Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 181–187, 1983. © Ankho International. Printed in the U.S.A.

Microsomal Ethanol Oxidizing System (MEOS): Interaction with Ethanol, Drugs and Carcinogens[1]

CHARLES S. LIEBER

Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine (CUNY), New York, NY 10468

LIEBER, C. S. *Microsomal ethanol oxidizing system (MEOS): Interaction with ethanol, drugs and carcinogens.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 181–187, 1983.—Several studies in our unit showed that in men, baboons, rats and deermice, blood ethanol clearance is significantly accelerated at ethanol concentrations higher than the levels needed to effectively saturate the low K_m forms of ADH present in animals, thereby incriminating a high K_m non-ADH system such as microsomal ethanol oxidizing system (MEOS). Furthermore, kinetics of blood ethanol clearance were consistent with the K_m of MEOS. After chronic ethanol consumption, there was an increase in rates of ethanol elimination and in the activity of MEOS. There was an associated rise in microsomal cytochrome P-450, including a form (different from that induced by phenobarbital and methylcholanthrene) which had a high affinity for ethanol in a reconstituted system. The role of a non-ADH pathway of ethanol metabolism and its increase after chronic ethanol consumption was most conclusively shown in ADH-negative deermice. Microsomal induction was also associated with enhanced metabolism of other drugs, resulting in metabolic drug tolerance. In addition, there was increased activation of known hepatotoxic agents (such as CCl_4 and acetaminophen) which may explain the enhanced susceptibility of alcoholics to the toxicity of solvents and commonly used drugs. There was enhanced activation of procarcinogens, sometimes at concentrations much lower than those required for other microsomal inducers. Moreover, catabolism of retinoic acid was accelerated possibly contributing to hepatic vitamin A depletion. In conclusion, after chronic ethanol consumption, enhanced MEOS activity and concomitant cytochrome P-450 changes may contribute to accelerated ethanol and drug metabolism and associated activation of hepatotoxic agents and carcinogens.

Acetaminophen Carcinogens CCl_4 Drugs Ethanol Hepatotoxic agents
Microsomal ethanol oxidizing system Microsomes Retinoic acid Tolerance (metabolic) Vitamin A

MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)

Discovery of MEOS and Chemical Characterization of MEOS-Mediated Ethanol Oxidation

The first indication of an interaction of ethanol with the microsomal fraction of the hepatocyte was provided by the morphologic observation that in rats, ethanol feeding results in a proliferation of the smooth endoplasmic reticulum (SER) [24,25]. This observation raised the possibility that, in addition to its oxidation by ADH in the cytosol, ethanol may also be metabolized by the microsomes. A microsomal system capable of methanol oxidation had been described [57], but its capacity for ethanol oxidation was extremely low. Furthermore, this system could not oxidize long-chain aliphatic alcohols such as butanol and was sensitive to the catalase inhibitors azide and cyanide. Therefore, Ziegler concluded that this system is clearly different from the cytochrome P-450 dependent system and involves the H_2O_2-mediated ethanol peroxidation by catalase [93]. However, a microsomal ethanol oxidizing system with a rate of ethanol oxidation ten times higher than reported by Orme-Johnson and Ziegler [57] was described [40, 41, 47]. The system required NADPH and O_2 and was relatively insensitive to catalase inhibition. Furthermore, the MEOS was differentiated from the system reported by Orme-Johnson and Ziegler [57] and from catalase by its ability to oxidize long-chain aliphatic alcohols [81], which are not substrates for catalase. The striking increase in the non-ADH fraction of ethanol metabolism with increasing ethanol concentrations [19, 49, 84] is consistent with the known K_m for MEOS. The in vitro K_m of MEOS agrees well with the corresponding value of the pyrazole-insensitive pathway of 9 mM in vivo [43] and with a similar value in isolated hepatocytes [48].

Differentiation of MEOS from ADH and Catalase

Differentiation of MEOS in total microsomes from alcohol dehydrogenase was achieved by subcellular localization, pH optimum in vitro (7.4 versus 10), cofactor requirements, and effects of inhibitors such as pyrazole [44,46]. Studies

[1]Original studies reported here were supported by the Veterans Administration and DHHS grant AA-03508.

with inhibitors have also indicated that a major fraction of the ethanol oxidizing activity in microsomes is independent of catalase [41, 44, 46]. A clear dissociation of the NADPH-dependent from an H_2O_2-mediated ethanol oxidation in microsomes was observed using pyrazole, which inactivates catalase in vivo [46], and azide, which inhibits catalase in vitro [41]. Thus, under experimental conditions with complete abolition of the peroxidatic activity of catalase, the NADPH-dependent ethanol oxidation still proceeded at a significant rate; this again dissociates the NADPH-dependent MEOS activity from a process involving catalase-H_2O_2. Subsequently, MEOS was solubilized and separated from alcohol dehydrogenase and catalase activities by diethylaminoethyl cellulose column chromatography [50, 79, 80]. Differentiation of MEOS from ADH in the column fractions was shown by the failure of NAD to promote ethanol oxidation at pH 9.6, by cofactor requirements (NADPH and O_2), by the apparent K_m for ethanol (7 to 9 mM), and by the insensitivity of MEOS to the ADH inhibitor pyrazole. MEOS was also distinguished from a process involving catalase-H_2O_2 by the lack of catalatic activity, by the apparent K_m for oxygen (8.3 μM), by the insensitivity to the catalase inhibitors azide and cyanide, and by the inability of an H_2O_2 generating system (glucose-glucose oxidase) to sustain ethanol oxidation in the isolated column fraction [80]. Thus, using specific and sensitive methods, MEOS activity could be clearly differentiated from an enzymatic process involving peroxidatic activity of catalase. In addition, MEOS activity could be dissociated from microsomal NADPH oxidase activity [22], which generates H_2O_2 in microsomes [16].

