PHYSIOLOGICAL REVIEWS
Vol. 77, No. 2, April 1997
Printed in U.S.A.

Cytochrome *P*-4502E1: Its Physiological and Pathological Role

CHARLES S. LIEBER

Alcohol Research and Treatment Center and Section of Liver Disease and Nutrition, Bronx Department of Veterans Affairs Medical Center and Mount Sinai School of Medicine, New York, New York

I. Introduction 518
II. Microsomal Ethanol-Oxidizing System and Its Differentiation From Alcohol Dehydrogenase and Catalase 519
III. "Ethanol-Specific" Cytochrome *P*-450 519
A. Purification 519
B. Reconstitution of microsomal ethanol-oxidizing activity 521
C. Role of hydroxyl radicals 521
D. Regulation of cytochrome *P*-4502E1 expression 521
E. Polymorphism of cytochrome *P*-4502E1 523
IV. Enzymology of Cytochrome *P*-4502E1 524
A. Cofactors 524
B. Role of lipids and other nutrients 524
C. Substrates 526
D. Inducers and inhibitors 527
V. Tissue Distribution 528
A. Liver 528
B. Extrahepatic tissue 529
VI. Physiological Functions 530
A. Starvation and ketone metabolism 530
B. Obesity, diabetes, and lipid metabolism 530
VII. Role of Cytochrome *P*-4502E1 in the Metabolism of Ethanol and Associated Pathology 530
A. Role of cytochrome *P*-4502E1 in ethanol oxidation after chronic ethanol consumption 530
B. Pathogenic role of cytochrome *P*-4502E1 in alcoholic liver disease and associated oxidative stress 531
C. Role of cytochrome *P*-4502E1 in carcinogenesis 533
D. Role of cytochrome *P*-4502E1 in energy balance 533
VIII. Markers of Cytochrome *P*-4502E1 Induction and Their Clinical Applications 534
IX. Ultrastructural Changes and Associated Induction of Other Microsomal Enzymes 534
X. Conclusions and Future Applications 535

Lieber, Charles S. Cytochrome *P*-4502E1: Its Physiological and Pathological Role. *Physiol. Rev.* 77: 517–544, 1997.—The role of the microsomal ethanol-oxidizing system (MEOS) in hepatic ethanol metabolism is reviewed, with focus on its constitutive, ethanol-inducible cytochrome *P*-4502E1 (2E1). The MEOS was purified and reconstituted using 2E1, phospholipids, and cytochrome *P*-450 reductase and shown to oxidize ethanol to acetaldehyde, mainly as a monooxygenase and secondarily via hydroxyl radicals, with transcriptional and posttranscriptional regulation. Polymorphism of 2E1 was recognized, and enzymology (including cofactors, role of lipids, inducers, and inhibitors) as well as cellular and tissue distribution were chartered. Physiological functions involve lipid metabolism and ketone utilization in starvation, obesity, and diabetes. The most significant role of 2E1 is its adaptive response to high blood ethanol levels with a corresponding acceleration of ethanol metabolism. The associated free radical production, however, contributes to liver injury in the alcoholic. Most importantly, 2E1 has a unique capacity to activate many xenobiotics (85 of which are listed) to hepatotoxic or carcinogenic products. Induction of 2E1 also results in enhanced production of acetaldehyde, a highly reactive and toxic metabolite. The proliferation of the endoplasmic reticulum associated with 2E1 induction is also accompanied by enhanced activity of other cytochrome *P*-450s, resulting in accelerated metabolism of, and tolerance to, other drugs, as well as increased degradation of retinol and its hepatic depletion. Some substrates and metabolites, however, are innocuous and may eventually be used as markers of heavy drinking. Recently discovered effective 2E1 inhibitors also have great therapeutic potential.

0031-9333/97 $15.00 Copyright © 1997 the American Physiological Society

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

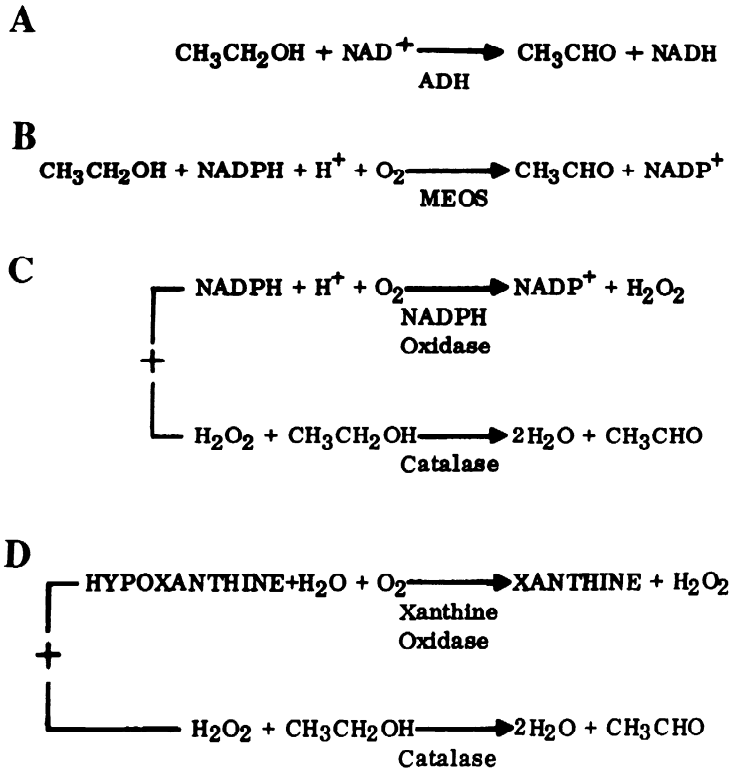

FIG. 1. Ethanol oxidation by alcohol dehydrogenase (ADH) and NAD (*A*), hepatic microsomal ethanol-oxidizing system (MEOS) and NADPH (*B*), a combination of NADPH oxidase and catalase (*C*), and xanthine oxidase and catalase (*D*). [From Lieber (177).].

I. INTRODUCTION

Ethanol is not only produced by yeast but can also be found in mammals in trace amounts. Bacterial fermentation in the gut is one way in which it is generated (151). However, ethanol is primarily an exogenous compound that is readily absorbed from the gastrointestinal tract. Only 2–10% of ethanol absorbed is eliminated through the kidneys and lungs; the rest is oxidized in the body, principally in the liver. The rate of ethanol removal from the blood is indeed remarkably decreased or halted by hepatectomy or procedures damaging the liver (310). Until three decades ago, it was believed that there was only one significant pathway for ethanol metabolism, involving multiple forms of alcohol dehydrogenase (ADH), an enzyme of the cytosol that catalyzes the conversion of ethanol to acetaldehyde, coupled with the reduction of NAD^+ to NADH (Fig. 1). It was known that catalase, located in the peroxisomes, is capable of oxidizing ethanol in vitro in the presence of an H_2O_2-generating system (126) (Fig. 1), but under physiological conditions, catalase appeared to play no major role. Some features of ethanol metabolism, however, such as the adaptive increase after chronic consumption, could not be explained on the basis of ADH or catalase, therefore raising the suspicion of the existence of another pathway. The first indication of a possible interaction of ethanol with the endoplasmic reticulum of the hepatocyte (also called microsomal fraction when obtained by ultracentrifugation) was provided by the morphological observation that in rats and humans, ethanol feeding results in a proliferation of the smooth endoplasmic reticulum (SER) (103, 104, 156). This increase in SER resembled that seen after the administration of a wide variety of hepatotoxins (206), barbiturates, and other therapeutic agents (46) and food additives (157). Because many of the substances that induce a proliferation of the SER are metabolized, at least in part, by the cytochrome *P*-450 enzyme system that is located in the SER, the possibility that ethanol may also be metabolized by a similar process was raised. Such a system was then demonstrated in liver microsomes in vitro and found to be inducible by chronic ethanol feeding in vivo (183, 184). This new, inducible, pathway of ethanol metabolism in liver microsomes explained the metabolic tolerance to ethanol in the alcoholic. It was found to involve an inducible cytochrome *P*-450, subsequently called cytochrome *P*-4502E1 (2E1), which was given its proper place in a large number of *P*-450 products of a cytochrome *P*-450 gene superfamily (222–224). Cytochrome *P*-4502E1 was shown to play a key role in the hepatotoxicity of ethanol and that of other xenobiotics. The critical role of the ethanol-induced increase of this microsomal cytochrome, and hence of the enhanced vulnerability of the alcoholic to the hepatotoxicity of many xenobiotics, was recognized more than 2 decades ago (87, 173), reaffirmed 10 years later (174), and is now generally accepted, with diagnostic and therapeutic applications of 2E1 metabolites and inhibitors being developed.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

II. MICROSOMAL ETHANOL-OXIDIZING SYSTEM AND ITS DIFFERENTIATION FROM ALCOHOL DEHYDROGENASE AND CATALASE

The existence of a distinct microsomal ethanol oxidizing pathway was first denied on the basis that it might merely reflect either ADH or catalase contamination of the microsomes. Indeed, when hepatic microsomes are prepared by standard techniques, there can be some contamination with cytosolic ADH and/or peroxisomal catalase. A series of experiments, however, established that the microsomal ethanol-oxidizing system (MEOS) was distinct from both ADH and catalase, dependent on cytochrome *P*-450 and, as for the other cytochrome *P*-450-dependent microsomal monooxygenation reactions, that it utilized NADPH and oxygen (Fig. 1). Differentiation of MEOS in total rat liver microsomes from ADH was achieved by subcellular localization, pH optimum in vitro (7.4 vs. 10), and cofactor requirements (Fig. 1).

Studies with inhibitors have also indicated that a major fraction of the ethanol-oxidizing activity in microsomes is independent of catalase (184, 187, 194). 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 (184), and azide, which is highly effective in inhibiting catalase in vitro (184). Such dissociation was found using preparations with the same rates of H_2O_2-mediated and NADPH-mediated ethanol metabolism (187). Under experimental conditions with almost complete abolition of the peroxidatic activity of catalase, the NADPH-dependent ethanol oxidation still proceeded at a significant rate; this again dissociated the NADPH-dependent MEOS activity from a process involving catalase-H_2O_2. Subsequently, MEOS was solubilized and separated from ADH and catalase activities by DEAE-cellulose column chromatography (Fig. 2) (207, 303, 304). 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 Michaelis constant (K_m) for ethanol (7–9 mM), and by the relative insensitivity of the MEOS to the ADH inhibitor pyrazole. The 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 (304). Thus, with the use of specific and sensitive methods, MEOS activity could be clearly differentiated from an enzymatic process involving peroxidatic activity of catalase. These results as well as other reports (94, 95, 207) failed therefore to support the concept that a catalase-H_2O_2 system accounts for the oxidation of ethanol in the microsomes.

It had also been reported that microsomes from acatalasemic mice failed to oxidize ethanol (325), but this claim was subsequently retracted (326). Indeed, hepatic microsomes of acatalasemic mice subjected to heat inactivation displayed decreased catalatic activity, but NADPH-dependent MEOS remained active and unaffected (188, 306). 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 (326). 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.

Of particular interest regarding the nature of MEOS are studies with different alcohols as substrates. In some microsomal preparations, NADPH-dependent oxidation of methanol and ethanol, but not of longer chain alcohols, was reported (231) and found to be exquisitely sensitive to the catalase inhibitors azide and cyanide. Therefore, Ziegler (351) concluded that this system was clearly different from a cytochrome *P*-450-dependent one and involves H_2O_2-mediated ethanol peroxidation by catalase. Such and similar other observations were considered as evidence for an obligatory role of catalase in microsomal alcohol oxidation (29, 311, 351), especially since catalase reacts peroxidatically primarily with methanol and ethanol, but not with alcohols of longer aliphatic chains (37). However, in other studies, the NADPH-dependent microsomal alcohol-oxidizing system was found capable of metabolizing methanol, ethanol, *n*-propanol, and *n*-butanol to their respective aldehydes in hepatic microsomal preparations as well as in reconstituted systems that contained the microsomal components cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase, and phospholipids and exhibited no ADH or catalase activity (304, 305, 307). This system was also demonstrated in acatalasemic mice (306). Furthermore, the involvement of cytochrome *P*-450 in MEOS was measured in the presence of carbon monoxide, and its inhibiting effect was reversed by lights of wavelengths 430 to 460 nm (65). These various experiments, including the action spectra, showed the involvement of a cytochrome *P*-450 in the activity of MEOS.

III. "ETHANOL-SPECIFIC" CYTOCHROME *P*-450

A. Purification

An "ethanol-specific" *P*-450 was first suggested by the increase of a cytochrome *P*-450 species showing high affinity for cyanide after ethanol administration (45, 88,

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

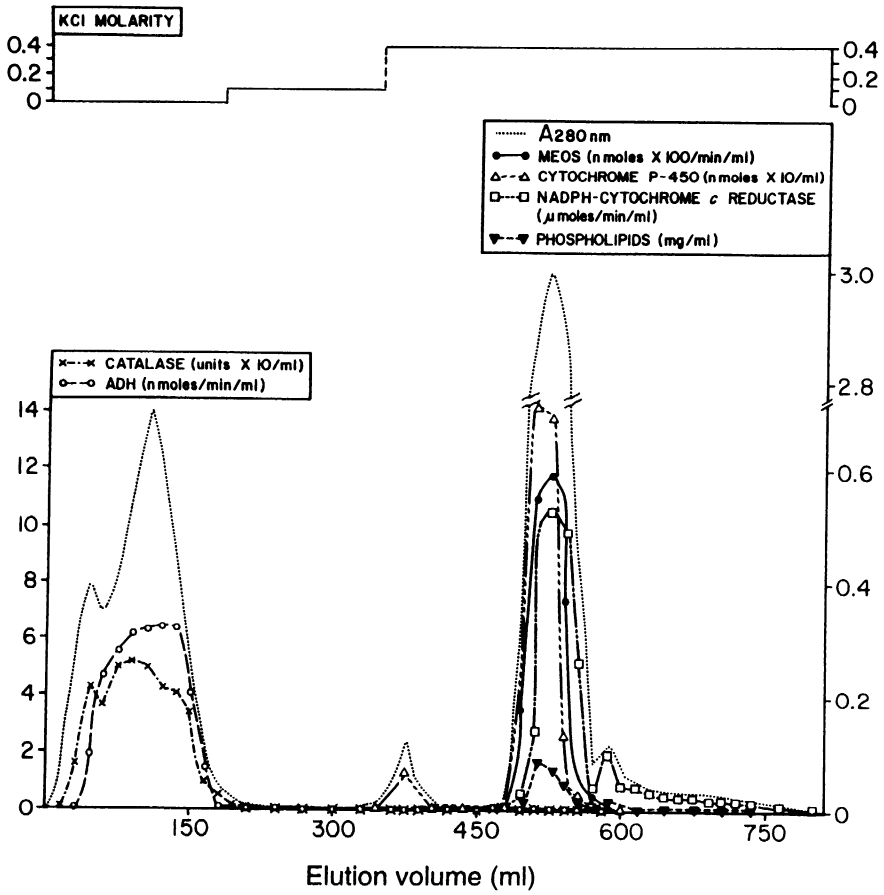

FIG. 2. Separation of MEOS from ADH and catalase activities by ion-exchange column chromatography on DEAE-cellulose. Sonicated microsomes from rats fed laboratory chow were further solubilized by treatment with sodium deoxycholate and put onto a DEAE-cellulose column. Separation of enzyme activities was achieved by a stepwise increase of KCl gradient. [From Teschke et al. (304).]

117). Evidence in favor of an increase of a special species of cytochrome *P*-450 after ethanol treatment was also derived from inhibitor studies (320). More direct proof was obtained from studies of microsomal proteins (227) after chronic ethanol feeding. The rise in cytochrome *P*-450 involved a hemoprotein different from those induced by phenobarbital or 3-methylcholanthrene treatment. Moreover, sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed an increase of a microsomal protein of 53,400 Da. The partially purified cytochrome *P*-450 from ethanol-fed rats was more active for alcohol oxidation than the control preparation in the presence of NADPH-cytochrome *P*-450 reductase and dioleoyl lecithin.

Studies by Joly and co-workers (116, 118) 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. An ethanol-inducible form of *P*-450, called then LM3a, purified from rabbit liver microsomes (101, 147) catalyzed ethanol oxidation at rates much higher than other *P*-450 isoenzymes and also had an enhanced capacity to oxidize 1-butanol, 1-pentanol, aniline (214), acetaminophen (215), CCl_4 (101), acetone (142), and *N*-nitrosodimethylamine (NDMA) (342). Similar results have been obtained with a hepatic *P*-450 cytochrome isoenzyme (then called *P*-450j), purified from ethanol- or isoniazid-treated rats (255, 257). Evidence was also provided for the existence of a *P*-450j-like isoenzyme in humans (281, 336, 337).

