protein is not known, since the sequence was deduced from a cDNA clone (Song et al., 1986). Underlined P-450-B and P-450-C residues indicate those common to P-450-ALC. (From Lasker et al., 1987a.)
liver P-450IIE1 mRNA was associated with increased liver microsomal P-450IIE1 protein content and enhanced rates of microsomal ethanol oxidation and p-nitrophenol hydroxylation. Quantitatively, the increase in translatable P-450IIE1 mRNA levels after ethanol treatment closely paralleled the elevation of P-450IIE1 protein and associated catalytic activities. It remains to be established whether such increases in P-450IIE1 messenger result from transcriptional activation of the P-450IIE1 gene or mRNA stabilization.

Role of MEOS in ethanol metabolism

MEOS has a relatively high Km for ethanol (8-10 mM compared with 0.2-2 mM for ADH) and thus normally ADH accounts for the bulk of ethanol oxidation at low blood ethanol levels; however, this may not be true at high ethanol levels and/or after chronic use of alcohol. MEOS may play a highly significant role in ethanol oxidation under these circumstances, in part because of its inducibility (Lieber & DeCarli, 1970, 1972; Papengetal., 1970; Grunnet et al., 1973; Matsuzaki et al., 1981). While data obtained with inhibitors are suggestive of MEOS involvement, this cannot be considered conclusive since inhibitors are not sufficiently specific. For example, the ADH inhibitor pyrazole has been shown to inhibit MEOS also (Teschke et al., 1976; Takagi et al., 1986). However, a mutant decemouse strain that lacks ADH nevertheless actively oxidizes ethanol (Burnett & Felder, 1978, 1980; Shigeta et al., 1984), providing clear evidence that non-ADH pathways can be important in vivo. The availability of this strain has also stimulated research on the respective roles of MEOS and catalase in non-ADH ethanol metabolism (Shigeta et al., 1984; Glassman et al., 1985; Takagi et al., 1986; Handler et al., 1986; Kato et al., 1987). Catalase has generally been assumed to play a minor role, owing principally to the limited intracellular production of H2O2 (Bovers et al., 1972). Handler & Thurman (1987a) reported, in the contrary, that fatty acids (particularly palmitate) stimulate both ADH and ethanol uptake, and suggested a corresponding role for catalase in ethanol metabolism under physiological conditions. It must be pointed out, however, that this phenomenon was observed only with 4-methylpyrazole present. In the absence of 4-methylpyrazole, extra H2O2 generation with fatty acids in the intact perfused liver has been reported to be small for all fatty acids tested, and even totally absent for palmitate (Foerster et al., 1981). Indeed, unless 4-methylpyrazole is added, the rate of ethanol metabolism is reduced in the presence of fatty acids (Williamson et al., 1969; Berry et al., 1983), and β-oxidation of fatty acids is inhibited when either ethanol (Williamson et al., 1969) or butanol (Handler & Thurman, 1987b) is metabolized via ADH. Thus, under physiological conditions with ADH present, the presence of fatty acids stimulates H2O2 generation, or that this peroxisomal pathway represents a significant contribution to ethanol metabolism. Even in the absence of ADH, other studies provide evidence which argues against a major role for this pathway. Indeed, in hepatocytes from ADH-decremice, azide, which does not significantly affect other ethanol pathways in vivo at low concentrations (Teschke et al., 1974, 1976), has little non-specific effects (Kato et al., 1987). Azide is a potent metabolic poison and cannot be used in vivo. The other commonly used catalase inhibitor, 3-amino-1,2,4-triazole (AT), is used in vivo, but it also inhibits microsomal enzymes (Kato, 1967) and ADH (Feytmans & Leighton, 1973). The current controversy over whether catalase plays a role in non-ADH metabolism originates, to some degree, from the failure to take into account the multiple effects of AT. Thus, the small depression in ethanol metabolism sometimes reported after AT administration was found to quantitatively match a moderate inhibition in MEOS activity (Kato et al., 1987). Another study (Handler et al., 1986) reported that 1.5 h after AT administration to decemice, ethanol elimination was inhibited by 85%, while after 6 h, ethanol elimination was almost fully restored. While this contradicts virtually every report on the effects of AT in either decemice (Shigeta et al., 1984; Takagi et al., 1986; Kato et al., 1987) or rats (Kinard et al., 1956; Tephly et al., 1964; Rouche et al., 1972), it does illustrate the kind of problem associated with inhibitors: secondary effects of unknown magnitude are likely. The ADH-decremice offered the possibility of studying non-ADH pathways without the use of inhibitors. Even when ADH is present (in the ADH strain), non-ADH pathways (mostly MEOS) participated significantly in ethanol metabolism at all concentrations tested and played a major role at high levels. These studies are consistent with the recent observation by Kato et al. (1987) that pretreatment of l-butanol, ethanol elimination in ADH-decremice was inhibited by 50% compared to controls. This is the first observation of the effective inhibition of ethanol metabolism by a MEOS inhibitor in vivo, and suggests a significant role for MEOS in intact animals. By determining the fate of 1H from [1-1H]-ethanol, the contribution of non-ADH pathways to ethanol oxidation was found in rats (Takagi et al., 1985; Glassman et al., 1985). The latter observation, however, was not confirmed (Sussick & Zannoni, 1987), nor was there a decrease in blood ethanol disappearance in scorbutic animals (Dow & Goldberg, 1975).