It has also been reported that microsomes from acatalasemic mice fail to oxidize ethanol [88], but this claim has been subsequently retracted [89]. Indeed, hepatic microsomes of acatalasemic mice subjected to heat inactivation displayed decreased catalatic activity, but NADPH-dependent MEOS remained active and unaffected [45,82]. Even without heat inactivation, in the acatalasemic strain, the NADPH-dependent metabolism was much more active than the H_2O_2-mediated one, whereas microsomes of control mice displayed equal rates of H_2O_2-and NADPH-dependent ethanol oxidation [89]. These results therefore support the conclusion that hepatic microsomes of normal and acatalasemic mice contain a NADPH-mediated ethanol oxidizing system that is catalase-independent.

Nature of MEOS Activity

The reconstitution of the ethanol oxidizing activity with the three microsomal components cytochrome P-450, NADPH- cytochrome c reductase, and lecithin was demonstrated [54]. Successful reconstitution of MEOS was confirmed by Miwa and coworkers [52].

The activity of the reconstituted microsomal ethanol oxidizing system (reconstituted MEOS) showed a dependency upon cytochrome P-450 and the reductase and required synthetic phospholipids (such as lecithin) for its maximal activity. The K_m of the reconstituted MEOS for ethanol was 10 mM, which is similar to the K_m measured in crude microsomes and the MEOS fraction isolated by column chromatography [40, 41, 80]. This reconstituted system required NADPH as a cofactor, did not react to an H_2O_2 generating system, and was insensitive to the catalase inhibitor azide. These characteristics were also similar to those observed in crude microsomes. The activity was dependent on the amount of cytochrome P-450 present. The involvement of reduced cytochrome P-450 in MEOS was further shown by action spectra [13]. How MEOS mediates ethanol oxidation is still the subject of debate. Part of the mechanism could involve generation of hydroxyl radicals, which in turn might be "scavenged" by ethanol, since other compounds that interact with hydroxyl radicals inhibit MEOS activity [5,56]. In addition to cytochrome P-450, ethanol can also be oxidized by NADPH-cytochrome P-450 reductase. It has been shown by Ohnishi and Lieber [54] that the reductase mediated ethanol oxidation is much less than the one catalyzed by the ethanol induced form (used in saturating amounts), but is equal to the oxidation catalyzed by non induced P-450. It has also been shown that superoxide dismutase inhibits the reductase mediated reaction (Winston *et al.* unpublished observation). Indeed, there is now evidence that the reductase operates via the hydroxyl radical mechanism [91] and therefore can be expected to be dismutase sensitive whereas the P-450 mechanism (that is inducible by ethanol) can be expected to be dismutase insensitive. Thus, when one uses non-induced cytochrome P-450 [23], the reductase mediated inhibition by dismutase may be more apparent then when the more active ethanol induced cytochrome P-450 [56] is used.

RELATIVE IMPORTANCE OF ADH AND NON-ADH PATHWAYS

There are now several lines of evidence indicating that a non-ADH pathway significantly contributes to ethanol oxidation in the liver. This includes the incomplete inhibition of ethanol metabolism using ADH inhibitors and the pattern of labeling of acetaldehyde derived from stereospecific labeled ethanol [65] and the increased rate of ethanol metabolism at high ethanol concentrations well above those needed to saturate alcohol dehydrogenase.

Actually, estimates of the magnitude of the non-ADH pathway obtained by measurements of residual ethanol metabolism after inhibition with the ADH inhibitor pyrazole or 4-methyl-pyrazole are underestimations, in view of the fact that these inhibitors also reduce the activity of the microsomal alcohol oxidizing system [83]. This is also illustrated by the fact that Rognstad [64], using isotopic probes to assess rates of ethanol metabolism, found the contribution of the non-ADH pathway to be 35 per cent under conditions that yielded somewhat lower results in the presence of pyrazole. In comparing the rate of utilization of deuterated and non-deuterated ethanol by liver cells, Rognstad concluded not only that a significant non-ADH pathway exists, but also that it is unlikely that catalase contributes to its operation.

Increasing ethanol metabolism with rising ethanol concentrations was found not only in the presence of an ADH inhibitor but also in its absence, in isolated hepatocytes [19,49], in isolated perfused livers [17], and in vivo in man [14, 37, 69, 70], in rats [14], and in baboons [58, 69, 70].

The fact that ethanol metabolism increases with rising ethanol concentrations well above the level needed to fully saturate ADH suggests the involvement of a non-ADH pathway at least in those species (rat, baboon) devoid of the anodic high-K_m ADH. Moreover, the acceleration of ethanol metabolism at higher ethanol concentrations explains sporadic observations, which now have been confirmed [14, 37, 69, 70], that ethanol disappearance from the blood is not linear at high ethanol concentrations that fully saturate the

ADH pathway. Finally, the persistence of a substantial rate of ethanol metabolism in deermice congenitally devoid of ADH, in association with a high MEOS activity [3,78], illustrates most elegantly the in vivo importance of non-ADH ethanol metabolism.