Next, a purified human protein was obtained in a catalytically active form, with a high turnover rate for ethanol and other specific substrates (158). In a new nomenclature for cytochromes *P*-450 (CYP) products of a *CYP* gene superfamily, it was proposed that the ethanol-inducible form be designated as *P*-450IIE1 (222), subsequently changed to *P*-4502E1 (2E1). The designation 2E1 should be reserved for this specific *P*-450 alcohol monooxygenase. However, other microsomal cytochrome *P*-450 isoenzymes can also contribute to ethanol oxidation (159, 261). Thus the term *MEOS* should be maintained when one refers to the overall capacity of the microsomes to

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

oxidize ethanol rather than to that fraction of the activity that is specifically catalyzed by 2E1.

The rat *CYP2E1* gene was isolated, characterized, and localized to chromosome 7 (322) and the human gene to chromosome 10 (321). In rabbits, two genes may be involved (128).

B. Reconstitution of Microsomal Ethanol-Oxidizing Activity

Reconstitution of the ethanol-oxidizing activity with the three microsomal components cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase, and phospholipids was demonstrated (227). In these experiments, the cytochrome *P*-450 was partially purified by protease treatment and subsequently by column chromatography on DEAE-cellulose using a stepwise KCl gradient according to Comai and Gaylor (45). The enzyme NADPH-cytochrome *P*-450 reductase was partially purified, essentially by the method of Levin et al. (171). Successful reconstitution of MEOS was confirmed using highly purified microsomal cytochrome *P*-450 from phenobarbital-treated rats (213) and ethanol-treated rabbits (147) and isoniazid- and ethanol-treated rats (255, 257), respectively.

The reconstituted MEOS showed a dependency on cytochrome *P*-450 and the reductase and required phospholipids for its maximal activity. The K_m for ethanol of the reconstituted enzyme system was 10 mM, which is similar to the K_m measured in microsomes and in the semipurified MEOS fraction isolated by column chromatography (183, 184, 304). This reconstituted system required NADPH as a cofactor, did not react to an H_2O_2-generating system, and was relatively insensitive to the catalase inhibitor azide. These characteristics were also similar to those observed in microsomes. The activity was dependent on the amount of cytochrome *P*-450 present.

C. Role of Hydroxyl Radicals

In addition to ethanol oxidation via 2E1 through a typical monooxygenase mechanism, ethanol can be oxidized by liver microsomes to acetaldehyde through hydroxyl radicals (·OH), originating from iron-catalyzed degradation of H_2O_2 (33). Such a nonenzymatic pathway can be partially responsible for the formation of hydroxyethyl radicals (137), an intermediate step between ethanol and its product acetaldehyde. This could also represent the basis of the reported activity of ethanol as ·OH scavenger in some experimental conditions (35, 228). Other evidence indicates that the production of such radicals might be due to an oxidizing species possibly bound to cytochrome *P*-450 and sufficiently reactive to abstract a proton from the alcohol α-carbon (4). Such a mechanism is also consistent with the reported inhibition of microsomal ethanol oxidation by adding iron chelators, such as 2-keto-4-thiomethylbutyric acid (35). Ingelman-Sundberg and Johansson (101) initially proposed that microsomal ethanol oxidation does not involve a specific cytochrome *P*-450-dependent mechanism but rather results from ·OH formed primarily in iron-catalyzed Haber-Weiss and Fenton reactions. Microsomal ethanol oxidation under in vivo conditions would, according to this concept, require the presence of nonheme iron and endogenous iron chelators. However, it was soon realized that this ·OH-dependent mechanism represents only a minor contribution toward microsomal ethanol oxidation under normal conditions (34). Its importance increased under experimental conditions promoting microsomal ·OH production, such as adding ferric EDTA, whereas it decreased under conditions that minimize production of ·OH (34). It was found that whereas ·OH may participate in the activity of a reconstituted ethanol-oxidizing system, superoxide was not involved (228), although increased NADPH- and NADH-dependent production of superoxide and hydroxyl radical by microsomes has been demonstrated after chronic ethanol treatment (244).

It appears, therefore, that at least two mechanism contribute to microsomal metabolism of ethanol, a lesser one involving ·OH radicals produced in a Fenton-type reaction from endogenously formed H_2O_2 and the other major one being cytochrome-*P*-450-mediated monooxygenation (as shown by its strong depression by *P*-450 inhibitors) and apparently independent from ·OH (1, 3, 251), with 2E1 possibly playing a role in the formation of hydroxyethyl radicals during ethanol metabolism. Indeed, the generation of alcohol-derived free radicals was specifically enhanced following induction of 2E1 by chronic ethanol feeding, whereas antibodies against 2E1 inhibited that process (2).

D. Regulation of Cytochrome *P*-4502E1 Expression

The rat hepatic *CYP2E1* gene is transcriptionally activated within 1 day after birth (319). This activation is accompanied by a demethylation of cytosine residues located within the 5′-flanking region of the gene, suggesting that methylation of specific residues in the *2E1* gene is responsible for the lack of transcription of the *2E1* gene in fetal liver. Cytochrome *P*-4502E1 remains relatively stable during the remainder of the life span, and a tissue-specific relation between the hypomethylation of the human *CYP2E1* gene and its hypoexpression was observed (17). CYP2E1 can be strikingly induced by a variety of substrates. Indeed, one of the most characteristic features that distinguishes 2E1 from other ethanol-metabolizing pathways is its inducibility, demonstrated in several species, including humans (312) (Fig. 3). However, the regulation of 2E1 expression is complex, involving transcrip-

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

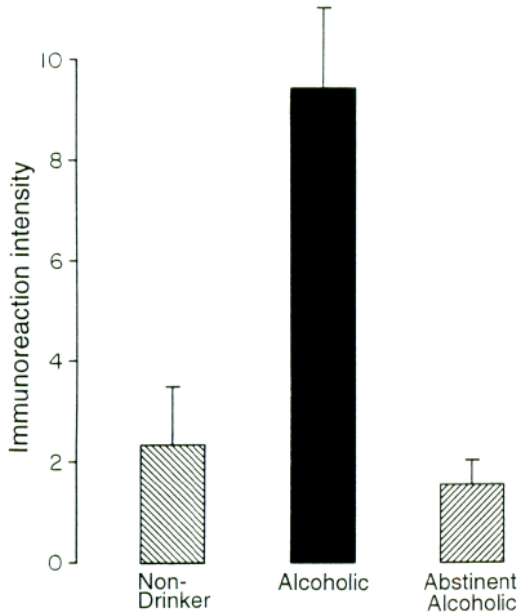

FIG. 3. Hepatic cytochrome *P*-4502E1 levels in alcoholics and nondrinkers. Cytochrome *P*-4502E1 was quantitated by scanning of Western blots of microsomes obtained from percutaneous liver biopsies, using anti-2E1 antibodies. [Data from Tsutsumi et al. (312).]

tional, posttranscriptional, and posttranslational events. Investigations using rabbits (and some involving rats) appeared to have ruled out transcriptional activation of the *2E1* gene or stabilization of the 2E1 mRNA as possible mechanisms, since ethanol and similar agents (acetone, imidazole, 4-methylpyrazole, pyrazole, and pyridine) had little effect on 2E1 transcript content in liver (113, 129, 243, 281, 282). A posttranslational mechanism, namely protein stabilization, was thus proposed, since *1*) ethanol and imidazole were found to prevent the rapid decrease in 2E1 enzyme level that occurs in rat hepatocytes upon primary culture (62) and *2*) acetone treatment was shown to prolong the in vivo half-life of 2E1 in rat liver by eliminating the fast-phase component associated with the enzyme's normal degradation (283). Similar experiments were also presented with ethanol, and a role for ubiquitin conjugation in the rapid degradation of 2E1 was proposed (253). A hormone- and substrate-regulated intracellular degradation of 2E1 (involving Mg^{2+}-ATP-activated rapid proteolysis in the endoplasmic reticulum membranes) was also suggested (63). Furthermore, in a HepG2 cell line, which stably and constitutively expresses the coding sequences of human cytochrome 2E1, addition of ethanol (2–100 mM) for 2 days resulted in an increased 2E1 content as determined by Western blotting, whereas Northern blot analysis showed no raised 2E1 mRNA. These results indicated that ethanol probably increases the content of 2E1 in this experimental model by stabilization of the protein against degradation (28). Similarly, a FGC-4 hepatoma cell line exhibited basal levels of 2E1 apoprotein that were inducible by ethanol treatment, but there was no observed rise in 2E1 mRNA levels, suggesting that 2E1 is increased by ethanol through posttranscriptional mechanisms (204).

Other studies support the role of enhanced de novo enzyme synthesis and/or increased mRNA in the 2E1 induction process. Kubota et al. (153) found that induction of 2E1 protein in hamsters by ethanol and pyrazole was associated with an increase in translatable 2E1 mRNA, while Kim and Novak (134) observed increased rates of [^{14}C]leucine incorporation into 2E1 protein after treatment of rats with pyridine, a phenomenon attributed to the enhancement, by pyridine, of 2E1 mRNA translational efficiency (135). Diehl et al. (51) described elevated levels of hepatic 2E1 mRNA in alcohol-treated rats. Enhanced levels of both hepatic 2E1 protein and mRNA were also found in actively drinking patients. The latter study also showed that 2E1 protein distribution, assessed by the immunohistochemical staining of liver sections with anti-2E1 antibodies (102, 312, 315), was found to be analogous to that of the mRNA in control subjects (the level in perivenular cells was greater than that in midzonal cells, which was greater than that in periportal cells) (Fig. 4),

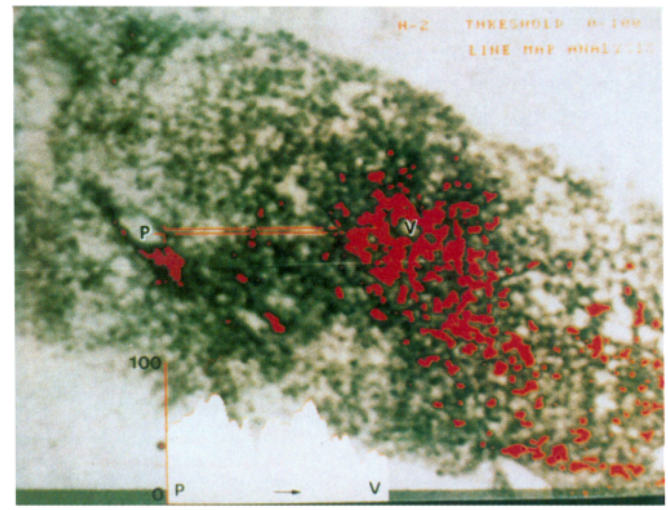

FIG. 4. Image analysis of cytochrome *P*-4502E1 (2E1) mRNA/cDNA hybrids in a human liver section. After in situ hybridization with a 2E1 cDNA, liver specimen (from a control individual) was subjected to computerized image analysis to quantitate 2E1 mRNA/cDNA hybridization signals and to determine mRNA signal density gradient in hepatic acinus. Area measurements were made on red portions of section, which represent acinar regions positive for 2E1 mRNA transcripts. Nonspecific coloring of portal triad (P) (because of abundant methyl green-stained nuclei) was excluded from area measurements by manual tracing and deletion. Hybridization signal densities within small red rectangle overlaid between a portal and terminal hepatic venule (V) were measured along width by horizontal scanning actual computer-drawn line map corresponding to this scan spanning P → V area is illustrated in white below. The section 2E1 mRNA hybridization signal intensity has been plotted inversely on *y*-axis, where 100 denotes no specific signal and 0 denotes most intense signal. Magnification, ×150. [From Takahashi et al. (296).]

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

whereas recent drinkers exhibited marked elevations in enzyme content in both perivenular and midzonal hepatocytes. Moreover, cellular levels of 2E1 protein and its mRNA were significantly correlated (296), indicating that mRNA stabilization and/or transcriptional activation are involved in ethanol-mediated 2E1 induction in humans. Furthermore, by measuring both the synthesis and the degradation of radiolabeled 2E1 protein at induced high steady-state levels in the rat, ethanol appeared to stimulate rates of de novo 2E1 synthesis but had no effect on rates of enzyme degradation (313). It is therefore reasonable to assume that the enhancement of de novo 2E1 synthesis noted in ethanol-treated rats results from increased steady-state levels of 2E1 message and/or the increased efficiency with which this mRNA is translated.

The dose of the inducer is also of importance, because 2E1 induction appears to occur via two steps: *1*) posttranslational mechanism at low ethanol concentrations and *2*) an additional transcriptional one at high ethanol levels (9, 254). However, large interindividual variations were noted in the 2E1-specific mRNA content of hepatic tissue from controls (up to 11-fold) and patients with advanced hepatic disease (up to 44-fold). Cytochrome *P*-4502E1 mRNA levels were strikingly decreased in patients with both hepatocellular and cholestatic forms of cirrhosis (78).

The conflicting results with regard to the 2E1 induction process may stem in part from differences among species, the duration and/or manner of inducer treatment, and/or distinct mechanisms of induction by the various compounds, since the commonly used 2E1 inducing agents other than ethanol, such as acetone, pyrazole, or pyridine, may differ in their mode of action. Obviously, the clinically most relevant results are those obtained with alcohol in humans who exhibited a clear-cut 2E1 mRNA increase in conjunction with 2E1 induction (296).

Finally, it was observed that in rats continuously infused with ethanol, blood alcohol concentrations were not constant, but fluctuated in a rather regular pulsatile manner in which peak levels were obtained approximately every 6 days (9). The mechanisms by which pulsatile blood alcohol levels occur are unknown. One speculation is that 2E1 could be involved in the process. Indeed, there is also a circadian variation of ethanol metabolism (240, 287, 334), which is particularly manifested and perhaps even altered in the alcoholic (119) and may be related to a diurnal variation in the relative amounts of endoplasmic reticulum within the hepatic lobule (38).

E. Polymorphism of Cytochrome *P*-4502E1

Several polymorphic sites in the 5′-flanking region of the human cytochrome *2E1* gene have been reported. Indeed, it contains several restriction fragment length polymorphisms that may affect transcriptional regulation or the functional activity of the expressed protein (89, 96, 236, 317, 318, 328). Two nucleotide exchanges are within restriction sites (*Pst* I and *Rsa* I) and were found to be in complete linkage disequilibrium. The *Rsa* I sites in the regulatory 5′-flanking region described in Japanese subjects were found to affect the rate of transcription when coupled to a reporter gene in an in vitro expression system (89, 328). Hayashi et al. (89) classified the genotypes as type A (homozygous "normal" alleles; *c1,c1*), type C (homozygous alleles with the nucleotide exchanges; *c2,c2*), and type B (heterozygous, having a normal and an altered allele; *c1,c2*). The rare mutant allele (termed the *c2* allele) that lacks the *Rsa* I restriction site is associated with higher transcriptional activity, protein levels, and enzyme activity than the more common wild-type allele (*c1* allele) (89, 316, 328).

The frequency of the *Rsa* I-lacking *c2* allele varies in different populations (286). The highest frequency has been observed in the Taiwanese (0.28) and Japanese (0.19–0.27), whereas in African-Americans, European-Americans, and Scandinavians, the frequency is much lower, ranging between 0.01 and 0.05 (286). The *Rsa* I polymorphism has been investigated in the Japanese population by two groups; one study showed a positive association of the *c2* allele with alcoholic liver disease (316), and the other study showed an association between the *c1* (wild-type) allele and alcoholic liver disease (201). A smaller study has also been performed in North American Caucasian men (26); it did not show an association between alcoholic liver disease and the *Rsa* I polymorphism, whereas Pirmohamed et al. (241) found that the *c2* allele frequency in patients with alcoholic liver disease was significantly higher than in control subjects. The discrepancies between the different studies may be related to clinical dissimilarities of the patients investigated as well as to differences in the gene frequencies of the populations studied. Polymorphism of *2E1* may also play an important role in cigarette smoking-related hepatocarcinogenesis. In a recent, as yet unconfirmed, study, a much greater risk was observed for those smokers who were also habitual alcohol drinkers and carried the *c1/c1* genotype (346). However, *Rsa* I polymorphism did not affect lung cancer risk (330).

Dra I polymorphism of *2E1* has been reported to be associated with lung cancer risk in the Japanese (318). However, observations in the United States (123), Finland (96), and Brazil (290) have not supported these findings. There was also no significant difference in *c2* gene frequency between alcoholic and nonalcoholic controls (108). Similarly, Maezawa et al. (202) found no significant difference in the frequency of the *2E1* genotype between alcoholics and healthy subjects, concluding that polymorphisms of the gene does not influence the risk of developing alcoholism in the Japanese.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

In summary, regulation of 2E1 expression is complex, involving transcriptional, posttranscriptional, and posttranslational events with polymorphism playing a role. Although alcohol consumption results in a striking induction of hepatic 2E1, a 6- to 20-fold variability (or more) of 2E1 protein or activity has been described, not related to liver disease or cancer, nor to alcohol drinking or smoking. Other environmental factors, diet, or genetic factors may explain the large interindividual variations observed (197).