Role of MEOS in the metabolism and toxicity of xenobiotics

Elucidation of the cytochrome-P-450-dependent pathway of ethanol oxidation has improved not only our understanding of the metabolic adaptation to ethanol, but also of a number of interactions of ethanol with xenobiotics. Interaction of alcohol and drugs occurs at many sites, as reviewed elsewhere (Lieber, 1982, 1985). This paper will focus on the
interaction of ethanol with cytochrome-P-450-dependent microsomal drug metabolism (Fig. 3).

**Acute interactions**

The main effect of the presence of ethanol is the inhibition of hepatic drug metabolism (Fig. 3c) as reviewed elsewhere (Lieber, 1982). One mechanism involved appears to be direct competition between ethanol and other drugs for a common metabolic process involving cytochrome P-450 (Lieber, 1982). Ethanol may also interfere with microsomal drug metabolism indirectly by decreasing the supply of NADPH; in addition, it may affect glucuronidation which was found to be inhibited by 50% (Moldeus et al., 1978). In contrast, other drug detoxification processes such as acetylation (Hutchings et al., 1984) and sulphation (Sundheimer & Brendel, 1984) are unaltered by ethanol.

The acute interference of ethanol with drug metabolism may have some important practical consequences. For instance, it has been shown that acute oral administration of ethanol results in prolongation of the clearance of meprobamate from the blood (Rubin et al., 1970). Alcohol acts synergistically with meprobamate to depress performance tasks and driving-related skills such as time estimation, attention, reaction time, body steadiness, ocoulomotor control, and alertness.

Benzodiazepines are the most frequently used minor tranquilizers. Although earlier studies showed no synergism between alcohol and diazepam, the majority of recent investigations clearly indicate that the combined use of these drugs is dangerous. The combination of ethanol and barbiturates represents a particular danger, since the lethal dose for barbiturates is nearly 50% lower in the presence of alcohol than when the drug is used alone (Bogan & Smith, 1967). A
major reason for this is that alcohol inhibits the enzymes that metabolize barbiturates, so that the drugs are present in the blood stream in abnormally high concentrations. Synergism and tolerance may occur directly in the brain tissue as well as in the liver.

Ethanol also interacts with narcotics. Several epidemiologic studies suggest that the combined use of morphine and alcohol potentiates the effects of both drugs and increases the probability of death. Experimentally, acute administration of ethanol and methadone results in increased brain and liver concentrations of the latter (Borowsky & Lieber, 1978). Ethanol also interacts with industrial solvents. Occupational exposure to xylene is widespread in the manufacture and after work. Since the excretion of xylene is delayed by its high solubility and storage in lipid-rich tissues, the simultaneous blood stream in abnormally high concentrations. Synergism and tolerance may occur directly in the brain tissue.

Logic studies suggest that the combined use of morphine and Ethanol also interacts with industrial solvents. Occupational liver concentrations of the latter (Borowsky & Lieber, 1978) result in an alteration of ethanol and methadone, resulting in increased brain and liver concentrations of the latter (Borowsky & Lieber, 1978). Ethanol also interacts with industrial solvents. Occupational exposure to xylene is widespread in the manufacture and after work. Since the excretion of xylene is delayed by its high solubility and storage in lipid-rich tissues, the simultaneous blood stream in abnormally high concentrations. Synergism and tolerance may occur directly in the brain tissue.