ALTERATION IN THE METABOLISM OF ETHANOL AFTER CHRONIC ETHANOL CONSUMPTION

Regular drinkers tolerate large amounts of alcoholic beverages, mainly because of central nervous system adaptation. In addition, alcoholics develop increased rates of blood ethanol clearance, that is, metabolic tolerance [32,86]. Experimental ethanol administration also results in an increased rate of ethanol metabolism [14, 41, 51, 58, 85]. The progressive acceleration of ethanol metabolism after chronic ethanol consumption is not to be confounded with the rise that occurs after an acute dose of ethanol (the so-called "swift increase" in alcohol metabolism), which appears to result from a stress-associated adrenalin discharge [92]. The mechanism of the chronic acceleration is still the subject of discussion. The contribution of ADH-related acceleration of ethanol metabolism is discussed in detail by others in these proceedings. Therefore I shall focus here on the non-ADH related acceleration of ethanol metabolism.

Following chronic ethanol consumption, MEOS significantly increases in activity [40,41]. This is associated with an increase in various constituents of the smooth fraction of the membranes involved in drug metabolism, such as phospholipids, cytochrome P-450 reductase, and cytochrome P-450 [26, 28, 71]. The increase of cytochrome P-450 was associated with the appearance of a distinct form of cytochrome P-450. A cytochrome P-450 species showing high affinity for cyanide has been reported to be preferentially induced by ethanol [9, 22, 27]. Evidence in favor of an increase of a special species of cytochrome P-450 after ethanol treatment was also derived from inhibitor studies [87]. More direct proof was obtained from studies of microsomal proteins [55]. The rise in cytochrome P-450 involves a hemoprotein different from those induced by phenobarbital or 3-methylcholanthrene treatment both in rats [55] and in rabbits [33]. The partially purified cytochrome P-450 from ethanol-fed rats was more active for alcohol oxidation than the control preparation in the presence of an excess of NADPH-cytochrome c reductase and L-α-dioleoyl lecithin [55]. There was no significant difference in the capacity of partially purified NADPH-cytochrome c reductase from either ethanol-fed rats or controls to promote ethanol oxidation in the presence of cytochrome P-450 and L-α-dioleoyl lecithin [55].

Studies by Joly and coworkers [29,30], also showed that chronic ethanol administration to rats is associated with the appearance of a form of cytochrome P-450 with spectral and catalytic properties different from those of the cytochrome P-450 of control, phenobarbital-treated, and methylcholanthrene-treated rats.

That chronic ethanol feeding results in an increased activity in liver tissue of a non-ADH and non-catalase pathway was also shown in liver slices and in isolated hepatocytes. Ethanol oxidation was enhanced in isolated liver tissue by increasing the ethanol concentration employed in vitro from 10 to 30 mM. Of particular interest was the observation that this phenomenon was more pronounced in ethanol-fed rats than in their pair-fed controls [83]. To test whether or not MEOS is involved in this adaptive increase, ADH and catalase activities were inhibited by pyrazole and sodium azide respectively. The activity of the non-ADH and non-catalase pathway, which is most likely due to MEOS, was significantly higher in ethanol-fed rats than in controls. In addition, the difference between the two groups was more striking at 30 mM than at 10 mM. Similarly, when a relatively constant blood ethanol level is maintained through continuous infusion in the baboon, the acceleration of ethanol metabolism with higher blood levels is more pronounced in alcohol-fed than in control animals [58]. All these data indicate that a non-ADH pathway, most likely MEOS, represents a major mechanism for the acceleration of ethanol metabolism at high ethanol concentrations. A similar change was shown in man: in volunteers, alcohol consumption resulted in a progressive acceleration of blood ethanol clearance, particularly at high ethanol concentrations [70].

INTERACTION OF ETHANOL WITH CYTOCHROME P- 450-DEPENDENT MICROSOMAL DRUG METABOLISM

The microsomal ethanol oxidizing system (MEOS) shares with other microsomal drug metabolizing systems many properties, including utilization of cytochrome P-450, NADPH, and O_2. This sharing explains many alcohol-drug interactions. Furthermore, the induction of MEOS by chronic alcohol consumption is associated with corresponding increases in activity of other microsomal drug-metabolizing systems, with important clinical sequelae.

Acute Interactions

The main effect of the acute presence of ethanol is inhibition of drug metabolism. The main mechanism involved appears to be competition for a partially common detoxification process including competition for binding with cytochrome P-450 [68]. Other mechanisms could include the release of steroid hormones that may inhibit some microsomal drug metabolizing enzymes [6]. In some instances, the NADH generated by the oxidation of ethanol might also inhibit citric acid cycle activity [38] and possibly deplete intermediates that might be necessary for the generation of cytosolic NADPH [62].

The complexity of ethanol-drug interactions at the microsomal level is exemplified by the fact that for some drug metabolizing systems (such as aniline hydroxylase), inhibition occurs at low ethanol concentrations, whereas for others (such as aminopyrine demethylase) high ethanol concentrations are required [67]. Furthermore, in the latter case, low ethanol concentrations were even stimulatory [7], possibly because of enhanced NADH and the likelihood that NADH may serve as an electron donor for microsomal drug-detoxifying systems [8].

Chronic Interaction: Enhanced Drug Metabolism (Metabolic Drug Tolerance)

Repeated ethanol administration results in increased activities of a variety of microsomal drug-detoxifying enzymes [1, 4, 28, 51, 66]. On some occasions, some effects are already observed after a single ethanol dose [59]. The increase in the activity of hepatic microsomal drug-detoxifying enzymes and in the content of cytochrome P-450 induced by ethanol ingestion offers a likely explanation for the observation that ethanol consumption enhances the rate of drug clearance in vivo. Indeed, it has been shown that the rate of drug clearance from the blood is enhanced in alcoholics [31]. Of course, this could be caused by a variety of factors other than ethanol, such as the congeners and the use of other drugs

so commonly associated with alcoholism. Controlled studies showed, however, that administration of pure ethanol with non-deficient diets either to rats or man (under metabolic ward conditions) resulted in a striking increase in the rate of blood clearance of meprobamate and pentobarbital [51]. Similarly, increases in the metabolism of aminopyrine [90], propranolol [60] and rifamycin [18] were found. Furthermore, the capacity of liver slices from animals fed ethanol to metabolize meprobamate was also increased [51], which clearly showed that ethanol consumption affects drug metabolism in the liver itself, independent of drug excretion or distribution or hepatic blood flow.