IV. ENZYMOLOGY OF CYTOCHROME *P*-4502E1

A. Cofactors

Microsomes as well as purified and semipurified 2E1 catalyze the oxidation of ethanol to acetaldehyde and that of a large number of exogenous and endogenous substrates (Table 1), thereby explaining 2E1's physiological and toxicological role. As for other monooxygenase systems, optimal incubation conditions for maximal rates of metabolism require the presence of NADPH, NADPH-cytochrome *P*-450 reductase, and lipids. No substantial metabolism is detected when any of these components are omitted. A marked enhancement of metabolism (up to 4-fold) was obtained in the presence of various concentrations of phospholipids, a commonly used one being dilauroylphosphatidylcholine. With NDMA as a substrate, formaldehyde formation was directly proportional to the concentration of 2E1 and time of incubation for at least 10 min. The pH optimum for the reaction for that substrate was between 6.5 and 6.8 (172). Similar results were also obtained with ethanol and higher alcohols as substrates, using a reconstituted system consisting of semipurified 2E1 and reductase (227) except that the optimal pH was 7.4. The apparent K_m values of this system for ethanol and NADPH were 11.4 and 0.045 mM, respectively. Reduction of oxygen to 4% decreased the microsomal ethanol-oxidizing activity by 45% compared with the reaction in air. In such a system, it was found that introduction of 40% CO inhibited the reaction by 34%. Replacement of air by nitrogen abolished the activity almost completely. Heating of this system to 90°C for 20 min destroyed the enzymatic activity. Substantial activity was observed in the presence of NADPH, whereas with NADH, activity was <50% that with NADPH. Other cofactors (such as $NADP^+$ and NAD^+) were not active. The catalase inhibitor azide did not inhibit the activity of the system at 0.1 mM, but at higher concentrations, there was a nonspecific inhibitory effect.

The ADH inhibitor pyrazole did not decrease the reconstituted microsomal ethanol-oxidizing activity at the concentration of 2 mM. However, 4-methylpyrazole (4-MP) is both an inhibitor in vitro and an inducer in vivo (see sect. IV*D*). When catalase was added to the system and incubated with NADPH, acetaldehyde production increased, but this was completely abolished by 0.1 mM azide. When NADPH was replaced by a H_2O_2-generating system, the reconstituted system could not oxidize ethanol without added catalase. Furthermore, this ethanol oxidation was completely abolished by 0.1 mM sodium azide. There was no significant difference in the capacity of partially purified NADPH-cytochrome *P*-450 reductase from either ethanol-treated rats or controls to promote alcohol oxidation in the presence of the cytochrome *P*-450 (227). However, the amount of NADPH-cytochrome *P*-450 reductase may become rate limiting (99). Ratios of *P*-450-reductase-lipid of 1:1:20 are considered optimal (256).

B. Role of Lipids and Other Nutrients

The induction effect of chronic ethanol consumption or liver microsomes may be modulated, in part, by the dietary content in lipids (114). The quantity and degree of saturation of dietary lipids also affect levels of 2E1 (344, 345). The various liver *P*-450s require the addition of phospholipids for catalytic activity to be reconstituted in vitro. Traditionally, dilauroylphosphatidylcholine ([12:0/12:0] PC) has been used for that purpose with various *P*-450s, including highly purified 2E1 from human liver microsomes (158, 337). However, [12:0/12:0] PC is not the only active phospholipid. It was shown before in reconstituted systems with semipurified *P*-450 enzymes (227) that these preparations were quite active with a less saturated phospholipid, namely, dioleoylphosphatidylcholine ([18:1/18:1] PC).

Whereas the effects of high-fat diets on hepatic 2E1 were paralleled by similar changes in the corresponding mRNA in one study (347), inconclusive results were found in another (344). It has been reported that dietary corn oil (20%) also affects the constitutive levels of 2E1 protein in microsomes, but not the catalytic activity of this enzyme (344). Yun et al. (347) also reported that a 2E1-promoted enzyme activity (aniline 4-hydroxylation) in hepatic and renal microsomes was elevated about two- to threefold by feeding a high-fat diet for 3 days and that the rises in enzyme activities were also accompanied by a comparable increase in immunoreactive 2E1 protein and its mRNA. It is not clear at this time whether these results can be specifically ascribed to the high-fat diet rather than to a difference in fatty acids. A diet rich in polyunsaturated fatty acid, especially linoleic acid, can enhance MEOS induction by chronic ethanol intake (114). The relative increase of 2E1-dependent MEOS activity and 2E1-dependent hydroxylation of *p*-nitrophenol was higher in microsomes isolated from rats treated with the corn oil diet (rich in linoleate) compared with the tallow-fed rats (295). It must be pointed out, however, that when ethanol was

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

TABLE 1. *Substrates metabolized/activated by cytochrome P-4502E1: 1997 update*

Substrate	Reference No.
Alcohols, aldehydes, ketones, and nitriles	
Acetaldehyde	302
Butanol	144
2-Butanone	301
	21
Ethanol	227
	144
Glycerol*	335
Isopropanol	214
Methanol	214
Propanol	144
Pentanol	144
1-Phenylethanol (to acetophenone)	15
Aromatic compounds	
Acetaminophen	246
Aniline	144
Benzene	111
Bromobenzene	331
Caffeine (to theophylline and theobromine)	298
	216
	297
Capsaicin	292
Chlorzoxazone	238
3-Hydroxypyridine	135
Isoniazid	255
Phenol	146
Pyridine	136
p-Nitrophenol	139
	299
	210
Pyrazole	43
Styrene	85
Tamoxifen	288
Theophylline 8-hydroxylation (at high concn.)	86
	350
Toluene	16
Ethers	
Diethyl ether	19
Methyl *t*-butyl ether	22
1,1,2,3,3,3-Hexafluoropropyl methyl ether	149
*Fatty acids**	
Arachidonic acid ω-1 and ω-2 hydroxylation	155
	252
Lauric acid ω-1 hydroxylation	7
	42
	73
Halogenated and nonhalogenated alkanes and alkenes	
Acetoacetate*	142
Acetol	144
Acetone*	144
Acetonitrile (+ catalase)	67
Acrylonitrile	85
	125
1,3-Butadiene	59
Chloroform (to glutathione conjugate)	300
	301
	85
Chloroform (low-affinity component)	217
Halogenated and nonhalogenated alkanes and alkenes—Continued	
Chloromethane	83
Dibromoethane	83
1,1-Dichloro-2,2,2-trifluoroethane	323
2,2-Dichloro-1,1,1-trifluoroethane	196
Dichloromethane	83
1,1-Dichloroethane	160
1,1-Dichloroethylene	160
	58
1,2-Dichloropropane	83
N,N-Dimethylacetamide	276
N,N-Dimethylformamide	40
Enflurane	98
	294
Enflurane (in vivo in humans)	132
1,2-Epoxy-3-butene	266
Ethane	301
Ethyl carbamate	83
Ethylene dichloride	83
Halothane	82
Hexane	301
β,β-Iminodipropionitrile	77
Methoxyflurane	98
	131
Methyl formate	235
Methylenechloride	83
N-Methylformamide	100
Pentane	300
Seroflurane	98
	130
1,1,1,2-Tetrafluoroethane	229
	291
1,1,2,2-Tetrafluoro-1-1(2,2,2-trifluoroethoxy)ethane	91
Thioacetamide	41
Tirapazamine	127
1,1,1-Trichloroethane	83
Trichloroethylene	83
Vinyl chloride	83
Vinyl bromide	83
Nitrosamines, azocompounds	
Azoxymethane	279
N,N-Diethylnitrosamine	344
N,N-Dimethylnitrosamine	344
Methylazoxymethanol	279
N-Nitroso-2,3-dimethylmorpholine	136
N-Nitrosomethylbenzylamine	234
N-Nitrosopyrrolidine	203
N-Nitroso*bis*(2-oxopropyl)amine	124
Reducible substrates	
t-Butylhydroxyperoxide	327
Carbon tetrachloride	83
Chromium [Cr(VI)]	208
Cumyl hydroperoxide	327
13-Hydroperoxy-9,11-octadecadienoic acid	327
15-Hydroperoxy-5,8,11,13-eicosatetraenoic acid	327
Oxygen	81

* Endogenous compounds.

given with linoleate as the only source of dietary lipid, the ethanol induction of these parameters was greater with diets containing 2 or 5% of total calories as linoleate than with diets containing 10% of total calories as linoleate. In contrast, when ethanol was given with high-fat (35% of total calories) diets, the ethanol induction of these same parameters was slightly greater when linoleate provided 10% of total calories than when it provided 3% of

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

calories (115). Thus the apparent effect of dietary linoleate on the induction, by ethanol, of microsomal drug-metabolizing enzymes is markedly different when linoleate is given as the only source of dietary lipid as opposed to when it is given with other dietary lipids, and conclusions on the effect of ethanol on hepatic microsomal drug-biotransformation enzymes, drawn from studies with dietary models in which linoleate provides the only source of dietary lipid, cannot be extended to dietary models of more complex lipid composition. Furthermore, other results (191) indicated a comparable five- to sixfold ethanol-dependent induction of 2E1, MEOS, and *p*-nitrophenol hydroxylation in rats fed low-fat (5%) and high-fat (35%) diets. A tendency to an extra increase of MEOS activity was observed in the latter group, without any concomitant extra rise in 2E1. This was taken as an indication that non-2E1 metabolizing *P*-450s participated in the oxidation of ethanol. Indeed, the Lieber-DeCarli feeding regimen also increased *P*-450 4A1 specific activity (199, 220), which may contribute to a decrease in arachidonic acid (8, 270), possibly in association with increased phospholipase A activity (8, 220).

The difference in ethanol metabolism could also be due, at least in part, to an increase in the catalytic activity of 2E1 in a microsomal environment modified by the fat content of the diet. In any event, in addition to varying fat content of the diets, some investigations also varied the essential fatty acids, a deficiency of which may have an independent and confounding effect. When one compared the effects of high- and low-fat diets with administration of sufficient essential fatty acids to both groups, no difference in levels of 2E1 was found, nor did the response to the induction by ethanol differ (191), whereas, as already mentioned, MEOS activity was increased significantly in rats fed the high-fat diet.

It should be noted that, in addition to lipids, dietary proteins can also affect microsomal functions (212). Furthermore, decreased carbohydrate content increased MEOS activity by 72% (309).

C. Substrates

In addition to ethanol, many other substrates for 2E1 have been identified (141), and an updated list is given in Table 1. Some of these are endogenous substrates, illustrating the physiological role of 2E1 (see sect. VI). Most, however, are exogenous compounds. Not infrequently, the metabolites produced in the microsomes are more toxic than the precursors. Much of the medical significance of MEOS (and its ethanol-inducible 2E1) results not only from the oxidation of ethanol but also from the unusual and unique capacity of 2E1 to activate many xenobiotic compounds to their toxic metabolites, often free radicals, thereby contributing to the ethanol-induced pathology (see sect. VII).

Of the various 2E1 substrates, the demethylation of NDMA and the hydroxylation of *p*-nitrophenol are most effective for monitoring the level of this enzyme. Recently, a highly sensitive, simple assay for the determination of 4-nitrocatechol formed during the *P*-450-dependent hydroxylation of *p*-nitrophenol has been developed. The assay utilizes high-performance liquid chromatography with electrochemical detection, which makes it possible to measure 0.5 pmol of 4-nitrocatechol injected, 30 times less than previously reported for an ultraviolet detector (210). It has also been demonstrated, using cDNA-expressed human enzymes, that the conversion of *p*-nitrophenol is catalyzed predominantly (at least 85%) by 2E1 (299).

The 6-hydroxylation of chlorzoxazone has also been suggested as a probe for 2E1 phenotyping in humans (238) (see sect. VIII). Carriere et al. (27) reported that recombinant human 2E1 and 1A1 (expressed in yeast cells) were involved in the metabolism of chlorzoxazone, whereas Yamazaki et al. (341) found no such activity for recombinant 1A2 expressed in *Escherichia coli*. However, cDNA expression in mammalian cells of 1A2 was associated with chlorzoxazone oxidation (230). In addition, the level of human hepatic 1A2 is about twofold higher than that of 2E1 (85, 272). Because human 1A2 has a lower Michaelis constant for chlorzoxazone than 2E1, a role of 1A2 in 6-hydroxychlorzoxazone formation in vivo cannot be excluded (230).

In addition to oxidative reactions, 2E1 is also an efficient catalyst of reductive reactions. The formation of trichloromethyl radicals from CCl_4 (enhanced by ethanol treatment) was reported by Reinke et al. (250). Vaz et al. (327) described the efficient reduction of hydroperoxides by cytochrome *P*-4502E1. These reactions were conducted under anaerobic conditions and produced hydrocarbons and either aldehydes or ketones from the original hydroperoxide. Lipid hydroperoxides were proposed as the physiological substrates.

Included in the list of substrates for reductive reactions is molecular oxygen. The NADPH-dependent reduction of oxygen by cytochrome *P*-450 (the "oxidase activity of *P*-450") is well documented. Peroxide formation is thought to occur by the release and subsequent dismutation of superoxide. Direct release of peroxide from the two-electron reduced enzyme can also occur (81, 83). The reduction of oxygen is particularly relevant for 2E1. This form of *P*-450 is usually isolated in the high-spin state (144), but Gillam et al. (79) report a mixed spin state for 2E1 expressed in *E. coli*. If the enzyme is also high spin in the endoplasmic reticulum, it may be readily reduced by an electron provided by NADPH-cytochrome *P*-450 reductase (in the absence of substrate). Indeed, microsomes from ethanol-fed animals exhibit much greater rates of NADPH oxidation than microsomes from untreated animals (49, 61, 68, 152, 185). Enhanced oxidase activity

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

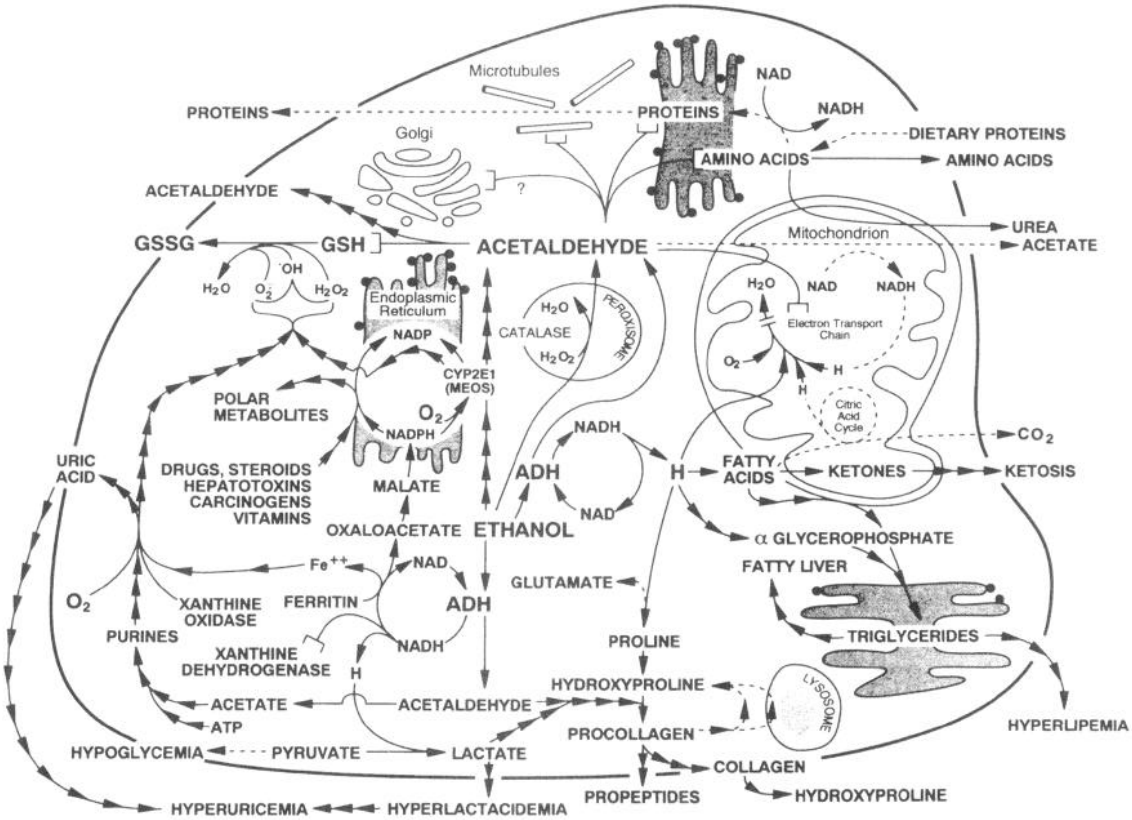

FIG. 5. Oxidation of ethanol in hepatocyte. Many disturbances in intermediary metabolism and toxic effects can be linked to *1*) ADH-mediated generation of NADH; *2*) induction of activity of microsomal enzymes, especially MEOS containing 2E1; and *3*) acetaldehyde, product of ethanol oxidation. GSH, reduced glutathione; GSSG, oxidized glutathione; dashed lines, pathways that are depressed by ethanol; solid lines, stimulation or activation; -[, interference or binding.

would result in the increased production of both superoxide anion and hydrogen peroxide which, in the presence of chelated iron, can produce reactive hydroxyl radicals (49, 68) (Fig. 5), but it is still not demonstrated that a comparable uncoupling of 2E1 occurs in vivo.