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Although most acute ethanol–xenobiotic interactions involve alterations in the disposition of such compounds by ethanol, the converse may also occur. For instance, it has been reported that ethanol metabolism may be affected by oral administration of cimetidine at therapeutic doses (Feely & Sager, 1982) and indeed, cimetidine was found to inhibit gastric ADH activity and thereby to enhance bioavailability of ethanol (Caballeria & Lieber, 1987).

Chronic interactions

Tolerance. It is well known that chronic alcohol abusers develop tolerance to ethanol. Such tolerance is due, in part, to central nervous system adaptation (pharmacodynamic tolerance). In addition, there is metabolic tolerance that results from an increased capacity to oxidize ethanol. This occurs, in part, because of the adaptive increase in activity of non-ADH pathways involved in ethanol metabolism, most likely MEOS, as discussed before (Fig. 3d).

In addition to tolerance to ethanol, alcoholics tend to display other enzyme adaptations, which has generally been attributed to central nervous system adaptation. However, metabolic adaptation must also be considered. Indeed, the induction of MEOS activity after chronic alcohol consumption ‘spills over’ to various other drug-metabolizing systems in liver microsomes, thereby accelerating drug metabolism in general (Fig. 3e). As a consequence, the administration of ethanol to volunteers under metabolic ward conditions resulted in a striking increase in the rate of blood clearance of mebrobamate and pentobarbital (Misra et al., 1971). Similarly, increases in the metabolism of aminopyrine, tobutamidine, propranolol, and rifampicin due to ethanol administration have been described. Experimentally, this effect of chronic ethanol consumption may be modulated in part by the dietary content in carbohydrates (Teske et al., 1981), lipids (Joly & Hetu, 1975), and proteins (Mitchell et al., 1981).

Hepatotoxicity. Chronic alcohol intake has been shown to enhance susceptibility to hepatotoxic agents: the general increase in microsomal enzyme activities also applies to those that convert exogenous substrates to toxic compounds and it particularly affects those xenobiotics for which the ethanol-inducible form of cytochrome P-450 (P-450IIE1) has a high affinity. For instance, CCI4, exerts its toxicity after conversion to an active metabolite in the microsomes. As discussed before, P-450IIIE1 is particularly active in that regard and indeed alcohol pretreatment markedly lowers the CCI4 hepatotoxicity (Hasumura et al., 1974). Thus, the clinical observation of the enhanced susceptibility of alcoholics to the hepatotoxic effect of CCI4 may be due, at least in part, to increased metabolic activation of this compound. Liver toxicity of bromobenzene was also found to increase following chronic alcohol consumption (Hetu et al., 1983). It is likely that a large number of other toxic agents of this nature will be found to display a selective injurious action in the alcoholic patient. This pertains not only to industrial solvents, but also to a variety of prescribed drugs. For instance, the increased hepatotoxicity of isoniazid observed in alcoholics may well be due to increased microsomal production of an active metabolite of the acetyl derivative of the drug (Timbrell et al., 1980). The capacity of isoniazid to induce the ethanol-specific form of cytochrome P-450 has been noted before. Similarly, chronic ethanol administration enhances phenylbutazone hepatotoxicity, possibly because of increased biotransformation (Beskid et al., 1980).

The above mechanism underlying hepatotoxicity also pertains to some over-the-counter medications. Acetaminophen (paracetamol, N-acetyl-p-aminophenol), widely used as an analgesic and an antipyretic, can be metabolized by the microsomal cytochrome P-450 system; the latter bio-transformation yields an active metabolite highly toxic to the liver. The practical implications are obvious in view of the widespread use of drugs known to be inducers of microsomal drug-metabolizing activities. Because alcohol induces microsomal drug-metabolizing systems, it was to be expected that a history of alcohol consumption might favour the hepatotoxicity of acetaminophen; this has been suggested by various case reports (Wright & Prescott, 1973; Seel et al., 1986). Experimentally, enhanced covalent binding of reactive metabolite(s) of acetaminophen to liver microsomes from ethanol-fed rats was observed to be associated with increased hepatotoxicity (Sato et al., 1981). Furthermore, in a reconstituted system, P-450IIIE1 was found to have a higher capacity to oxidize acetaminophen (as discussed above) than the C7P-450 form, which readily formed a conjugate with reduced glutathione (Morgan et al., 1983). For all these reasons, it is likely that the enhanced hepatotoxicity of acetaminophen observed after chronic ethanol consumption is due, at least in part, to increased microsomal production of reactive metabolite(s) of this drug. However, experimentally, unlike pretreatment with alcohol, which (as discussed above) accelerates the rate of metabolism, the presence of ethanol in part prevents the acetaminophen-induced hepatotoxicity, most likely because of inhibition of the bio-transformation of acetaminophen to reactive metabolites (Sato & Lieber, 1981; Altomare et al., 1984a,b). Thus, the greatest vulnerability of the alcoholic to acetaminophen is not necessarily during drinking, when ethanol may compete with acetaminophen for its microsomal metabolism, but rather after alcohol withdrawal, at which time there is no inhibitory effect of alcohol anymore, but the induction of the microsomal metabolism still persists.