Thus, a recovering alcoholic's previous history of alcohol abuse can be a key factor in prescribing decisions, because even after withdrawal alcoholics need doses different from those required by non-drinkers to achieve therapeutic levels of certain drugs, such as warfarin, diphenylhydantoin, tolbutamide, and isoniazid as well as a number of sedatives and tranquilizers.

AGGRAVATION OF HEPATOTOXICITY THROUGH MICROSOMAL "INDUCTION"

Accelerated microsomal ethanol metabolism results in enhanced production of acetaldehyde and exacerbation of its various toxic manifestations discussed in detail elsewhere [39]. The damage may possibly be compounded by the increased generation of active radicals by the "induced" microsomes following chronic ethanol consumption. It is well known that the microsomal pathway, which requires O_2 and NADPH, is capable of generating lipid peroxides. Enhanced lipid peroxidation, possibly mediated by acetaldehyde [12], has been proposed as a mechanism for ethanol-induced fatty liver [11], but its role has been controversial [2, 10, 20, 74]. Theoretically, increased activity of microsomal NADPH oxidase following ethanol consumption [42,63] could result in enhanced H_2O_2 production, thereby also favoring lipid peroxidation.

Increased microsomal activity may also enhance the oxygen requirements, thereby aggravating whatever hypoxia may be present. Furthermore, some compounds acquire hepatotoxicity only after metabolism or "activation," by the enzymes of the endoplasmic reticulum. One such compound is carbon tetrachloride, the hepatotoxicity of which is greatly increased after chronic alcohol consumption, at least partly because of enhanced activation by microsomes [21].

It is likely that a larger number of other toxic agents will be found to display a selective injurious action in the alcoholic. This pertains not only to industrial solvents, but also to a variety of prescribed drugs. For instance, the observed increased hepatotoxicity of isoniazid in alcoholics [53] may well be a result of increased production by the microsomes of an active metabolite of the acetyl derivative of the drug.

The same mechanism of hepatotoxicity also pertains to some "over the counter" medications. Acetaminophen (paracetamol, N-acetyl-p-aminophenol), widely used as an analgesic and an antipyretic, is generally safe when taken in recommended doses. However, acetaminophen in large doses (that is, in cases of suicide attempts) has been shown to produce fulminant hepatic failure. Scott and Stewart [75] noted that "the majority of cases of paracetamol overdose are accompanied by some alcohol." Experimentally, after chronic ethanol feeding, enhanced covalent binding of reactive metabolite(s) of acetaminophen to microsomes from ethanol-fed rats was observed. These alterations were associated with the enhanced hepatotoxicity in ethanol-fed rats as evidenced by light and electronmicroscopy as well as increased serum glutamic oxaloacetic transaminase levels and glutamic dehydrogenase levels in the blood. However, to what extent ethanol, when still present, competes with microsomal metabolism of acetaminophen remains to be evaluated clinically. Experimentally, unlike pretreatment with alcohol, which accentuates toxicity, the presence of ethanol in fact prevented the acute acetaminophen-induced hepatotoxicity, most likely because of the inhibition of the biotransformation of acetaminophen to reactive metabolites [73]. In any event, it is also likely that various other compounds, which are relatively innocuous in the normal individual, may exhibit enhanced toxicity in the alcoholic. This may include some potential carcinogens. It has been shown that, at least in female rats, chronic ethanol consumption results in increased hepatic microsome-mediated mutagenicity of benzo(a) pyrene [76]. It was also shown that chronic alcohol consumption increases intestinal activation of a variety of procarcinogens and mutagens [77]. Chronic ethanol consumption also enhances the hepatic metabolism and activation of diemethylnitrosamine [15]. The potential relationship of the effect of chronic ethanol consumption on microsomal enzymes, chemical mutagenesis and carcinogenesis and the observed enhanced incidences of cancer in the alcoholic have been recently reviewed [47].

Micronutrients such as vitamins may also serve as substrates for the microsomes and the "induction" of the microsomes may therefore alter vitamin requirements and even affect the integrity of the liver. Indeed it has been found recently that alcoholics commonly have very low vitamin A levels in their livers [34]. In experimental animals, ethanol administration was shown to depress hepatic vitamin A levels, even when administered with adequate diets [72]. When dietary vitamin A was virtually eliminated, the rate of depletion of vitamin A from endogenous hepatic storage was two to three times faster in ethanol-fed rats than in controls, possibly because of accelerated degradation of retinoic acid by the induced microsomes [73]. In rats, severe vitamin A depletion was associated with the appearance of multivesicular lysosomes [35]. Such lesions were commonly seen in alcoholic patients with low hepatic vitamin A levels. Thus, vitamin A depletion may contribute to the liver lesions of the alcoholic. Vitamin A supplementation is sometimes used to correct the problems of night blindness and sexual dysfunctions of the alcoholic. Such therapy might also be useful with regard to the liver pathology. The therapeutic usage of vitamin A, however, is complicated by the fact that excessive amounts of vitamin A are known to be hepatotoxic and that the alcoholic has an enhanced susceptibility to this effect [36]. In control rats, amounts of vitamin A equivalent to those commonly used for the treatment of the alcoholic were found to be without significant effects on the liver, but in animals chronically fed alcohol, signs of toxicity developed, such as striking morphologic and functional alterations of the mitochondria [36]. Enhanced toxicity was not associated with an increased vitamin A level in the liver. In fact, because alcohol administration tends to decrease vitamin A levels in the liver, even after vitamin A supplementation, alcohol-fed animals had vitamin A levels in the liver that were not higher than normal values. Nevertheless, toxicity developed. One possible explanation, still to be proven, is that vitamin A toxicity may be mediated at least in part by the enhanced production of a toxic metabolite, as in the case of xenobiotic agents.