Cytochrome *P*-4502E1 exhibits a unique ability to potentiate an iron-catalyzed Fenton-type reaction in a reconstituted system, and increased rates of hydroxyl radical-mediated metabolism of substrates such as ethanol and dimethyl sulfoxide were observed for microsomes isolated from animals with induced 2E1 (49, 61, 68, 152, 237). The increased capacity to produce active oxygen species was manifested by an increased rate of microsomal lipid peroxidation by microsomes or liposomes enriched in 2E1 (61, 152, 237). Antibody to 2E1 inhibited ~65% of microsomal peroxide production while almost completely inhibiting NADPH-dependent lipid peroxidation (61). These results suggest that the participation of 2E1 is necessary for lipid peroxidation to be observed.

Garro et al. (75) showed an enhancement of NDMA metabolism and activation to a mutagen following chronic ethanol consumption, and this nitrosamine was shown to be selectively metabolized by 2E1 at low concentrations of substrate (343). This may play a role in ethanol-promoted carcinogenesis (see sect. VII*C*).

D. Inducers and Inhibitors

Cytochrome *P*-4502E1 is inducible not only by ethanol, but also by some endogenous substrates such as acetone (145). Isoniazid has been identified as an inducer of 2E1 in the rat (255), and similar induction may have been observed in humans, where there was actually a biphasic effect that probably results from induction (by protein stabilization) with inhibition of catalytic activity while isoniazid is present (348). Pyrazole and 4-MP, 2E1 inhibitors, also increase levels when added to hepatocyte cultures (62, 340). These inhibitors were used to determine how soon after birth chemicals such as pyrazole or 4-MP can induce 2E1. As mentioned in section III*D*, 2E1 is not present in fetal rat liver, and activation of the gene occurs within hours after birth. In liver microsomes from saline-treated control rats, there was a progressive increase in

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

2E1 levels, up to 8–14 days after birth. Injecting pyrazole and 4-MP on *day 0* and *day 1* after birth resulted in increases (compared with control values) in 2E1 content. Northern blot analysis indicated a progressive increase in 2E1 mRNA levels, reaching a maximum at ~8 days after birth for saline-treated pups. Pyrazole or 4-MP did not increase 2E1 mRNA levels over values for the controls, suggesting a posttranscriptional effect (339).

Malotilate, a hepatoprotectant, also suppresses *CYP2E1* expression (133). Its structural analogue, YH439, displayed even much more striking transcriptional inhibition of *CYP2E1* (110). 3-Amino-1,2,4-triazole, a well-established inhibitor of catalase, also inhibits 2E1 activity (140). Gannett et al. (74) described the irreversible inactivation of rat liver 2E1 by the natural product from red peppers dihydrocapsaicin. Because 2E1 is responsible for the bioactivation of many carcinogens (see sect. VII*C*), the inhibition of 2E1 by compounds such as dihydrocapsaicin could explain inhibition of tumorigenesis by some foods. Phenethyl isothiocyanate, present in cruciferous vegetables such as cabbage and brussel sprouts, is an effective inhibitor of microsomal demethylation of NDMA (105). Disulfiram inhibits the carcinogenicity of 1,2-dimethylhydrazine (70) and the hepatotoxicity of $CHCl_3$, CCl_4, acetaminophen, and NDMA (18). Disulfiram is reduced to diethyldithiocarbamate, an effective inhibitor of 2E1 in rat (23) and human (84) microsomes. Diallyl sulfide, a component of garlic oil, exerts a potent inhibitory effect on the induction of colon and liver cancer induced by chemical carcinogens (20, 90), and it also inhibits 2E1 activity (154).

Allylmercaptan (AM) and allylmethylsulfide (AMS) suppressed constitutive and pyrazine-inducible levels of 2E1 similar to allylsulfide (AS). Immunoblot analyses of hepatic microsomes, using an anti-2E1 antibody, showed that AS, AM, and AMS significantly suppressed constitutive levels of 2E1 apoprotein after 24, 48, and 72 h, respectively. Time-dependent induction of 2E1 by pyrazine was also completely blocked by treatment of animals with AS throughout the experimental period, as evidenced by immunoblot analysis. The levels of 2E1 apoprotein in the hepatic microsomes isolated from animals treated with both AMS and pyrazine were comparable to those in control hepatic microsomes at 1–3 days posttreatment (154).

Diallyl sulfide, diallyl sulfoxide, and diallyl sulfone are competitive inhibitors, and the latter is a suicide inhibitor of 2E1 (18).

V. TISSUE DISTRIBUTION

A. Liver

1. Hepatocytes

In livers of rats as well as of humans, it was shown with immunohistochemistry that 2E1 is normally localized within the perivenular region, or zone 3, of the liver lobule (24, 102, 312, 315). The induction of 2E1 by chronic alcohol consumption occurs primarily within the some lobular region, although some enzyme induction does take place in the midzonal and periportal areas as well. In situ hybridization with a human 2E1 cDNA probe showed that the distribution of 2E1 mRNA transcripts in liver tissue from control (nondrinking) subjects was primarily perivenular in nature and reflected the distribution of the enzyme. A threefold increase of 2E1 transcripts was observed in hepatic acinar regions of actively drinking subjects, all of whom also exhibited substantial induction of 2E1 protein. Although this ethanol-mediated increase in 2E1 mRNA was distributed throughout the acinus, hepatocytes of the perivenular zone contained the highest 2E1 transcript levels (296). This localization is consistent with the propensity of centrilobular liver damage to develop in alcohol abusers after exposure to various hepatotoxins, including ethanol itself (177).

In addition to the endoplasmic reticulum, cytochrome 2E1 is also present in the plasma membrane surface of rat hepatocytes. Immunofluorescence with either rhodamine or fluorescein revealed a positive staining on the surface of most of the hepatocyte preparations when incubated with anti-2E1 but not preimmune immunoglobulin G. In general, the surface staining appeared to be uniform, suggesting an even distribution of 2E1 in the plasma membrane. Hepatocytes from the perivenular zone of the liver displayed a more intense fluorescence on their surface compared with periportal hepatocytes (338). The intensity of staining of the band in the plasma membrane was 14% that of the microsomes from preparations from 4-MP-treated rats and 26% of the microsomes from control preparations. The 2E1 in the plasma membrane was catalytically active with a low concentration of NDMA.

2. Kupffer cells

Chronic ethanol feeding results in an induction of 2E1 in rat Kupffer cells (138) (Fig. 6). The relative induction was of the same magnitude as in hepatocytes, but the 2E1 content was 10 times lower than in hepatocytes of the same animals. Immunocytochemical studies revealed that 2E1 is present in the endoplasmic reticulum of Kupffer cells in vivo and that it is also present in isolated Kupffer cells. There was also an induction of 2E1-dependent benzene hydroxylation by acetone in rat liver that was found to be more pronounced in Kupffer cells than in hepatocytes (143).

The induction of 2E1 in Kupffer cells by ethanol may have various consequences. It probably increases the intracellular levels of acetaldehyde, the toxic metabolite of ethanol. Kupffer cells have been shown to metabolize ethanol (333), and the relative contribution of 2E1 to overall

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

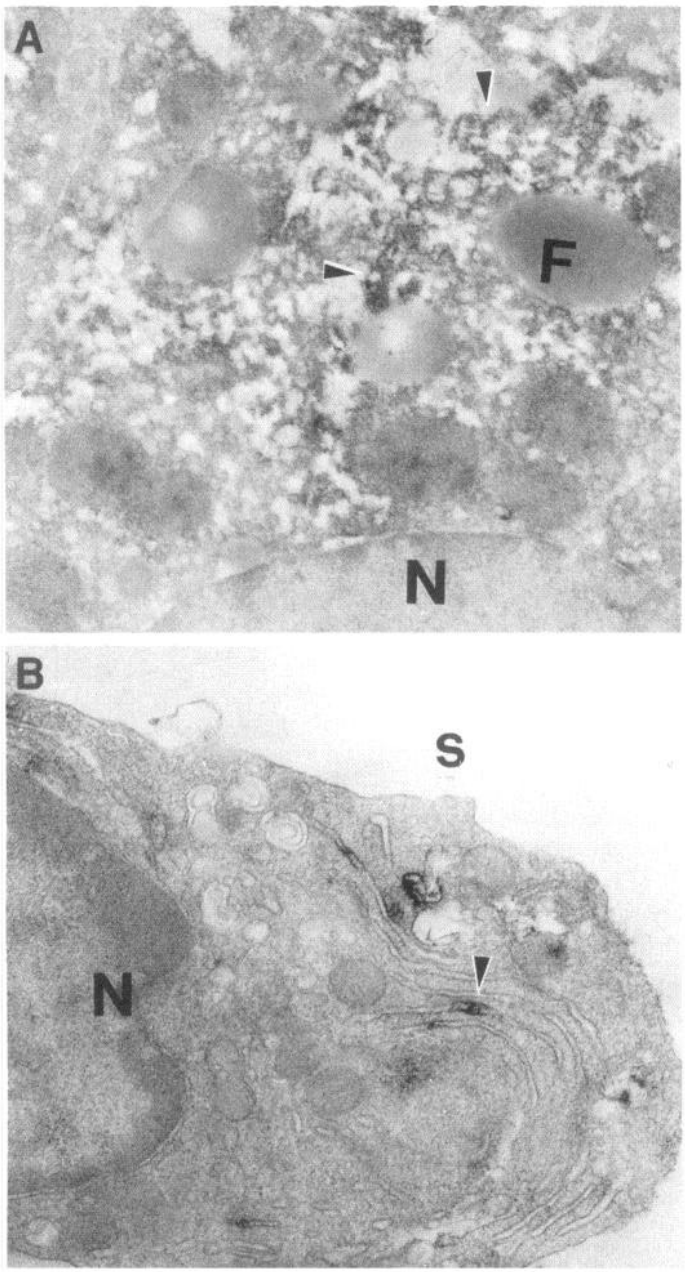

FIG. 6. Immunoelectron micrographs showing 2E1 staining in hepatocyte (*A*) and Kupffer cell from an ethanol-fed rat (*B*). Cytochrome *P*-4502E1 staining in hepatocyte is intense, and staining is in close association with proliferating endoplasmic reticulum (arrowheads). Kupffer cell exhibits a sparse 2E1 staining, which also appears to be associated with endoplasmic reticulum (arrowhead). N, nucleus; F, fat droplet; S, sinusoid. Magnification: *A*, ×18,000; *B*, ×20,000. [From Koivisto et al. (138).]

acetaldehyde production of these cells is probably much higher than in hepatocytes. In murine Kupffer cells, cytochrome *P*-450 inhibitors have been shown to inhibit ethanol metabolism by >50%, whereas ADH and catalase inhibitors decreased it only by <10% (333). Also, the finding that chronic ethanol feeding tends to increase ethanol metabolism in Kupffer cells (60) supports the hypothesis that ethanol-inducible 2E1 may be the major pathway of ethanol metabolism in these cells.

B. Extrahepatic Tissue

Although apparently not present under basal conditions (at least according to the immunohistochemical detection methods used), 2E1 was induced by ethanol to readily discernible levels in cheek mucosa, tongue, esophagus, and forestomach squamous epithelia, as well as in proximal colon surface epithelia in the rat. In contrast, immunoreactive 2E1 was not observed within epithelial cells of stomach fundic and antral mucosa, ileum, distal colon, or rectum, regardless of whether these tissues were obtained from alcohol-treated rats or their corresponding controls (273). Such results indicate that constitutive expression of 2E1 and its induction by alcohol in rat alimentary tract tissues is not a generalized phenomenon but is confined to only certain organs (or regions thereof) of the digestive canal (273). Interestingly, the sensitivity of the 2E1 immunohistochemical detection method used with rat alimentary tract tissues exceeded that of protein blotting. For instance, 2E1 immunostaining was clearly visible in esophageal mucosa epithelial cells from ethanol-treated rats but not on protein blots of microsomes prepared from whole esophageal mucosa (273). This phenomenon may partly explain why other investigators who used microsomal protein blotting failed to detect 2E1 in the alimentary tract (55).

The results of the study of Shimizu et al. (273) may provide a molecular basis for the acknowledged cocarcinogenic and cytotoxic effects of ethanol in alimentary tract and other extrahepatic tissues. For example, acrylonitrile is known to cause extensive gastrointestinal damage and tumors in rats. It is metabolized by intestinal mucosal cells, and 2E1 appears to be the major isoform involved (289).

Cytochrome *P*-4502E1 was detected on immunoblots of kidney and nasal mucosa microsomes (55), and it was increased in kidney as well as nasal microsomes by chronic ethanol treatment (54). A posttranscriptional regulation of mouse renal 2E1 by testosterone was also described (233).

Basal levels of 2E1 were 10–20 times lower in lung and kidney than in liver. Among the organs tested, lung appeared to be the tissue least sensitive to induction even after ethanol inhalation. After ethanol intoxication, immunostaining was increased in the alveolar cells of the lung and in the proximal convoluted tube of the kidney. The 2E1 activities decreased to control values in the three tissues tested within 24 h after cessation of intoxication (198). The presence in human lung microsomes of cytochrome *P*-450 immunochemically related to human liver 2E1 was also demonstrated (332).

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

Other studies have also shown that 2E1 is present in specific cells (76) and specific areas of the brain (278).

Induction of cytochromes in rabbit bone marrow (265) and lymphocytes (245) and in poorly controlled insulin-dependent diabetics (284) has also been reported and could therefore facilitate in vivo monitoring of this *P*-450.

VI. PHYSIOLOGICAL FUNCTIONS

A. Starvation and Ketone Metabolism

Cytochrome *P*-4502E1 is inducible by fasting in the rat (148), whereas it is decreased in liver microsomes of house musk shrew (*Suncus murinus*) (219). The increase may be due, at least in part, to ketones. Indeed, in rats (30), rabbits (142), and humans (249), acetone appears to be actively utilized, being metabolized by a microsomal acetone monooxygenase identified as 2E1 (32, 112). Acetone is both an inducer and a substrate of 2E1 (141, 343). The existence of a gluconeogenic pathway for acetone was shown by the incorporation of [^{14}C]acetone into glucose and amino acids during fasting and diabetic ketoacidosis (232, 248, 249). The conversion of acetone to acetol and then to methylglyoxal, both intermediates in the gluconeogenic pathway, was demonstrated in vitro (30, 31, 142). The contribution of 2E1 to the biotransformation of acetone was shown in vivo by the increase in blood acetone after the 2E1 inhibitors diallyl sulfide, diallyl sulfoxide, and diallyl sulfone (39). It is noteworthy, as already suggested by Reichard et al. (248), that acetone may be a significant gluconeogenic precursor in fasting humans, accounting for 10% of the gluconeogenic demands of humans fasted for 21 days. The mechanisms of the induction by fasting, acetone, and ethanol have been compared by Miller and Yang (209).

B. Obesity, Diabetes, and Lipid Metabolism

The obese overfed rat exhibits substantially higher levels of 2E1 and total cytochrome *P*-450 in liver microsomes compared with nonobese animals (260). Microsomal ethanol oxidation, acetaminophen activation, and *p*-nitrophenol hydroxylation (monooxygenase activities catalyzed by 2E1) are also enhanced in obese rats, reflecting their induced hepatic 2E1 content (247). Hepatic 2E1 content was also markedly elevated in diabetic rats to a degree similar to that of rats given acetone and slightly greater than that in ethanol-treated rats (282). The elevated levels of hepatic 2E1 found in spontaneously diabetic rats were highly correlated with plasma concentrations of the ketone β-hydroxybutyrate, suggesting that the 2E1 induction in diabetes is associated directly or indirectly with increased plasma levels of ketone bodies (12). Furthermore, cytochrome 2E1 is elevated in lymphocytes from poorly controlled insulin-dependent diabetics (284), and insulin downregulates 2E1 expression at the posttranscriptional level in a rat hepatoma cell line (48). Moreover, in hepatic and renal microsomes of rats with streptozocin-induced diabetes, 2E1 was induced (274). The mechanism of 2E1 induction in chemically induced and spontaneously diabetic rats was studied by Dong et al. (57).

Cytochrome *P*-4502E1, reconstituted with cytochrome b_5 and NADPH-cytochrome *P*-450 reductase, metabolizes lauric, stearic, oleic, linoleic, linolenic, and arachidonic acids. Two major metabolites, accounting for 78% of the total metabolism, were produced with arachidonic acid. The first one was identified as monohydroxylated eicosatetraenoic acids (HETEs), with the major one being 19-hydroxyeicosatetraenoic acid (19-HETE). The second metabolite was the ω-2 hydroxylated metabolite (18-HETE) (13, 155). Thus, when 2E1 is induced, it could contribute significantly to the formation of the ω-1 and ω-2 hydroxylated metabolites of arachidonic acid. Accordingly, the ratio of 20:4/18:2 was significantly lower in alcohol-fed rats compared with their pair-fed controls. Cytochrome *P*-4502E1 inhibitors inhibited the above effect of alcohol (216).