Activation of carcinogens. Alcohol abuse is associated with an increased incidence of upper alimentary and respiratory tract cancers and many factors have been incriminated in the co-carcinogenic effect of ethanol (Lieber et al., 1986). One of the mechanisms is the effect of ethanol on enzyme systems involved in cytochrome-P-450-dependent carcinogen activation. Ethanol has a unique effect on the chemical carcinogen NDMA: it induces a microsomal NDMA demethylase which functions at low NDMA concentrations (Garro et al., 1981). This is in contrast to other microsomal enzyme indu-
cers such as phenobarbital, 3-methylcholanthrene and polychlorinated biphenyls. These compounds induce other NDMA demethylases — the activity of which is detectable only at relatively high NDMA concentrations — while repressing the activity of low Km NDMA demethylases (Guttmann & Garon, 1977). Some of these effects may be due to the induction of a unique species of cytochrome P-450 by ethanol, which differentially affects the activation of various carcinogens; indeed a selective affinity for NDMA has been demonstrated with the ethanol-inducible form of cytochrome P-450 (Yang et al., 1985). Alcohols are also commonly heavy smokers and epidemiologically, a synergistic effect of alcohol consumption and smoking has been described, as reviewed elsewhere (Lieber et al., 1986). Interactions at various levels are possible: chronic ethanol consumption was found to enhance the mutagenicity of tobacco pyrolysates (Garro et al., 1981). More recently benzoflavone, a tobacco-like inducer, was also found to induce a liver cytochrome P-450 which is structurally different, but catalytically similar to P-450EII. This new P-450 could be involved in some of the pathological effects associated with combined habitual alcohol and tobacco use (Lasker et al., 1987b).

**Steroids and vitamins.** Ethanol also affects microsomal metabolism of exogenous and endogenous steroids, as discussed in detail elsewhere (Lieber, 1982); the effects include enhanced testosterone degradation and conversion to oestrogens, as well as decreased testicular steroid synthesis. Furthermore, ethanol alters the metabolism of structurally related vitamins, such as vitamin D (Gascon-Barre, 1982). These and other micronutrients may serve as substrates for the microsomal enzymes; the induction of the microsomal oxidative activities may therefore alter vitamin requirements and even affect the integrity of liver and other tissues. It has been known that vitamin A as well as retinol-binding protein levels (McClain et al., 1979) are decreased in patients with alcoholic cirrhosis. These complications have usually been attributed to malnutrition or to hepatic injury. However, it has been found that already at the early fatty liver stage, alcohols commonly have very low hepatic vitamin A concentration despite normal circulating vitamin A levels and the absence of obvious dietary vitamin A deficiency (Leo & Lieber, 1982). In experimental animals, ethanol administration was shown to depress hepatic vitamin A levels, even when administered with diets containing adequate amounts of vitamin A (Sato & Lieber, 1981). When dietary vitamin A was virtually eliminated, the depletion rate of hepatic vitamin A stores was two to three times faster in ethanol-administered rats (Sato & Lieber, 1981) than normal (Leo et al., 1982). Hepatic vitamin A depletion neverthe-
The interrelationships of alcohol dehydrogenase and the aldehyde dehydrogenases in the metabolism of ethanol in liver

MICHAEL C. HARRINGTON, GARY T. M. HENEHAN and KEITH F. TIPTON
Department of Biochemistry, Trinity College, Dublin 2, Republic of Ireland

The oxidation of ethanol in liver yields acetaldehyde which is then further oxidized by the action of aldehyde dehydrogenase (aldehyde: NAD oxidoreductase, EC 1.2.1.3) to form acetate. Acetaldehyde may be involved in a number of the effects of ethanol (for review see Jenkins & Thomas, 1981).

In liver, which is the predominant site of ethanol metabolism, aldehyde dehydrogenase has been shown to be present in multiple forms with different substrate specificities, subcellular locations and inhibitor sensitivities (for review see Forte-Robert & Pietruszko, 1985) and the absence of one of these has been shown to result in the alcohol-sensitivity, 'flushing' reaction (see Goedde et al., 1979, 1985).