CONCLUSIONS

Ethanol oxidation, once thought to be a simple, one enzyme mediated reaction, has now been shown to be a complex process affected by various enzyme systems. Even after ADH inhibition or in animals genetically deficient in ADH, substantial ethanol oxidation persists, apparently reflecting primarily the activity of a microsomal ethanol oxidizing system (MEOS). The latter has now been shown to be distinct from ADH and catalase, and it has been reconstituted with purified cytochrome P-450, NADPH-cytochrome P-450 reductase and synthetic phospholipids. The activity requires O_2 and NADPH dependent, and is inhibited by CO, a light reversible effect. After chronic ethanol consumption, the activity of MEOS rises, with increase of a species of cytochrome P-450 distinct from that induced by other drugs. This is associated with accelerated ethanol metabolism, particularly at high ethanol concentrations, consistent with the high K_m for MEOS (8–10 mM). In addition to this role in the development of metabolic tolerance to ethanol, recognition of the microsomal effects of ethanol have contributed to our understanding of the acute and chronic interactions between ethanol and the metabolism of other microsomal substrates such as drugs, steroid hormones, hepatotoxic agents, carcinogens and even some nutrients such as vitamin A.

REFERENCES

1. Ariyoshi, T., E. Takabatake and H. Remmer. Drug metabolism in ethanol-induced fatty liver. *Life Sci* **9:** 361–369, 1970.
2. Bunyan, J., M. A. Cawthrone, A. T. Diplock and J. Green. Vitamin E and hepatotoxic agents. 2. Lipid peroxidation and poisoning with orotic acid, ethanol and thioacetamide in rats. *Br J Nutr* **23:** 309–317, 1969.
3. Burnett, K. G. and M. R. Felder. Ethanol metabolism in Peromyscus genetically deficient in alcohol dehydrogenase. *Biochem Pharmacol* **28:** 1–8, 1980.
4. Carulli, N., F. Manenti, M. Gallo and G. F. Salviolli. Alcohol-drugs interaction in man: Alcohol and tolbutamide. *Eur J Clin Invest* **1:** 421–424, 1971.
5. Cederbaum, A. I., E. Dicker, E. Rubin and G. Cohen. The effect of dimethylsulfoxide and other hydroxyl radical scavengers on the oxidation of ethanol by rat liver microsomes. *Biochem Biophys Res Commun* **78:** 1254–1262, 1977.
6. Chung, H. and D. R. Brown. Mechanism of the effect of acute ethanol on hexobarbital metabolism. *Biochem Pharmacol* **25:** 1613–1616, 1976.
7. Cinti, D. L., R. Grundin and S. Orrenius. The effect on drug oxidation in vitro and the significance of ethanol-cytochrome P-450 interaction. *Biochem J* **134:** 367–375, 1973.
8. Cohen, B. S. and R. W. Estabrook. Microsomal electron transport reactions III. Cooperative interactions between reduced diphosphopyridine nucleotide and reduced triphosphophyridine nucleotide-linked reactions. *Arch Biochem Biophys* **143:** 54–65, 1971.
9. Comai, K. and J. L. Gaylor. Existence and separation of three forms of cytochrome P-450 from rat liver microsomes. *J Biol Chem* **248:** 4947–4955, 1973.
10. Comporti, M., E. Burdino and F. Raja. Fatty acids composition of mitochondrial and microsomal lipids of rat liver after acute ethanol intoxication. *Life Sci* **10:** 855–866, 1971.
11. DiLuzio, N. R. and A. D. Hartman. Role of lipid peroxidation on the pathogenesis of the ethanol-induced fatty liver. *Fed Proc* **26:** 1436–1442, 1967.
12. DiLuzio, N. R. and T. E. Stege. The role of ethanol metabolites in hepatic lipid peroxidation. In: *Alcohol and the Liver*, edited by M. M. Fisher and J. G. Rankin. New York: Plenum Press, 1977, pp. 45–62.
13. Fabry, T. L. and C. S. Lieber. The photochemical action spectrum of the microsomal ethanol oxidizing system. *Alcoholism: Clin Exp Res* **3:** 219–222, 1979.
14. Feinman, L., E. Baraona, S. Matsuzaki, M. Korsten and C. S. Lieber. Concentration dependence of ethanol metabolism in vivo in rats and man. *Alcoholism: Clin Exp Res* **2:** 381–385, 1978.
15. Garro, A. J., H. K. Seitz and C. S. Lieber. Enhancement of dimethylnitrosamine metabolism and activation to a mutagen following chronic ethanol consumption. *Cancer Res* **41:** 120–124, 1981.
16. Gillette, J. R., B. B. Brodie and B. N. LaDu. The oxidation of drugs by liver microsomes: On the role of TPNH and oxygen. *J Pharmacol Exp Ther* **119:** 532–540, 1957.
17. Gordon, E. R. The utilization of ethanol by the isolated perfused rat liver. *Can J Physiol Pharamcol* **46:** 609–616, 1968.
18. Grassi, G. G. and C. Grassi. Ethanol-antibiotic interactions at hepatic level. *J Clin Pharmacol Biopharmacol* **11:** 216–225, 1975.
19. Grunnet, N., B. Quistroff and H. I. D. Thieden. Rate-limiting factors in ethanol oxidation by isolated rat liver parenchymal cells. *Eur J Biochem* **40:** 275–282, 1973.
20. Hashimoto, S. and R. O. Recknagel. No chemical evidence of hepataic lipid peroxidation in acute ethanol toxicity. *Exp Mol Path* **8:** 225–242, 1968.
21. Hasumura, Y., R. Teschke and C. S. Lieber. Increased carbon tetrachloride hepatotoxicity, and its mechanism, after chronic ethanol consumption. *Gastroenterology* **66:** 415–422, 1974.
22. Hasumura, Y., R. Teschke and C. S. Lieber. Hepatic microsomal ethanol oxidizing system (MEOS): Dissociation from reduced nicotinamide adenine dinucleotide phosphate-oxidase and possible role of form I or cytochrome P-450. *J Pharmacol Exp Ther* **194:** 469–474, 1975.
23. Ingelman-Sundberg, M. and I. Johansson. The mechanism of cytochrome P-450-dependent oxidation of ethanol in reconstituted membrane vesicles. *J Biol Chem* **256:** 6321–6326, 1981.
24. Iseri, O. A., L. S. Gottlieb and C. S. Lieber. The ultrastructure of ethanol-induced fatty liver. *Fed Proc* **23:** 579, 1964.
25. Iseri, O. A., C. S. Lieber and L. S. Gottlieb. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Am J Pathol* **48:** 535–555, 1966.
26. Ishii, H., J.-G. Joly and C. S. Lieber. Effect of ethanol on the amount and enzyme activities of hepatic rough and smooth microsomal membranes. *Biochim Biophys Acta* **291:** 411–420, 1973.
27. Joly, J.-G., H. Ishii and C. S. Lieber. Microsomal cyanide-binding cytochrome: Its role in hepatic ethanol oxidation. *Gastroenterology* **62:** 174, 1972.
28. Joly, J.-G., H. Ishii, R. Teschke, Y. Hasumura and C. S. Lieber. Effect of chronic ethanol feeding on the activities and submicrosomal distribution of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase and the demethylases for aminopyrine and ethylmorphine. *Biochem Pharmacol* **22:** 1532–1535, 1973.
29. Joly, J.-G., C. Hetu, P. Mavier and J. P. Villeneuve. Mechanisms of induction of hepatic drug-metabolizing enzymes by ethanol. I. limited role of microsomal phosphalipids. *Biochem Pharmacol* **25:** 1995–2001, 1976.
30. Joly, J.-G., J.-P. Villeneuve and P. Mavier. Chronic ethanol administration induces a form of cytochrome P-450 with specific spectral and catalytic properties. *Alcoholism: Clin Exp Res* **1:** 17–20, 1977.
31. Kater, R. M., G. Roggin, F. Tobon, P. Zieve and F. L. Iber. Increased rate of clearance of drugs from the circulation of alcoholics. *Am J Med Sci* **258:** 35–39, 1969.
32. Kater, R. M. H., N. Carulli and F. L. Iber. Differences in the rate of ethanol metabolism in recently drinking alcoholic and nondrinking subjects. *Am J Clin Nutr* **22:** 1608–1617, 1969.