Lauric acid ω-1 hydroxylation was also demonstrated in human liver microsomes, and this activity was highly correlated with chlorzoxazone 6-hydroxylation (42). In turn, alcohol consumption enhances fatty acid ω-oxidation via induction of cytochrome *P*-4504A1 (200).

A purified system containing phospholipid, NADPH-cytochrome *P*-450 reductase, and 2E1 also oxidized glycerol to formaldehyde. However, glycerol is not a direct substrate for oxidation to formaldehyde by 2E1, but it is a substrate for an oxidant derived from interaction of iron with H_2O_2 generated by cytochrome *P*-450 (44).

VII. ROLE OF CYTOCHROME *P*-4502E1 IN THE METABOLISM OF ETHANOL AND ASSOCIATED PATHOLOGY

A. Role of Cytochrome *P*-4502E1 in Ethanol Oxidation After Chronic Ethanol Consumption

The fact that ethanol metabolism increases with rising ethanol concentrations well above the level needed to fully saturate the low K_m ADH, especially following chronic ethanol consumption, suggested the involvement of a non-ADH pathway (259). The MEOS was suggested, because it significantly increases in activity under these conditions (see sect. III*D*). Moreover, the acceleration of ethanol metabolism at higher ethanol concentrations explains that the disappearance of ethanol from the blood

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

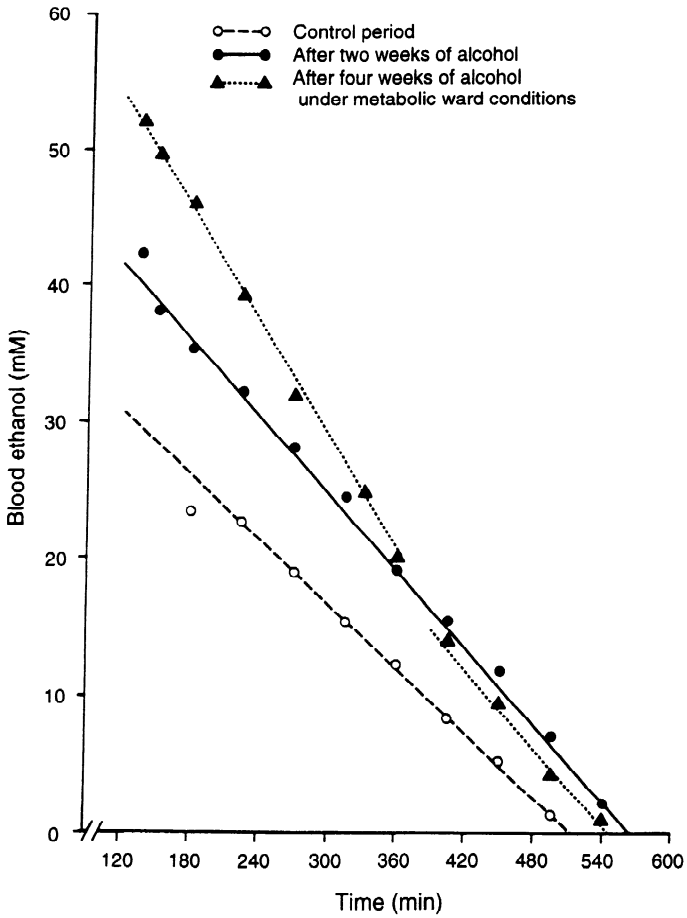

FIG. 7. Effect of chronic ethanol consumption on blood ethanol elimination curve in a human volunteer. Dose of ethanol administration was 1.0 g/kg in control experiment, 1.3 g/kg after 2 wk of alcohol, and 1.5 g/kg after 4 wk of alcohol. [Data from Salaspuro and Lieber (259).]

is not linear at high ethanol concentrations that fully saturate the ADH pathway (69, 170, 258, 259) (Fig. 7). Kinetic analysis in vivo indicated that ethanol elimination is best described by a two-compartment open model with parallel first-order and Michaelis-Menten elimination kinetics. The dose dependency of the elimination rate was attributed to contributions of the ADH pathway at lower blood ethanol concentration and of non-ADH first-order elimination pathways at higher blood ethanol concentrations (72). The contribution of the first order pathway increased with increases in the blood ethanol concentration and even exceeded that of the ADH pathway at high blood ethanol concentration. Finally, a mutant deermouse strain that lacks the hepatic low-K_m ADH (ADH−) nevertheless actively oxidizes ethanol (6, 25, 120–122, 271), and studies with stable isotopes indicated that this effect is mediated principally by the MEOS (6). Indeed, the rise in MEOS activity could account for all of the increases in ethanol metabolism of hepatocytes isolated from ADH− deermice (5) and for a major fraction of the increase in blood ethanol clearance in vivo (186). When ethanol is given parenterally, the MEOS rather than gastric ADH is the major pathway of ethanol oxidation in ADH− deermice, whereas both pathways contribute significantly to the metabolism of orally administered ethanol (107). That chronic ethanol feeding results in an increased activity of a non-ADH and noncatalase pathway in the liver was also shown in studies of liver microsomes, liver slices, and isolated hepatocytes. Ethanol oxidation was enhanced in isolated liver tissue by increasing the alcohol 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 (308). To test whether 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 noncatalase pathway 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 was more pronounced in ethanol-fed than in control animals (239). All these data indicate that a non-ADH pathway, most likely MEOS, represents a major mechanism for the acceleration of ethanol metabolism at high alcohol concentrations (Fig. 8*C*). A similar change was shown in human volunteers: alcohol consumption resulted in a progressive acceleration of blood alcohol clearance, particularly at high alcohol concentrations (Fig. 7) (259). In vitro, in primary cultures of human hepatocytes, ethanol increased 2E1, detected immunochemically (150).

B. Pathogenic Role of Cytochrome *P*-4502E1 in Alcoholic Liver Disease and Associated Oxidative Stress

In view of the capacity of 2E1 to generate reactive oxygen intermediates, such as superoxide radicals (Fig. 5) (see sect. IV*C*) (47) and the known toxicity of these free oxygen radical species, 2E1 plays a key role in the pathogenesis of liver injury. Indeed, Dai et al. (47) carried out experiments to stably and constitutively express the coding sequence of the human 2E1 in HepG2 cells, a human hepatoma-derived cell line, infected with recombinant retroviral vector. Results indicated that the human 2E1 is especially reactive in production of reactive oxygen intermediates and in catalysis of lipid peroxidation. There is increasing evidence that ethanol toxicity is linked to the increased production of reactive oxygen intermediates as evidenced by enhanced lipid peroxidation. DiLuzio (52, 53) was one of the first to report that ethanol produced increased lipid peroxidation in the liver and that the ethanol-induced fatty liver could be prevented by antioxidants.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

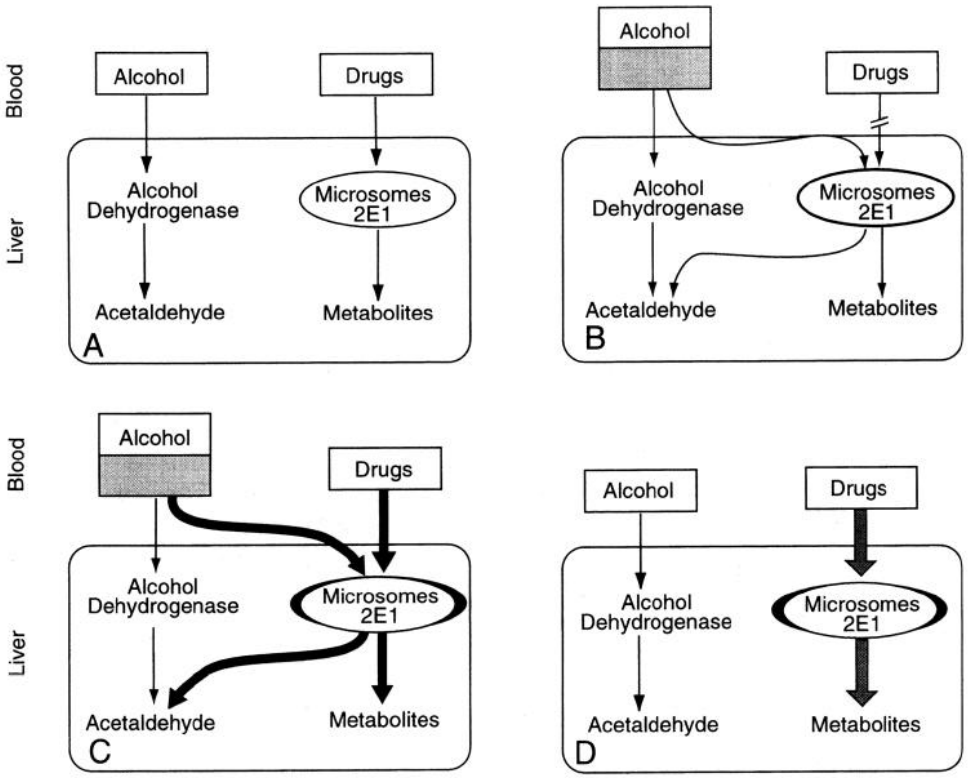

FIG. 8. Hepatic ethanol-drug interactions. *A*: ethanol metabolism at low concentration. *B*: microsomal drug metabolism is inhibited in presence of high concentrations of ethanol, in part through competition for a common microsomal detoxification process. *C*: microsomal induction after long-term alcohol consumption contributes to accelerated ethanol metabolism at high blood ethanol levels. *D*: increased drug metabolism and activation of xenobiotics (due to microsomal induction) persist after cessation of long-term alcohol consumption.

Several mechanisms by which ethanol could promote oxidative stress have been suggested (33), including prominently increased generation of oxygen- and ethanol-derived free radicals at the microsomal level, especially through the intervention of the ethanol-inducible 2E1 (226). Indeed, 2E1 reduces O_2 to superoxide anion and H_2O_2 in the absence of substrates for hydroxylation (81, 101) and even in their presence, since 2E1 is a leaky enzyme.

Membranous lipids are not the sole target of free radical attack (277). The increased microsomal generation of active oxygen derivatives could also contribute to ethanol toxicity through radical-mediated inactivation of metabolic enzymes (50), including 2E1 itself (148).

Furthermore, 2E1 activates a score of xenobiotics to highly hepatotoxic compounds. This pertains for instance to CCl_4. It is known that CCl_4 exerts its toxicity after conversion to an active compound (most likely trichloromethyl radical) in the microsomes, and alcohol pretreatment remarkably stimulates the toxicity of CCl_4 (87), with perivenular predominance, which can be explained by the selective presence and induction of 2E1 in that zone of the liver lobule (102, 312, 315). Indeed, the formation of trichloromethyl radicals from CCl_4 enhanced by ethanol treatment was reported by Reinke et al. (250). A large number of other organic compounds were found to show such a selective injurious action in the liver as well as other tissues of the alcoholic. These include other industrial solvents such as bromobenzene (92) and vinylidene chloride (275), as well as anesthetics such as enflurane (314) and halothane (294). Ethanol also markedly increased the activity of microsomal low-K_m benzene-metabolizing enzymes (218) and aggravated the hemopoietic toxicity of benzene. Thus commonly used industrial solvents and anesthetics, considered safe in normal subjects, may acquire unusual hepatotoxicity in heavy drinkers. Enhanced metabolism (and toxicity) pertains also to a variety of prescribed drugs, including isoniazid and phenylbutazone (14).

The same mechanism of hepatotoxicity also applies to some over-the-counter medications: therapeutic amounts of acetaminophen (2.5–4 g/day) can cause severe hepatic injury in alcoholics (267). In animals given ethanol for long periods, hepatotoxic effects peak after its withdrawal (263) (Fig. 8*D*) when ethanol is no longer competing (Fig. 8*B*) for the microsomal pathway but levels of the toxic metabolites are at their highest. Thus alcoholics are most vulnerable to the toxic effects of acetaminophen shortly after cessation of chronic drinking, when the still induced 2E1 activates acetaminophen to a highly toxic metabolite. The 2E1 induction also explains the increased vulnerability of these alcoholics to numerous other xenobiotics, as discussed in detail elsewhere (178, 179), including carcinogens (see sect. VII*C*).

Cytochrome *P*-4502E1 induction also results in strikingly increased oxidation of ethanol to acetaldehyde, a

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

highly reactive and toxic compound (184, 190). Acetaldehyde is rapidly metabolized to acetate, mainly by a mitochondrial low K_m aldehyde dehydrogenase, the activity of which is significantly reduced by chronic ethanol consumption (88). The decreased capacity of mitochondria of alcohol-fed subjects to oxidize acetaldehyde, associated with unaltered or even enhanced rates of ethanol oxidation (and therefore acetaldehyde generation because of MEOS induction; see above) results in an imbalance between production and disposition of acetaldehyde. The latter causes the elevated acetaldehyde levels observed after chronic ethanol consumption in humans (56) and in baboons, which revealed a tremendous increase of acetaldehyde in hepatic venous blood (182), reflecting high tissue levels. Acetaldehyde's toxicity is due, in part, to its capacity to form protein adducts. Indeed, acetaldehyde binds covalently to liver microsomal proteins (225), including 2E1 (11), and other macromolecules (195) such as collagen (10, 293). This protein binding results in an antibody response (97, 106), enzyme inactivation (280), and decreased DNA repair (64). It is also associated with a striking impairment of the capacity of the liver to utilize oxygen (182). Moreover, acetaldehyde promotes glutathione depletion, free radical-mediated toxicity, and lipid peroxidation (Fig. 5). The role of 2E1 in lipid peroxidation is complex. Some of the peroxidation products formed, for instance, organic hydroperoxides (such as cumyl hydroperoxide and tertiary butyl hydroperoxide), are also effective substrates. Cytochrome P-4502E1 may not only initiate lipid peroxidation (61, 152), but it may also continue to function in the elimination of hydroperoxides. However, the physiological significance of such reactions has not been demonstrated as yet.

Lipid peroxidation is not only a reflection of tissue damage; it may also play a pathogenic role, for instance, by promoting collagen production, as discussed elsewhere (181). In any event, the crucial role of lipid peroxidation in the pathogenesis of alcoholic liver injury was illustrated by the use of antioxidants, the most successful example being provided by the prevention, with polyenylphosphatidylcholine, of liver cirrhosis in the baboon (189, 193), and the attenuation of liver cirrhosis after CCl_4 or heterologous albumin in the rat (200), in association with a striking correction of the oxidative stress and lipid peroxidation (192).

Iron overload may play a contributory role, since chronic alcohol consumption results in increased iron uptake by hepatocytes (349) and because iron exposure accentuates the changes of lipid peroxidation and in the glutathione status of the liver cell induced by acute ethanol intoxication (324).

In addition to its role in increasing acetaldehyde production, the induced 2E1 may also contribute to acetaldehyde disposition. Indeed, Watanabe et al. (329) described conversion of aldehyde to carboxylic acid catalyzed by a cytochrome P-450 isozyme, and Terelius et al. (302) reported that liver microsomes from starved and acetone-treated rats catalyzed NADPH-supported metabolism of acetaldehyde at a rate eightfold higher than corresponding control microsomes; the maximum velocity was ~6 nmol · mg microsomal protein ^{1} · min ^{1}, and the apparent K_m 30 μM. The reaction was efficiently inhibited by anti-2E1 immunoglobulin G. Although of theoretical interest, such a system of acetaldehyde disposition is hardly of practical significance, since it represents only 2% of the amount of acetaldehyde formed by the microsomal system (184) and a much smaller fraction of that either produced or disposed of by the cytosolic and mitochondrial dehydrogenase systems.

In summary, the crucial role of 2E1 and its induction in the pathogenesis of alcoholic liver injury through free radical and acetaldehyde generation and promotion of adduct formation and peroxidation is now widely recognized (71, 221), as reviewed in more detail elsewhere (177, 178, 180). Accordingly, 2E1 inhibitors should be beneficial in preventing or improving ethanol-induced liver disorders. Indeed, this has now been demonstrated for some lipid changes (216). In view of the increasing number of 2E1 inhibitors now available (see sect. IV*D*), some of which are innocuous, this is obviously a promising field for future studies.

C. Role of Cytochrome *P*-4502E1 in Carcinogenesis

There is an association between alcohol misuse and an increased incidence of upper alimentary and respiratory tract cancers (190). Many factors have been incriminated, one of which is the effect of ethanol on the cytochrome P-450-dependent activation of procarcinogens to carcinogens. This effect has been demonstrated with the use of microsomes isolated from a variety of tissues, including the liver (the principal site of xenobiotic metabolism) (75), the lungs (269), and intestines (268, 269) (the chief portals of entry for tobacco smoke and dietary carcinogens, respectively), and the esophagus (66) (where ethanol consumption is a major risk factor in the development of cancer). Nitrosamines have been implicated. As already mentioned, nitrosamines are metabolized by 2E1 even at low concentrations (343), with a resulting increased mutagen production after 2E1 induction, which may contribute to the enhanced carcinogenesis.