There has been some controversy about the contributions of the different forms of the enzyme to the metabolism of the acetaldehyde derived from ethanol in liver. Our own studies (Harrington et al., 1987; Tipton et al., 1981) and those of others (Parilla et al., 1974; Rognstad & Clark, 1974; Corrall et al., 1976; Dawson, 1983) indicate that it is the form of the enzyme associated with the mitochondrial matrix which will play the major role in this process. In human liver, however, it has been suggested that a form present in the cytosol may also be involved to a significant extent in acetaldehyde metabolism (Jenkins & Peters, 1980; Jenkins & Thomas, 1981).

Table 1 compares the kinetic parameters towards acetaldehyde of the different aldehyde dehydrogenase activities detected in subcellular fractions prepared from human liver (Harrington et al., 1987). The very much higher value of the apparent specificity constant, V/K<sub>n</sub>, shown by one of the mitochondrial forms of the enzyme indicates that its activity would predominate in the oxidation of acetaldehyde under physiological conditions. Mitochondrial fractionation studies in other tissues (Tottmar et al., 1973; Tipton et al., 1981) have indicated that the form of the enzyme with the low K<sub>n</sub> value towards acetaldehyde is associated with the mitochondrial matrix, whereas the other form is bound to the outer membrane of that organelle.

Such interpretations of enzyme kinetic behaviour are based on the assumption that all forms of the enzyme are equally saturated with the coenzyme, NAD<sup>+</sup>, that the acetaldehyde formed is equally accessible to them and that product inhibition or other possible effectors do not differentially affect their activities. They would, however, be consistent with the observation that the alcohol-sensitivity reaction is associated with individuals lacking the low-K<sub>n</sub> mitochondrial form of the enzyme (Yoshida et al., 1984; Goedde et al., 1985).

The functions of the other aldehyde dehydrogenase forms present in liver are still not completely clear (see Tipton et al., 1987). However, the cytoplasmic enzyme appears to be involved in the metabolism of the aldehydes derived from the metabolism of a number of physiologically important amines including the catecholamines (Harada et al., 1982; Helander & Tottmar, 1986) and histamine (Henehan et al., 1985; Gitomer & Tipton, 1983). It has been suggested that interference with this process, either by direct competition between aldehydes or by reduction in the availability of oxidized NAD, may account for some of the adverse reactions seen when individuals lacking the mitochondrial form of the enzyme (Goedde et al., 1979, 1985) or treated with an aldehyde dehydrogenase inhibitor, such as disulfiram (Harada et al., 1982b) or cyanamide (Deitrich et al., 1976), ingest ethanol (Gitomer & Tipton, 1983; Henehan et al., 1985).

In an attempt to define the roles of the different aldehyde dehydrogenases more directly we have extended the approach, developed by Dawson (1983), of studying the metabolism of ethanol in subcellular fractions prepared from rat liver by the method of deDuve et al. (1955). Fractions were incubated, at 37°C with 30 mM-[1-14C]ethanol in a medium containing (mM): 210, sucrose; 14, KCl; 12, sodium pyruvate; 10, glucose; 8, potassium phosphate buffer; 3.8 Hepes buffer; 2.5, ADP; 1.5, MgCl<sub>2</sub>; 3 u. of hexokinase and 0.6, NAD<sup>+</sup>, at a pH of 7.2. The metabolites produced after 60 min are summarized in Table 2. No significant formation of NADH occurred during this time and the oxidation of ethanol can be seen to have been accompanied by a matching conversion of the added pyruvate to lactate to regenerate the NAD<sup>+</sup> reduced. Analysis of the non-volatile labelled material produced during the reaction showed that over 95% was acetate.

In cytosol alone, ethanol metabolism occurred at a rate of 8.66 ± 0.83 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> (mean ± S.E.M., n = 6).

Table 1. Kinetic parameters of aldehyde dehydrogenase activities in subcellular fractions from human liver

<table>
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<tr>
<th>Fraction</th>
<th>k&lt;sub&gt;n&lt;/sub&gt; (mM)</th>
<th>V&lt;sub&gt;n&lt;/sub&gt; (nmol min&lt;sup&gt;-1&lt;/sup&gt; mg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>V&lt;sub&gt;n&lt;/sub&gt;/K&lt;sub&gt;n&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt; mg&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<td>Cytosol</td>
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<td>Microsomes</td>
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