33. Koop, D. R., E. T. Morgan, G. E. Tarr and M. J. Coon. Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of ethanol-treated rabbits. *J Biol Chem* **257:** 8472–8480, 1982.
34. Leo, M. A. and C. S. Lieber. Hepatic vitamin A depletion in alcoholic liver injury in man. *N Engl J Med* **37:** 597–601, 1982.
35. Leo, M. A., M. Sato and C. S. Lieber. Effect of hepatic vitamin A depletion on the liver in men and rats. *Gastroenterology* **84:** 562–572, 1983.
36. Leo, M. A., M. Arai, M. Sato and C. S. Lieber. Hepatotoxicity of moderate vitamin A supplementation in the rat. *Gastroenterology* **82:** 194–205, 1982.
37. Li, T.-K. and W. F. Bosron, W. P. Dafeldecker, L. G. Lang and B. L. Valle. Isolation of II-alcohol dehydrogenase of human liver: Is it a determinant of alcoholism: *Proc Natl Acad Sci USA* **74:** 4378–4381, 1977.
38. Lieber, C. S. Metabolism of ethanol. In: *Metabolic Aspects of Alcoholism,* edited by C. S. Lieber. Baltimore, MD: University Park Press, 1977, pp. 1–29.
39. Lieber, C. S. *Medical Disorders of Alcoholism: Pathogenesis and Treatment.* Philadelphia, PA: W. B. Saunders Co, 1982.
40. Lieber, C. S. and L. M. DeCarli. Ethanol oxidation by hepatic microsomes: Adaptive increase after ethanol feeding. *Science* **162:** 917–918, 1968.
41. Lieber, C. S. and L. M. DeCarli. Hepatic microsomal ethanol oxidizing system: In vitro characteristics and adaptive properties in vivo. *J Biol Chem* **245:** 2505–2512, 1970.
42. Lieber, C. S. and L. M. DeCarli. Reduced nicotinamide-adenine dinucleotide phosphate oxidase: Activity enhanced by ethanol consumption. *Science* **170:** 78–80, 1970.
43. Lieber, C. S. and L. M. DeCarli. The role of the hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism in vivo. *J Pharmacol Exp Ther* **181:** 279–287, 1972.
44. Lieber, C. S. and L. M. DeCarli. The significance and characterization of hepatic microsomal ethanol oxidation in the liver. *Drug Metab Dispos* **1:** 428–440, 1973.
45. Lieber, C. S. and L. M. DeCarli. Oxidation of ethanol by hepatic microsomes of acatalasemic mice. *Biochem Biophys Res Commun* **60:** 1187–1192, 1974.
46. Lieber, C. S., E. Rubin and L. M. DeCarli. Hepatic microsomal ethanol oxidizing system (MEOS): Differentiation from alcohol dehydrogenase and NADPH oxidase. *Biochem Biophys Res Commun* **40:** 858–865, 1970.
47. Lieber, C. S., H. K. Seitz, A. J. Garro and T. M. Worner. Alcohol as a co-carcinogen. In: *Frontiers in Liver Disease,* edited by P. D. Berk and T. C. Chalmers. New York: Thieme-Stratton, 1981, pp. 320–325.
48. Matsuzaki, S., R. Teschke, K. Ohnishi and C. S. Lieber. Acceleration of ethanol metabolism by high ethanol concentrations and chronic ethanol consumption: Role of the microsomal ethanol oxidizing system (MEOS). In: *Alcohol and the Liver,* edited by M. M. Fisher and J. G. Rankin. New York: Plenum Press, 1977, pp. 119–143.
49. Matsuzaki, S., E. Gordon and C. S. Lieber. Increased ADH independent ethanol oxidation at high ethanol concentrations in isolated rat hepatocytes: The effect of chronic ethanol feedings. *J Pharmacol Exp Ther* **217:** 133–137, 1981.
50. Mezey, E., J. J. Potter and W. D. Reed. Ethanol oxidation by a component of liver microsomes rich in cytochrome P-450. *J Biol Chem* **248:** 1183–1187, 1973.
51. Misra, P. S., A. Lefevre, H. Ishii, E. Rubin and C. S. Lieber. Increase of ethanol, meprobamate and pentobarbital metabolism after chronic ethanol administration in man and in rats. *Am J Med* **51:** 346–351, 1971.
52. Miwa, G. T., W. Levin, P. E. Thomas and A. Y. H. Lu. The direct oxidation of ethanol by a catalase-and alcohol dehydrogenase-free reconstituted system containing cytochrome P-450. *Arch Biochem Biophys* **187:** 464–475, 1978.
53. Mitchell, J. R. and D. J. Jollows. Metabolic activation of drugs in toxic substances. *Gastroenterology* **68:** 392–410, 1975.