D. Role of Cytochrome *P*-4502E1 in Energy Balance

Although ethanol is rich in energy (7.1 kcal/g), chronic consumption of substantial amounts of alcohol is not associated with the expected effect on body weight (176). Furthermore, isocaloric substitution of carbohy-

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

drates by ethanol under metabolic ward conditions resulted in weight loss, and addition of ethanol to an otherwise normal diet did not produce expected weight gain (242). This energy deficit cannot be explained merely on the basis of maldigestion or malabsorption but has been attributed, in part, to induction of the MEOS (a metabolic pathway which oxidizes ethanol without associated chemical energy production; Fig. 1). Alternate or additional mechanisms invoked include increased sympathetic tone and associated thermogenesis and/or enhanced ATP breakdown (with increased purine catabolism) secondary to the acetate produced from ethanol. Although attractive, all these hypotheses do not fully explain the lack of weight loss when alcohol is consumed with a very low-fat diet (5% of energy) (191), which suggests that an alteration in the energy utilization derived from fat plays a major role in the ethanol-induced energy deficit. One possible mechanism is uncoupling of oxidation with phosphorylation in mitochondria damaged by chronic ethanol consumption, in part because of the acetaldehyde generated (36, 202a), as also reviewed elsewhere (176, 178).

VIII. MARKERS OF CYTOCHROME *P*-4502E1 INDUCTION AND THEIR CLINICAL APPLICATIONS

As discussed in section VII*B*, 2E1 can activate commonly used anesthetics and therapeutic agents to highly toxic metabolites. Knowledge of the state of induction of 2E1 may therefore have some practical applications, particularly in situations in which induction of 2E1 can be suspected, for instance, in heavy drinkers, or individuals treated with agents capable of 2E1 induction (e.g., isoniazid) or physiological states possibly associated with such induction (e.g., diabetic ketoacidosis). Therefore, a noninvasive test of 2E1 would be of value in screening alcoholic patients requiring general anesthesia or in need of treatment with agents activated to toxins by 2E1, such as acetaminophen (see sect. VII*B*). Although direct hepatic 2E1 quantification requires a liver biopsy, noninvasive approaches may be feasible. Raucy et al. (245) reported that the 2E1 content from freshly isolated circulating lymphocytes correlated with hepatic 2E1 in rabbits administered ethanol. If verified in alcoholics, this would provide a noninvasive test for 2E1 induction. Furthermore, as already mentioned, 2E1 can metabolize chlorzoxazone (CZX) (238), commonly used as a muscle pain relaxant and analgesic. Thus the measurement of the metabolite of CZX (6-hydroxy-CZX) could serve as a marker of 2E1. In human liver microsomes, the hydroxylation of CZX correlated with the concentration of 2E1 and was inhibited by incubation with antibody to 2E1 (238). Furthermore, after administration of CZX, recently drinking alcoholics had increased plasma levels of 6-hydroxy-CZX; the levels of this metabolite following a repeat CZX challenge decreased during the withdrawal period from alcohol (80, 198). Current investigations are ongoing to determine how well such noninvasive measurements of 6-hydroxy-CZX correlate with actual hepatic 2E1 measurements in humans. In the rat, cytochromes *P*-4501A1 and 3A are also involved in CZX hydroxylation (109), but because of species differences, such studies cannot be directly extrapolated to humans.

IX. ULTRASTRUCTURAL CHANGES AND ASSOCIATED INDUCTION OF OTHER MICROSOMAL ENZYMES

As mentioned in section I, heavy ethanol consumption is accompanied by a proliferation of the membranes of the endoplasmic reticulum of the liver. This proliferation was associated with increased 2E1 as shown by immunoelectron microscopy not only in hepatocytes (Fig. 6*A*) but also in Kupffer cells (Fig. 6*B*), albeit to a lesser degree in the latter. The endoplasmic reticulum harbors scores of other microsomal enzymes, the activity of which is increased in association with the proliferation. Accordingly, the microsomal induction resulting from long-term alcohol consumption accelerates not only the oxidation of ethanol, but it also increases the metabolism of many other microsomal substrates, including the synthesis of triacylglycerols (264). Its main interaction, however, is with other xenobiotics because other cytochromes *P*-450s are increased in their activity toward multiple drugs, including phenytoin, tolbutamide, propranolol, and rifampin (177). Ethanol administration to volunteers under metabolic ward conditions resulted in a striking increase in the rate of blood clearance of meprobamate and pentobarbital sodium (211). The metabolic drug tolerance persists several days to weeks after the cessation of alcohol abuse, and the duration of recovery varies with each drug (93). During that period, the dosage of these drugs has to be increased to offset the increased breakdown. Furthermore, ethanol consumption has been shown to depress hepatic levels of vitamin A in humans (164) and in animals, even when given with diets containing large amounts of vitamin A (263), reflecting in part accelerated microsomal degradation of the vitamin. Indeed, new hepatic pathways of microsomal retinol metabolism, inducible by either ethanol or drug administration, have been discovered (162, 165). Furthermore, reconstituted systems with purified forms of cytochrome *P*-450 revealed that retinoic acid (161) and retinol (165) can serve as substrates for monooxygenation. Immunoblots performed with a monospecific antibody directed against human *P*-4502C8 (2C8) showed that appreciable amounts of this enzyme were present in human liver microsomes. The same antibody significantly inhibited retinol metabolism in liver micro-

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

somes and in a system reconstituted with 2C8, establishing the role of this 2C8 in retinol metabolism; 2C8 also converted retinoic acid to polar metabolites (163). When ethanol and phenobarbital were combined, a marked potentiation of the hepatic vitamin A depletion was observed (168). Because alcohol abuse is often associated clinically with overuse of other drugs, and because drug use may also be associated with severe hepatic vitamin A depletion (167), this potentiation may be meaningful in terms of vitamin A depletion in an appreciable segment of the population. Hepatic vitamin A depletion causes adverse effects (169). Excess vitamin A is also hepatotoxic (166), possibly because of toxic metabolites resulting from enhanced retinol degradation.

Contrasting with the inductive effect of long-term ethanol consumption, after short-term administration, inhibition of hepatic drug metabolism is found, primarily because of its direct competition for a common metabolic process involving the cytochrome *P*-450 and its cofactor (e.g., NADPH) (177) (Fig. 8*B*). Methadone provides a cogent example of this dual interaction. Whereas long-term ethanol consumption leads to increased hepatic microsomal metabolism of methadone and decreased levels in the brain and liver, short-term administration has the opposite effect: it inhibits microsomal demethylation of methadone and enhances brain and liver concentrations of the drug (16). These effects may be of clinical relevance, because ~50% of the patients taking methadone are alcohol abusers. The combination of ethanol with tranquilizers and barbiturates also results in increased drug concentrations in the blood, sometimes to dangerously high levels, as commonly observed in successful suicides, and discussed in detail elsewhere (177).

X. CONCLUSIONS AND FUTURE APPLICATIONS

Until three decades ago, it was believed that ethanol is oxidized in the liver mainly by ADH, with a minor contribution of catalase, two noninducible pathways that could not explain the metabolic tolerance to ethanol that develops in the heavy drinker. This tolerance is now understood on the basis of the discovery of MEOS comprising an ethanol-inducible cytochrome *P*-450 (2E1). The regulation of 2E1 expression is complex and still debated, but the 2E1 induction is associated with increased release of toxic oxygen free radicals and the highly reactive metabolite acetaldehyde, both playing a significant role in the pathogenesis of alcoholic liver injury. Of even greater significance is the extraordinary capacity of 2E1 to activate many xenobiotics to highly toxic products, thereby explaining the increased vulnerability of the heavy drinker to industrial solvents, anesthetics, commonly used medications, analgesics (acetaminophen), and carcinogens. The induction "spills over" to other microsomal *P*-450s, such as those involved in the degradation of retinol, thereby explaining its depletion. Cytochrome *P*-4502E1 is also involved physiologically in the metabolism of lipids and ketones (in starvation, obesity, and diabetes). This physiological role, however, appears to be minor compared with the toxic potential and, therefore, recently discovered effective 2E1 inhibitors may eventually provide useful tools for the prevention and treatment of the hepatotoxicity associated with heavy drinking, as suggested by successful pioneering experimental studies. Furthermore, some substrates and products of 2E1 metabolism are harmless and are being assessed as potential markers of excessive alcohol consumption and of relapse in alcoholics. Such clinical tests are needed for the early detection of 2E1 induction to ensure judicious guidance of therapy in the heavy drinkers.

Constructive comments and upgrading of Table 1 by Dr. I. B. Tsyrlov and editorial assistance of L. M. DeCarli are gratefully acknowledged. L. M. DeCarli's long-standing collaboration as well as those of a series of co-workers too numerous to be mentioned individually here were invaluable over the three decades during which the MEOS/2E1 field has evolved. S. Dickerson is thanked for the typing of the manuscript.

Original studies of the author were supported by National Institutes of Health Grants AA-05934, AA-07275, and AA-03508 and by the Department of Veterans Affairs and the Kingsbridge Research Foundation.

REFERENCES

1. ALBANO, E., A. TOMASI, L. GORIA-GATTI, AND M. U. DIANZANI. Spin trapping of free radical species produced during the microsomal metabolism of ethanol. *Chem. Biol. Interact.* 65: 223–234, 1988.
2. ALBANO, E., A. TOMASI, L. GORIA-GATTI, J. O. PERSSON, Y. TEREIUS, L. GORIA-GATTI, M. INGELMAN-SUNDBERG, AND M. U. DIANZANI. Role of ethanol-inducible cytochrome *P*450 (*P*450IIE1) in catalyzing the free radical activation of alphatic alcohols. *Biochem. Pharmacol.* 41: 1895–1902, 1991.
3. ALBANO, E., A. TOMASI, L. GORIA-GATTI, G. POLI, V. VANNINI, AND M. U. DIANZANI. Free radical metabolism of alcohol by rat liver microsomes. *Free Radical Res. Commun.* 3: 243–249, 1987.
4. ALBANO, E., A. TOMASI, AND M. INGELMAN-SUNDBERG. Spin trapping of alcohol derived radicals in microsomes and reconstituted systems by electron spin resonance. *Methods Enzymol.* 223: 117–127, 1994.
5. ALDERMAN, J., S. KATO, AND C. S. LIEBER. The microsomal ethanol oxidizing system mediates metabolic tolerance to ethanol in deermice lacking alcohol dehydrogenase. *Arch. Biochem. Biophys.* 27: 33–39, 1989.
6. ALDERMAN, J., T. TAKAGI, AND C. S. LIEBER. Ethanol metabolizing pathways in deermice: estimation of flux calculated from isotope effects. *J. Biol. Chem.* 262: 7497–7503, 1987.
7. AMET, Y., F. BERTHOU, T. GOASDUFF, J. P. SALAUN, L. LEBRETON, AND J. F. MENEZ. Evidence that cytochrome *P*450 2E1 is involved in the (ω-1)-hydroxylation of lauric acid in rat liver microsomes. *Biochem. Biophys. Res. Commun.* 203: 1168–1174, 1994.
8. ARAI, M., E. R. GORDON, AND C. S. LIEBER. Decreased cytochrome oxidase activity in hepatic mitochondria after chronic ethanol consumption and the possible role of decreased cytochrome aa_3 content and changes in phospholipids. *Biochim. Biophys. Acta* 797: 320–327, 1984.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

9. BADGER, T. M., J. HUANG, M. RONIS, AND C. K. LUMPKIN. Induction of cytochrome *P*450 2E1 during chronic ethanol exposure occurs via transcription of the *CYP 2E1* gene when blood alcohol concentrations are high. *Biochem. Biophys. Res Commun.* 190: 780–785, 1993.
10. BARAONA, E., W. LIU, X. L. MA, G. SVEGLIATI-BARONI, AND C. S. LIEBER. Acetaldehyde-collagen adducts in *N*-nitrosodimethylamine-induced liver cirrhosis in rats. *Life Sci.* 52: 1249–1255, 1993.
11. BEHRENS, U. J., M. HOERNER, J. M. LASKER, AND C. S. LIEBER. Formation of acetaldehyde adducts with ethanol inducible *P*-450IIE1 in vivo. *Biochem. Biophys. Res. Commun.* 154: 584–590, 1988.
12. BELLWARD, G. D., T. CHANG, J. H. RODRIGUES, J. H. MCNEIL, S. MAINES, D. E. RYAN, W. LEVIN, AND P. E. THOMAS. Hepatic cytochrome *P*-450J induction in the spontaneously diabetic BB rat. *Mol. Pharmacol.* 33: 140–143, 1987.
13. BERTHOU, F., T. GOASDUFF, Y. DREANO, AND J. F. MENEZ. Caffeine metabolism by hepatic microsomes of rats treated by inducers of CYP1A and CYP2E1 (Abstract). *Int. Symp. Microsomal Drug Oxidations 10th Toronto Canada 1994*, p. 606.
14. BESKID, M., J. BIALCK, J. DZIENISZEWSKI, J. SADOWSKI, AND J. TLALKA. Effect of combined phenylbutazone and ethanol administration on rat liver. *Exp. Pathol.* 18: 487–491, 1980.
15. BESTERVELT, L. L., A. D. VAZ, AND M. J. COON. Inactivation of ethanol-inducible cytochrome *P*-450 and other microsomal *P*-450 isozymes by *trans*-4-hydroxy-2-nonenal, a major product of membrane lipid peroxidation. *Proc. Natl. Acad. Sci. USA* 92: 3764–3768, 1995.
16. BOROWSKY, S. A., AND C. S. LIEBER. Interaction of methadone and ethanol metabolism. *J. Pharmacol. Exp. Ther.* 207: 123–129, 1978.
17. BOTTO, F., E. SEREE, S. E. KHYARI, G. DESOUSA, A. MASSACRIER, M. PLACIDI, P. CAU, W. PELLET, R. RAHMANI, AND Y. BARRA. Tissue specific expression and methylation of the human *CYP2E1* gene. *Biochem. Pharmacol.* 48: 1095–1103, 1994.
18. BRADY, J. F., H. ISHIZAKI, J. M. FUKUTO, M. C. LIN, A. FADEL, J. M. GUPAC, AND C. S. YANG. Inhibition of cytochrome *P*-450 2E1 by diallyl sulfide and its metabolites. *Chem. Res. Toxicol.* 4: 642–647, 1991.
19. BRADY, J. F., M. J. LEE, M. LI, H. ISHIZAKI, AND C. S. YANG. Diethyl ether as a substrate for acetone/ethanol-inducible cytochrome *P*-450 and as an inducer for cytochrome(s) *P*-450. *Mol. Pharmacol.* 33: 148–154, 1987.
20. BRADY, J. F., D. LI, H. ISHIZAKI, AND C. S. YANG. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer Res.* 48: 5937–5940, 1988.
21. BRADY, J. F., M. LI, H. ISHIZAKI, M. LEE, S. M. NING, F. XIAO, AND C. S. YANG. Induction of cytochromes *P*-450IIE1 and *P*-450IIB1 by secondary ketones and the role of *P*-450IIE1 in chloroform metabolism. *Toxicol. Appl. Pharmacol.* 100: 342–349, 1989.
22. BRADY, J. F., F. XIAO, S. M. NING, AND C. S. YANG. Metabolism of methyl *tertiary* butyl ether by rat liver microsomes. *Arch. Toxicol.* 64: 157–160, 1990.
23. BRADY, J. F., F. XIAO, M. H. WANG, Y. LI, S. M. NING, J. M. GAPAC, AND C. S. YANG. Effects of disulfiram on hepatic *P*-450IIE1, other microsomal enzymes, and hepatotoxicity in rats. *Toxicol. Appl. Pharmacol.* 108: 366–373, 1991.
24. BUCHLER, R., K. O. LINDROS, K. VON BOGUSLAWSKY, P. KARKKAINEN, J. MAKINEN, AND M. INGELMAN-SUNDBERG. Perivenous expression of ethanol-inducible cytochrome *P*-450IIE1 in livers from alcoholics and chronically ethanol-fed rats. *Alcohol Alcohol.* 1, *Suppl.*: 311–315, 1991.
25. BURNETT, K. G., AND M. R. FELDER. Ethanol metabolism in peromyscus genetically deficient in alcohol dehydrogenase. *Biochem. Pharmacol.* 28: 1–8, 1980.
26. CARR, L. G., J. Y. HARTLEROAD, Y. B. LIANG, C. MENDENHALL, T. MORITZ, AND H. THOMASSON. Polymorphism at the *P*-450IIE1 locus is not associated with alcoholic liver disease in caucasian men. *Alcohol Clin. Exp. Res.* 19: 182–184, 1995.
27. CARRIERE, V., T. GOASDUFF, D. RATANASAVANH, F. MOREL, J.-C. GAUTIER, A. GUILLOUZO, P. BEAUNE, AND F. BERTHOU. Both cytochromes *P*-450 2E1 and 1A1 are involved in the metabolism of chlorzoxazone. *Chem. Res. Toxicol.* 6: 852–857, 1993.
28. CARROCCIO, A., D. WU, AND A. I. CEDERBAUM. Ethanol increases content and activity of human cytochrome *P*-4502E1 in a transduced HepG2 cell line. *Biochem. Biophys. Res. Commun.* 203: 727–733, 1994.
29. CARTER, E. A., AND K. J. ISSELBACHER. The role of microsomes in the hepatic metabolism of ethanol. *Ann. NY Acad. Sci.* 179: 282–294, 1971.
30. CASAZZA, J. P., M. E. FELVER, AND R. L. VEECH. The metabolism of acetone in rat. *J. Biol. Chem.* 259: 231–236, 1984.
31. CASAZZA, J. P., AND J. L. FU. Measurement of acetol in serum. *Anal. Biochem.* 148: 344–348, 1985.
32. CASAZZA, J. P., AND R. L. VEECH. The production of 1,2 propanediol in ethanol treated rats. *Biochem. Biophys. Res. Commun.* 129: 426–430, 1985.
33. CEDERBAUM, A. I. Introduction: role of lipid peroxidation and oxidative stress in alcohol toxicity. *Free Radical Biol. Med.* 7: 537–539, 1989.
34. CEDERBAUM, A. I. Oxygen radical generation by microsomes: role of iron and implications for alcohol metabolism and toxicity. *Free Radical Biol. Med.* 7: 559–567, 1989.
35. 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.
36. CEDERBAUM, A. I., C. S. LIEBER, AND E. RUBIN. Effects of chronic ethanol treatment on mitochondrial functions. *Arch. Biochem. Biophys.* 165: 560–569, 1974.
37. CHANCE, B. An intermediate compound in the catalase–hydrogen reaction. *Acta Chem. Scand.* 1: 236–267, 1947.
38. CHEDID, A., AND V. NAIR. Diurnal rhythm in endoplasmic reticulum of rat liver: electron microscopic study. *Science Wash. DC* 175: 176–179, 1972.
39. CHEN, L., M. LEE, J.-Y. HONG, W. HUANG, E. WANG, AND C. S. YANG. Relationship between cytochrome *P*-450 2E1 and acetone catabolism in rats as studied with diallyl sulfide as an inhibitor. *Biochem. Pharmacol.* 48: 2199–2205, 1994.
40. CHIELI, E., M. SAVIOZZI, S. MENICAGLI, T. BRANCA, AND P. G. GERVASI. Hepatotoxicity and *P*-4502E1 dependent metabolic oxidation of *N,N*-dimethylformamide in rats and mice. *Arch. Toxicol.* 69: 165–170, 1995.
41. CHIELI, E., M. SAVIOZZI, P. PUCCINI, V. LONGO, AND P. G. GERVASI. Possible role of the acetone-inducible cytochrome *P*-450IIE1 in the metabolism and hepatotoxicity of thiobenzamide. *Arch. Toxicol.* 64: 122–127, 1990.
42. CLARK, S. E., S. J. BALDWIN, J. C. BLOOMER, A. D. AYRTON, R. S. SOZIO, AND R. J. CHENERY. Lauric acid as a model substrate for the simultaneous determination of cytochrome *P*-450 2E1 and 4A in hepatic microsomes. *Chem. Res. Toxicol.* 7: 836–842, 1994.
43. CLEJAN, L. A., AND A. I. CEDERBAUM. Oxidation of pyrazole by reconstituted systems containing cytochrome *P*-450 IIE1. *Biochim. Biophys. Acta* 1034: 233–237, 1990.
44. CLEJAN, L. A., AND A. I. CEDERBAUM. Role of cytochrome *P*-450 in the oxidation of glycerol by reconstituted systems and microsomes. *FASEB J.* 6: 765–770, 1992.
45. 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.
46. CONNEY, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317–366, 1967.
47. DAI, Y., J. RASHBA-STEP, AND A. I. CEDERBAUM. Stable expression of human cytochrome *P*-4502E1 in HepG2 cells: characterization of catalytic activities and production of reactive oxygen intermediates. *Biochemistry* 32: 6928–6937, 1993.
48. DEWAZIERS, I., M. GARLATTI, J. BOUGUET, P. H. BEAUNE, AND R. BAROUKI. Insulin down-regulates cytochrome *P*-450 2B and 2E expression at the post-transcriptional level in the rat hepatoma cell line. *Mol. Pharmacol.* 47: 474–479, 1995.
49. DICKER, E., AND A. I. CEDERBAUM. Hydroxyl radical generation by microsomes after chronic ethanol consumption. *Alcohol. Clin. Exp. Res.* 11: 309–314, 1987.
50. DICKER, E., AND A. I. CEDERBAUM. Increased oxygen radical dependent inactivation of metabolic enzymes by liver microsomes after chronic ethanol consumption. *FASEB J.* 2: 2901–2906, 1988.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

51. DIEHL, A. M., H. C. BISGAARD, B. T. KREN, AND C. J. STEER. Ethanol interferes with regeneration-associated changes in biotransforming enzymes: a potential mechanism underlying ethanol's carcinogenicity? *Hepatology* 13: 722–727, 1991.
52. DiLUZIO, N. R. Prevention of the acute ethanol induced fatty liver by the simultaneous administration of anti-oxidants. *Life Sci.* 3: 113–119, 1964.
53. DiLUZIO, N. R. The role of lipid peroxidation and antioxidants in ethanol induced lipid alterations. *Exp. Mol. Pathol.* 8: 394–402, 1968.
54. DING, X., AND M. J. COON. Induction of cytochrome *P*-450 isozyme 3a (*P*-450IIE1) in rabbit olfactory mucosa by ethanol and acetone. *Drug Metab. Dispos.* 18: 742–745, 1988.
55. DING, X., D. R. KOOP, B. I. CRUMP, AND M. J. COON. Immunochemical identification of cytochrome *P*-450 isozyme 3a [*P*-450ALC] in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Mol. Pharmacol.* 30: 370–378, 1986.
56. DI PADOVA, C., T. M. WORNER, R. J. K. JULKUNEN, AND C. S. LIEBER. Effects of fasting and chronic alcohol consumption on the first pass metabolism of ethanol. *Gastroenterology* 92: 1169–1173, 1987.
57. DONG, Z., J. HONG, Q. MA, D. LI, J. BULLOCK, F. J. GONZALEZ, S. S. PARK, H. V. GELBOIN, AND C. S. YANG. *Mechanism of Induction of Cytochrome P-450AC (P-450J) in Chemically Induced and Spontaneously Diabetic Rats.* Orlando, FL: Academic, 1988, p. 29–35.
58. DOWSLEY, T. F., P. G. FORKERT, L. A. BENESCH, AND J. L. BOLTON. Reaction of glutathione with the electrophilic metabolites of 1,1-dichloroethylene. *Chem. Biol. Interact.* 95: 227–244, 1995.
59. DUESCHER, R. J., AND A. A. ELFARRA. Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence for major roles by cytochromes *P*-450 2D6 and 2E1. *Arch. Biochem. Biophys.* 311: 342–349, 1994.
60. EARNEST, D. L., E. R. ABRIL, C. S. JOLLEY, AND D. C. ESKELSON. Kupffer cell function is modified by both diet and ethanol. *Adv. Biosci.* 86: 181–190, 1993.
61. EKSTROM, G., AND M. INGELMAN-SUNDBERG. Rat liver microsomal NADPH supported oxide activity and lipid peroxidation dependent on ethanol-inducible cytochrome *P*-450 (*P*-450IIE1). *Biochem. Pharmacol.* 38: 1313–1319, 1989.
62. ELIASSON, E., I. JOHANSSON, AND M. INGELMAN-SUNDBERG. Ligand dependent maintenance of ethanol inducible cytochrome *P*-450 in primary rat hepatocyte cell cultures. *Biochem. Biophys. Res. Commun.* 150: 436–443, 1988.
63. ELIASSON, E., S. MKRTCHIAN, AND M. INGELMAN-SUNDBERG. Hormone- and substrate-regulated intracellular degradation of cytochrome *P*-450 (2E1) involving MgATP-activated rapid proteolysis in the endoplasmic reticulum membranes. *J. Biol. Chem.* 267: 15765–15769, 1992.
64. ESPINA, N., V. LIMA, C. S. LIEBER, AND A. J. GARRO. In vitro and in vivo inhibitory effect of ethanol and acetaldehyde on O^6-methylguanine transferase. *Carcinogenesis* 9: 761–766, 1988.
65. FABRY, T. L., AND C. S. LIEBER. The photochemical action spectrum of the microsomal ethanol oxidizing system. *Alcohol. Clin. Exp. Res.* 3: 219–222, 1979.
66. FARINATI, F., Z. ZHOU, J. BELLAH, C. S. LIEBER, AND A. J. GARRO. Effect of chronic ethanol consumption on activation of nitrosopyrrolidine to a mutagen by rat upper alimentary tract, lung and hepatic tissue. *Drug Metab. Dispos.* 13: 210–214, 1985.
67. FEIERMAN, D. E., AND A. I. CEDERBAUM. Role of cytochrome *P*-450 IIE1 and catalase in the oxidation of acetonitrile to cyanide. *Chem. Res. Toxicol.* 2: 359–366, 1989.
68. FEIERMAN, D. E., G. W. WINSTON, AND A. I. CEDERBAUM. Ethanol oxidation by hydroxyl radicals: role of iron chelates, superoxide and hydrogen peroxide. *Alcohol. Clin. Exp. Res.* 9: 95–102, 1985.
69. FEINMAN, L., E. BARAONA, S. MATSUZAKI, M. KORSTEN, AND C. S. LIEBER. Concentration dependence of ethanol metabolism in vivo in rats and man. *Alcohol. Clin. Exp. Res.* 2: 381–385, 1978.
70. FIALA, E. S. Inhibition of carcinogen metabolism and action by disulfiram, pyrazole, and related compounds. In: *Inhibition of Tumor Induction and Development*, edited by M. S. Zadeck and M. Lipkin. New York: Plenum, 1981, p. 23–69.
71. FRENCH, S. W., K. WONG, L. JUI, E. ALBANO, A. L. HAGBJORK, AND M. INGELMAN-SUNDBERG. Effect of ethanol on cytochrome *P*-450 2E1 (CYP2E1) lipid peroxidation, and serum protein adduct formation in relation to liver pathology pathogenesis. *Exp. Mol. Pathol.* 58: 61–75, 1993.
72. FUJIMIYA, T., K. YAMAOKA, AND Y. FUKUI. Parallel first-order and Michaelis Menten elimination kinetics of ethanol: respective role of alcohol dehydrogenase (ADH), non-ADH and first order pathways. *J. Pharmacol. Exp. Ther.* 249: 311–317, 1989.
73. FUKUDA, T., Y. IMAI, M. KOMORI, M. NAKAMURA, E. KUSUNOSE, K. SATOOCHI, AND M. KUSUNOSE. Different mechanisms of regioselection of fatty acid hydroxylation by laurate (omega-1) hydroxylating *P*-450s, *P*-450 2C2 and *P*-450 2E1. *J. Biochem. Tokyo* 115: 338–344, 1994.
74. GANNETT, P. M., P. IVERSEN, AND T. LAWSON. The mechanism of inhibition of cytochrome *P*-450IIE1 by dihydrocapsaicin. *Bioorg. Chem.* 18: 185–198, 1990.
75. 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.
76. GENG, J., AND H. W. STROBEL. Identification of cytochromes *P*-450 1A2, 2A1, 2C7, 2E1 in rat glioma C6 cell line by RT PCR and specific restriction enzyme digestion. *Biochem. Biophys. Res. Commun.* 197: 1179–1184, 1993.
77. GENTER, M. B., N. J. DEAMER, Y. CAO, AND P. E. LEVI. Effects of *P*-450 inhibition and induction on the olfactory toxicity of β-iminodipropionitrile (IDPN) in the rat. *J. Biochem. Toxicol.* 9: 31–39, 1994.
78. GEORGE, J., C. LIDDLE, M. MURRAY, K. BYTH, AND G. C. FARRELL. Pretranslational regulation of *P*-450 genes is responsible for disease specific changes of individual *P*-450 enzymes among patients with cirrhosis. *Biochem. Pharmacol.* 49: 873–881, 1995.
79. GILLAM, E. M., Z. GUO, AND F. P. GUENGERICH. Expression of modified human cytochrome *P*-450 2E1 in *Escherichia coli*, purification, and spectral and catalytic properties. *Arch. Biochem. Biophys.* 312: 59–66, 1994.
80. GIRRE, C., D. LUCAS, E. HISPARD, C. MENEZ, S. DALLY, AND J. F. MENEZ. Assessment of cytochrome *P*-4502E1 induction in alcoholic patient by chlorzoxazone pharmacokinetics. *Biochem. Pharmacol.* 47: 1503–1508, 1994.
81. GORSKY, L. D., D. R. KOOP, AND M. J. COON. On the stoichiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome *P*-450. *J. Biol. Chem.* 259: 6812–6817, 1984.
82. GRUENKE, L. D., K. KONOPKA, D. R. KOOP, AND L. WASKELL. Characterization of halothane oxidation by hepatic microsomes and purified cytochrome *P*-450 using gas chromatographic mass spectrometric assay. *J. Pharmacol. Exp. Ther.* 246: 454–459, 1988.
83. GUENGERICH, F. P. Reactions and significance of cytochrome *P*-450 enzymes. *J. Biol. Chem.* 266: 10019–10022, 1991.
84. GUENGERICH, F. P., D. H. KIM, AND M. IWASAKI. Role of human cytochrome *P*-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.* 4: 168–179, 1991.
85. GUENGERICH, F. P., AND C. G. TURVY. Comparison of levels of several human microsomal cytochrome *P*-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J. Pharmacol. Exp. Ther.* 256: 1189–1194, 1991.
86. HA, H. P., J. CHEN, A. U. FREIBURGHAUS, AND F. FOLLATH. Metabolism of theophylline by cDNA expressed human cytochromes *P*-450. *Br. J. Clin. Pharmacol.* 39: 321–326, 1995.
87. HASUMURA, Y., R. TESCHKE, AND C. S. LIEBER. Increased carbon tetrachloride hepatotoxicity, and its mechanism, after chronic ethanol consumption. *Gastroenterology* 66: 226–234, 1974.
88. 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 1 of cytochrome *P*-450. *J. Pharmacol. Exp. Ther.* 194: 469–474, 1975.
89. HAYASHI, S., J. WATANABE, AND K. KANAME. Genetic polymorphism in the 5′-flanking region change transcriptional regulation of the human cytochrome *P*-450IIE1 gene. *J. Biochem.* 110: 559–565, 1991.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