54. Ohnishi, K. and C. S. Lieber. Reconstitution of the hepatic microsomal ethanol oxidizing system (MEOS) in control rats and after ethanol feeding. In: *Alcohol and Aldehyde Metabolizing Systems,* edited by R. G. Thurman, J. R. Williamson, H. Drott and B. Chance. New York: Academic Press, 1977, pp. 341–350.
55. Ohnishi, K. and C. S. Lieber. Reconstitution of the microsomal ethanol oxidizing system (MEOS): Qualitative and quantitative changes of cytochrome P-450 after chronic ethanol consumption. *J Biol Chem* **252:** 7124–7131, 1977.
56. Ohnishi, K. and C. S. Lieber. Respective role of superoxide and hydroxyl radical in the activity of the reconstituted microsomal ethanol-oxidizing system. *Arch Biochem Biophys* **191:** 798–803, 1978.
57. Orme-Johnson, W. H. and D. M. Ziegler. Alcohol mixed function oxidase activity of mammalian liver microsomes. *Biochem Biophys Res Commun* **21:** 78–82, 1965.
58. Pikkarainen, P. and C. S. Lieber. Concentration dependency of ethanol elimination rates in baboons: Effect of chronic alcohol consumption. *Alcoholism: Clin Exp Res* **4:** 40–43, 1980.
59. Powis, G. Effect of a single oral dose of methanol, ethanol and propanolol on the hepatic microsomal metabolism of foreign compounds in the rat. *Biochem J* **148:** 269–277, 1975.
60. Prichard, J. F. and D. W. Schneck. Effects of ethanol and phenobarbital on the metabolism of propranolol by 9000 g rat liver supernatant. *Biochem Pharmacol* **26:** 2453–2454, 1977.
61. Ratcliff, F. The effect of chronic ethanol administration on the responses to amylbarbitone sodium in the rat. *Life Sci* **8:** 1051–1061, 1969.
62. Reinke, L. A., F. C. Kauffman, S. A. Belinsky and R. G. Thurman. Interactions between ethanol metabolism and mixed-function oxidation in perfused rat liver: Inhibition of p-nitroanisole o-demethylation. *J Pharmacol Exp Ther* **213:** 70–78, 1980.
63. Reitz, R. C. A possible mechanism for the peroxidation of lipids due to chronic ethanol ingestion. *Biochim Biophys Acta* **380:** 145–154, 1975.
64. Rognstad, R. Isotopic probes into pathways of ethanol metabolism. *Arch Biochem Biophys* **163:** 544–551, 1974.
65. Rognstad, R. and D. G. Clark. Tritium as a tracer for reducing equivalents in isolated liver cells. *Eur J Biochem* **42:** 51–60, 1974.
66. Rubin, E. and C. S. Lieber. Hepatic microsomal enzymes in man and rat: Induction and inhibition by ethanol. *Science* **162:** 690–691, 1968.
67. Rubin, E., H. Gang, P. S. Misra and C. S. Lieber. Inhibition of drug metabolism by acute ethanol intoxication. A hepatic microsomal mechanism. *Am J Med* **49:** 801–806, 1970.
68. Rubin, E., C. S. Lieber, A. P. Alvares, W. Levin and R. Kuntzman. Ethanol binding to hepatic microsomes: Its increase by ethanol consumption. *Biochem Pharmacol* **20:** 229–231, 1971.
69. Salaspuro, M. P. and C. S. Lieber. Non-ADH pathway of alcohol metabolism: Its increase in activity at high ethanol concentrations and after chronic consumption. *Gastroenterology* **73:** A47, 1977.
70. Salaspuro, M. P. and C. S. Lieber. Non-uniformity of blood ethanol elimination: Its exaggeration after chronic consumption. *Ann Clin Res* **10:** 294–297, 1977.
71. Sato, N., T. Kamaga, M. Shiohiri, N. Hayashi, T. Matsumura, H. Abe and B. Hagihara. The levels of the mitochondrial and microsomal cytochromes in drinkers' livers. *Clin Chim Acta* **87:** 347–351, 1978.
72. Sato, M. and C. S. Lieber. Hepatic vitamin A depletion after chronic ethanol consumption in baboons and rats. *J Nutr* **111:** 2015–2023, 1981.
73. Sato, M. and C. S. Lieber. Increased metabolism of retinoic acid after chronic ethanol consumption in rat liver microsomes. *Arch Biochem Biophys* **213:** 557–564, 1982.
74. Scheig, R. and G. Klatskin. Some effects of ethanol and carbon tetrachloride on lipoperoxidation in rat liver. *Life Sci* **8:** 855–865, 1969.

75. Scott, C. R. and M. J. Stewart. Cysteamine treatment in paracetamol overdose. *Lancet* **1:** 452–453, 1975.
76. Seitz, H. K., A. J. Garro and C. S. Lieber. Sex dependent effect of chronic ethanol consumption in rats on hepatic microsome mediated mutagenicity of benzo(a) pyrene. *Cancer Let* **13:** 97–102, 1981.
77. Seitz, H. K., A. J. Garro and C. S. Lieber. Enhanced pulmonary and intestinal activation of procarcinogens and mutagens after chronic ethanol consumption in the rat. *Eur J Clin Invest* **11:** 33–38, 1981.
78. Shigeta, Y., S. Iida, M. A. Leo, F. Nomura, M. R. Felder and C. S. Lieber. Ethanol (E) metabolism in deermice lacking alcohol dehydrogenase (ADH). *Alcoholism: Clin Exp Res* **6:** 153, 1982.
79. Teschke, R., Y. Hasumura, J.-G. Joly, H. Ishii and C. S. Lieber. Microsomal ethanol-oxidizing system (MEOS): Purification and properties of a rat liver system free of catalase and alcohol dehydrogenase. *Biochem Biophys Res Commun* **49:** 1187–1193, 1974.
80. Teschke, R., Y. Hasumura and C. S. Lieber. Hepatic microsomal ethanol oxidizing system: Solubilization, isolation and characterization. *Arch Biochem Biophys* **163:** 404–415, 1974.
81. Teschke, R., Y. Hasumura and C. S. Lieber. Hepatic microsomal alcohol oxidizing system. Affinity for methanol, ethanol and propanol. *J Biol Chem* **250:** 7379–7404, 1975.
82. Teschke, R., Y. Hasumura and C. S. Lieber. Hepatic microsomal alcohol oxidizing system in normal and acatalasemic mice: Its dissociation from the peroxidatic activity of catalase-H_2O_2. *Molec Pharmacol* **11:** 841–849, 1975.
83. Teschke, R., S. Matsuzaki, K. Ohnishi, L. M. DeCarli and C. S. Lieber. Microsomal ethanol oxidizing system (MEOS): Current status of its characterization and its role. *Alcoholism: Clin Exp Res* **1:** 7–15, 1977.
84. Thieden, H. I. D. The effect of ethanol concentration on ethanol oxidation rate in rat liver slices. *Acta Chem Scand* **25:** 3421–3427, 1971.
85. Tobon, F. and E. Mezey. Effect of ethanol administration on hepatic ethanol and drug-metabolizing enzymes and on rates of ethanol degradation. *J Lab Clin Med* **77:** 110–121, 1971.
86. Ugarte, G., I. Pereda, M. E. Pino and H. Iturriaga. Influence of alcohol intake, length of abstinence and meprobamate on the rate of ethanol metabolism in man. *Q J Stud Alcohol* **33:** 698–705, 1972.
87. Ullrich, V., P. Weber and P. Wollenberg. Tetrahydrofurane-an inhibitor for ethanol-induced liver microsomal cytochrome P-450. *Biochem Biophys Res Commun* **64:** 808–813, 1975.
88. Vatsis, K. P. and M. P. Schulman. Absence of ethanol metabolism in 'acatalatic' hepatic microsomes that oxidize drugs. *Biochem Biophys Res Commun* **52:** 588, 1973.
89. Vatsis, K. P. and M. P. Schulman. An unidentified constituent of ethanol oxidation in hepatic microsomes. *Fed Proc* **33:** 554, 1974.
90. Vesell, E. S., J. G. Page and G. T. Passananti. Genetic and environmental factors affecting ethanol metabolism in man. *Clin Pharmacol Ther* **12:** 192–201, 1971.
91. Winston, G. W. and A. I. Cederbaum. Oxygen radical production by purified components of the microsomal mixed function oxidase system: Role in ethanol oxidation. *Alcoholism: Clin Exper Res* **6:** 318, 1982.
92. Yuki, T. and R. G. Thurman. The swift increase in alcohol metabolism. *Biochem J* **186:** 119–126, 1980.
93. Ziegler, D. M. Discussion in Microsomes and Drug Oxidation. In: *Microsomes and Drug Oxidation*, edited by R. W. Estabrook, J. R. Gillette and K. C. Liebman. Baltimore, MD: Williams and Wilkins, 1972, pp. 458–460.