90. HAYES, M. A., T. H. RUSHMORE, AND M. T. GOLDBERG. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydramine by diallylsulfide, a component of garlic oil. *Carcinogenesis* 8: 1155–1157, 1987.
91. HERBST, J., U. KOSTER, R. KERSSEBAUM, AND W. DEKANT. Role of *P*-4502E1 in the metabolism of 1,1,2,2-tetrafluoro-1(2,2,2-trifluoroethoxy)ethane. *Xenobiotica* 24: 507–516, 1994.
92. HETU, C., A. DUMONT, AND J. G. JOLY. Effect of chronic ethanol administration on bromobenzene liver toxicity in the rat. *Toxicol. Appl. Pharmacol.* 67: 166–167, 1983.
93. HETU, C., AND J. G. JOLY. Differences in the duration of the enhancement of liver mixed function oxidase activities in ethanol fed rats after withdrawal. *Biochem. Pharmacol.* 34: 1211–1216, 1985.
94. HILDEBRANDT, A. G., AND M. SPECK. Investigations on metabolism of ethanol in rat liver microsomes (Abstract). *Arch. Pharmacol.* 277, *Suppl.*: 165, 1973.
95. HILDEBRANDT, A. G., M. SPECK, AND I. ROOTS. The effects of substrates of mixed function oxidase on ethanol oxidation in rat liver microsomes. *Naunyn–Schmiedebergs Arch. Pharmacol.* 281: 371–382, 1974.
96. HIRVONEN, A., K. HUSGAFVEL-PURSIAINEN, S. ANTTILA, A. KARJALAINEN, AND H. VAINIO. The human *CYP2E1* gene and lung cancer: *Dra* I and *Rsa* I restriction fragment length polymorphisms in a Finnish study population. *Carcinogenesis* 14: 85–88, 1993.
97. HOERNER, M., U. J. BEHRENS, T. WORNER, AND C. S. LIEBER. Humoral immune response to acetaldehyde adducts in alcoholic patients. *Res. Commun. Chem. Pathol. Pharmacol.* 54: 3–12, 1986.
98. HOFFMAN, J., K. KONOPKA, C. BUCKHORN, D. R. KOOP, AND L. WASKELL. Ethanol inducible cytochrome *P*-450 in rabbits metabolizes enflurane. *Br. J. Anaesth.* 63: 103–108, 1989.
99. HONG, J.-Y., S. M. NING, B.-L. MA, M.-J. LEE, J. PAN, AND C. S. YANG. Roles of pituitary hormones in the regulation of hepatic cytochrome *P*-450IIE1 in rats and mice. *Arch. Biochem. Biophys.* 281: 132–138, 1990.
100. HYLAND, R., A. GESCHER, K. THUMMEL, C. SCHILLER, P. JHEETA, K. MYNETT, A. SMITH, AND J. MRAZ. Metabolic oxidation and toxification of *N*-methylformamide catalyzed by the cytochrome *P*-450 isoenzyme CYP2E1. *Mol. Pharmacol.* 41: 259–266, 1992.
101. INGELMAN–SUNDBERG, M., AND I. JOHANSSON. Mechanisms of hydroxyl radical formation and ethanol oxidation by ethanol inducible and other forms of rabbit liver microsomal cytochromes *P*–450. *J. Biol. Chem.* 259: 6447–6458, 1984.
102. INGELMAN–SUNDBERG, M., I. JOHANSSON, K. E. PENTTILA, H. GLAUMANN, AND K. O. LINDROS. Centrilobular expression of ethanol inducible cytochrome *P*-450 (IIE1) in rat liver. *Biochem. Biophys. Res. Commun.* 157: 55–60, 1988.
103. ISERI, O. A., L. S. GOTTLIEB, AND C. S. LIEBER. The ultrastructure of ethanol induced fatty liver (Abstract). *Federation Proc.* 23: 579, 1964.
104. ISERI, O. A., L. S. GOTTLIEB, AND C. S. LIEBER. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Am. J. Pathol.* 48: 535–555, 1966.
105. ISHIZAKI, H., J. F. BRADY, S. M. NING, AND C. S. YANG. Effect of phenethyl isothiocyanate on microsomal *N*-nitroxodimethylamine metabolism and other monooxygenase activities. *Xenobiotica* 20: 255–264, 1990.
106. ISRAEL, Y., E. HURWITZ, O. NIEMELA, AND R. ARNON. Monoclonal and polyclonal antibodies against acetaldehyde containing epitopes in acetaldehyde-protein adducts. *Proc. Natl. Acad. Sci. USA* 83: 7923–7927, 1986.
107. ITO, D., AND C. S. LIEBER. Ethanol metabolism in deermice: role of extrahepatic alcohol dehydrogenase. *Alcohol. Clin. Exp. Res.* 17: 919–925, 1993.
108. IWAHASHI, K., Y. MATSUO, H. SUWAKI, K. NAKAMURA, AND Y. ICHIKAWA. CYP2E1 and ALDH2 genotypes and alcohol dependence in Japanese. *Alcohol. Clin. Exp. Res.* 19: 564–566, 1995.
109. JAYYOSI, Z., D. KNOBLE, M. MUC, J. ERICK, P. E. THOMAS, AND M. KELLEY. Cytochrome *P*-450 2E1 is not the sole catalyst of chlorzoxazone hydroxylation in rat liver microsomes. *J. Pharmacol. Exp. Ther.* 273: 1156–1161, 1995.
110. JEONG, K.-S., I. J. LEE, B. J. ROBERTS, Y. SOH, J. K. YOO, J. W. LEE, AND B. J. SONG. Transcriptional inhibition of cytochrome *P*-4502E1 by a synthetic compound, YH439. *Arch. Biochem. Biophys.* 326: 137–144, 1996.
111. JOHANSSON, I. J., G. EKSTROM, B. SCHOLTE, D. PUZYCKI, H. JORNVALL, AND M. INGLEMAN-SUNDBERG. Ethanol, fasting, and acetone inducible cytochromes *P*-450 in rat liver: regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* 27: 1925–1934, 1988.
112. JOHANSSON, I. J., E. ELIASSON, C. NORSTEN, AND M. INGELMAN-SUNDBERG. Hydroxylation of acetone by ethanol- and acetone-inducible cytochrome *P*-450 in liver microsomes and reconstituted membranes. *FEBS Lett.* 196: 59–64, 1986.
113. JOHANSSON, I. J., AND M. INGELMAN-SUNDBERG. Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome *P*-450 (IIE1) in rat and rabbit liver microsomes. *Cancer Res.* 48: 5387–5390, 1988.
114. JOLY, J. G., AND C. HETU. Effects of chronic ethanol administration in the rat. Relative dependency on dietary lipids. I. Induction of hepatic drug-metabolism enzymes in vitro. *Biochem. Pharmacol.* 24: 1475–1480, 1975.
115. JOLY, J. C., AND C. HETU. Effects of chronic ethanol administration in the rat: relative dependency on dietary lipids. II. Paradoxical role of linoleate in the induction of hepatic drug metabolizing enzymes in vitro. *Can. J. Physiol. Pharmacol.* 55: 34–41, 1977.
116. 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 phospholipids. *Biochem. Pharmacol.* 25: 1995–2001, 1976.
117. JOLY, J. G., H. ISHII, AND C. S. LIEBER. Microsomal cyanide-binding cytochrome: its role in hepatic ethanol oxidation (Abstract). *Gastroenterology* 62: 174, 1972.
118. 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. *Alcohol. Clin. Exp. Res.* 1: 17–20, 1977.
119. JONES, B. M., AND A. PARADES. Circadian variation of ethanol metabolism in alcoholics. *Br. J. Addict.* 69: 3–10, 1974.
120. KATO, S., J. ALDERMAN, AND C. S. LIEBER. Ethanol metabolism in alcohol dehydrogenase deficient deermice is mediated by the microsomal ethanol oxidizing system, not by catalase. *Alcohol Alcohol.* 1, *Suppl.*: 231–234, 1987.
121. KATO, S., J. ALDERMAN, AND C. S. LIEBER. Respective roles of the microsomal ethanol oxidizing system (MEOS) and catalase in ethanol metabolism by deermice lacking alcohol dehydrogenase. *Arch. Biochem. Biophys.* 254: 586–591, 1987.
122. KATO, S., J. ALDERMAN, AND C. S. LIEBER. In vivo role of the microsomal ethanol oxidizing system in ethanol metabolism by deermice lacking alcohol dehydrogenase. *Biochem. Pharmacol.* 37: 2706–2708, 1988.
123. KATO, S., P. G. SHIELDS, N. E. CAPORASO, R. N. HOOVER, B. F. TRUMP, H. SUGIMURA, A. WESTON, AND C. C. HARRIS. Cytochrome *P*-450IIE1 genetic polymorphisms, radical variation, and lung cancer risk. *Cancer Res.* 52: 6712–6715, 1992.
124. KAZAKOFF, K., P. IVERSEN, T. LAWSON, J. BARON, F. P. GUENGERICH, AND P. M. POUR. Involvement of cytochrome *P*-450 2E1-like isoform in the activation of *N*-nitroso(2-oxopropyl)amine in the rat nasal mucosa. *Eur. J. Cancer B Oral Oncol.* 30B: 179–185, 1994.
125. KEDDERIS, G. L., R. BATRA, AND D. R. KOOP. Epoxidation of acrylonitrile by rat and human cytochromes *P*-450. *Chem. Res. Toxicol.* 6: 866–871, 1993.
126. KEILIN, D., AND E. F. HARTREE. Properties of catalase: catalysis of coupled oxidation of alcohols. *Biochem. J.* 39: 293–301, 1945.
127. KHAN, S., AND P. J. O'BRIEN. Molecular mechanisms of tirapazamine (SR 4233, Win 59075)-induced hepatocyte toxicity under low oxygen concentrations. *Br. J. Cancer* 71: 780–785, 1995.
128. KHANI, S. C., T. D. PORTER, AND M. J. COON. Isolation and partial characterization of the gene for cytochrome *P*-450 3a (*P*-450ALC) and a second closely related gene. *Biochem. Biophys. Res. Commun.* 150: 10–17, 1988.
129. KHANI, S. C., P. G. ZAPHIROPOULOS, V. S. FUJITA, T. D. PORTER, D. R. KOOP, AND M. J. COON. cDNA and derived amino acid sequence of ethanol-inducible rabbit liver cytochrome *P*-450 isozyme 3a (*P*-450ALC). *Proc. Natl. Acad. Sci. USA* 84: 638–642, 1987.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

130. KHARASCH, E. D. , A. S. ARMSTRONG, K. GUNN, A. ARTRU, K. COX, AND M. D. KAROL. Clinical sevoflurane metabolism and disposition. II. The role of cytochrome P-450 2E1 in fluoride and hexafluoroisopropanol formation. *Anesthesiology* 82: 1379–1388, 1995.
131. KHARASCH, E. D., D. C. HANKINS, AND K. E. THUMMEL. Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. *Anesthesiology* 82: 689–699, 1995.
132. KHARASCH, E. D., K. E. THUMMEL, D. MAUTZ, AND S. BOSSE. Clinical enflurane metabolism by cytochrome P-450 2E1. *Clin. Pharmacol. Ther.* 55: 434–440, 1994.
133. KIM, S. G., J. Y. KWAK, J. W. LEE, R. F. NOVAK, S. S. PARK, AND N. D. KIM. Malotilate, a hepatoprotectant, suppresses CYP2E1 expression in rats. *Biochem. Biophys. Res. Commun.* 200: 1414–1420, 1994.
134. KIM, S. G., AND R. F. NOVAK. Induction of rat hepatic P-450IIE1 (CYP 2E1) by pyridine: evidence for a role of protein synthesis in the absence of transcriptional activation. *Biochem. Biophys. Res. Commun.* 166: 1072–1079, 1990.
135. KIM, S. G., S. E. SHEHIN, J. C. STATES, AND R. F. NOVAK. Evidence for increased translational efficiency in the induction of P-450IIE1 by solvents: analysis of P-450IIE1 mRNA polyribosomal distribution. *Biochem. Biophys. Res. Commun.* 172: 767–774, 1990.
136. KIM, S. G., D. E. WILLIAMS, E. G. SCHUEETZ, P. S. GUZELIAN, AND R. F. NOVAK. Pyridine induction of cytochrome P-450 in the rat: role of P-450j (alcohol inducible form) in pyridine N-oxidation. *J. Pharmacol. Exp. Ther.* 246: 1175–1182, 1988.
137. KNECHT, K. T., R. G. THURMAN, AND P. R. MASON. Role of superoxide and trace transition metals in the production of α-hydroxyethyl radical from ethanol by microsomes from alcohol dehydrogenase-deficient deermice. *Arch. Biochem. Biophys.* 303: 339–348, 1993.
138. KOIVISTO, T., V. M. MISHIN, K. M. MAK, P. A. COHEN, AND C. S. LIEBER. Induction of cytochrome P-4502E1 by ethanol in rat Kupffer cells. *Alcohol. Clin. Exp. Res.* 20: 207–212, 1996.
139. KOOP, D. R. Hydroxylation of p-nitrophenol by rabbit ethanol inducible cytochrome P-450 isozyme 3a. *Mol. Pharmacol.* 29: 399–404, 1986.
140. KOOP, D. R. Inhibition of ethanol-inducible cytochrome P-450IIE1 by 3-amino-1,2,4-triazole. *Chem. Res. Toxicol.* 3: 377–383, 1990.
141. KOOP, D. R. Oxidative and reductive metabolism by cytochrome P-450 2E1. *FASEB J.* 6: 724–730, 1992.
142. KOOP, D. R., AND J. P. CASAZZA. Identification of ethanol-inducible P-450 enzyme 3a as the acetone and acetol monooxygenase of rabbit microsomes. *J. Biol. Chem.* 260: 13607–13612, 1985.
143. KOOP, D. R., A. CHERNOSKY, AND E. P. BRASS. Identification and induction of cytochrome P-450 2E1 in rat Kupffer cells. *J. Pharmacol. Exp. Ther.* 258: 1072–1076, 1991.
144. KOOP, D. R., AND M. J. COON. Ethanol oxidation and toxicity: role of alcohol P-450 oxygenase. *Alcohol. Clin. Exp. Res.* 10, *Suppl.*: 44s–49s, 1986.
145. KOOP, D. R., B. L. CRUMP, G. D. NORDBLOM, AND M. J. COON. Immunochemical evidence for induction of the alcohol oxidizing cytochrome P-450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole, and isoniazid. *Biochemistry* 82: 4065–4069, 1985.
146. KOOP, D. R., C. L. LAETHEM, AND G. G. SCHNIER. Identification of ethanol-inducible P-450 isozyme 3a (P-450IIE1) as a benzene and phenol hydroxylase. *Toxicol. Appl. Pharmacol.* 98: 278–288, 1989.
147. 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.
148. KOOP, D. R., AND D. J. TIERNEY. Multiple mechanisms in the regulation of ethanol inducible cytochrome P-450IIE1. *Bioessays* 9: 429–435, 1990.
149. KOSTER, U., P. SPEERSCHNEIDER, R. KERSSEBAUM, H. WITTMANN, AND W. DEKANT. Role of cytochrome P-450 2E1 in the metabolism of 1,1,2,3,3-hexafluoropropyl methyl ether. *Drug Metab. Dispos.* 22: 667–672, 1994.
150. KOSTRUBSKY, V. E., S. C. STROM, S. G. WOOD, S. A. WRIGHTON, P. R. SINCLAIR, AND J. F. SINCLAIR. Research report: ethanol and isopentanol increased CYP3A and CYP2E in primary cultures of human hepatocytes. *Arch. Biochem. Biophys.* 322: 516–520, 1995.
151. KREBS, H. A., AND J. R. PERKINS. The physiological role of liver alcohol dehydrogenase. *Biochem. J.* 118: 635–644, 1970.
152. KRIKUN, G., AND A. CEDERBAUM. Effect of chronic ethanol consumption on microsomal lipid peroxidation: role of iron and comparison between controls. *FEBS Lett.* 208: 292–296, 1986.
153. KUBOTA, S., J. M. LASKER. AND C. S. LIEBER. Molecular regulation of ethanol inducible cytochrome P-450-IIE1 in hamsters. *Biochem. Biophys. Res. Commun.* 150: 304–310, 1988.
154. KWAK, M. K., S. G. KIM, J. Y. KWAK, R. F. NOVAK, AND N. D. KIM. Inhibition of cytochrome P-4502E1 expression by organosulfur compounds allylsulfide allylmercaptan and allymethylsulfide in rats. *Biochem. Pharmacol.* 47: 531–539, 1994.
155. LAETHEM, R. M., M. BALAZY, J. R. FALCK, C. L. LAETHEM, AND D. R. KOOP. Formation of 19(S)-, 19(R)-, and 18(R)-hydroxyeicosatetraenoic acids by alcohol inducible cytochrome P-450 2E1. *J. Biol. Chem.* 26: 12912–12918, 1993.
156. LANE, B. P., AND C. S. LIEBER. Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. *Am. J. Pathol.* 49: 593–603, 1966.
157. LANE, B. P., AND C. S. LIEBER. Effects of butylated hydroxytoluene on the ultrastructure of rat hepatocytes. *Lab. Invest.* 16: 341–348, 1967.
158. LASKER, J. M., J. RAUCY, S. KUBOTA, B. P. BLOSWICK, M. BLACK, AND C. S. LIEBER. Purification and characterization of human liver cytochrome P-450 ALC. *Biochem. Biophys. Res. Commun.* 148: 232–238, 1987.
159. LASKER, J. M., M. TSUTSUMI, B. P. BLOSWICK, AND C. S. LIEBER. Characterization of benzoflavone (BF) inducible hamster liver cytochrome P-450 isozyme catalytically similar to cytochrome P-450-ALC (Abstract). *Hepatology* 7: 432, 1987.
160. LEE, R. P., AND P. G. FORKERT. In vitro biotransformation of 1,1-dichloroethylene by hepatic cytochrome P-450 2E1 in mice. *J. Pharmacol. Exp. Ther.* 270: 371–376, 1994.
161. LEO, M. A., S. IIDA, AND C. S. LIEBER. Retinoic acid metabolism by a system reconstituted with cytochrome P-450. *Arch. Biochem. Biophys.* 234: 305–312, 1984.
162. LEO, M. A., C. I. KIM. AND C. S. LIEBER. NAD^+-dependent retinol dehydrogenase in liver microsomes. *Arch. Biochem. Biophys.* 259: 241–249, 1987.
163. LEO, M. A., J. M. LASKER, J. L. RAUCY, C. I. KIM, M. BLACK, AND C. S. LIEBER. Metabolism of retinol and retinoic acid by human liver cytochrome P-450IIC8. *Arch. Biochem. Biophys.* 269: 305–312, 1989.
164. LEO, M. A., AND C. S. LIEBER. Hepatic vitamin A depletion in alcoholic liver injury. *N. Engl. J. Med.* 307: 597–601, 1982.
165. LEO, M. A., AND C. S. LIEBER. New pathway for retinol metabolism in liver microsomes. *J. Biol. Chem.* 260: 5228–5231, 1985.
166. LEO, M. A., AND C. S. LIEBER. Hypervitaminosis A: a liver lover's lament. *Hepatology* 8: 412–417, 1988.
167. LEO, M. A., N. LOWE, AND C. S. LIEBER. Decreased hepatic vitamin A after drug administration in humans and in rats. *Am. J. Clin. Nutr.* 40: 1131–1136, 1984.
168. LEO, M. A., N. LOWE, AND C. S. LIEBER. Potentiation of ethanol-induced hepatic vitamin A depletion by phenobarbital and butylated hydroxytoluene. *J. Nutr.* 117: 70–76, 1987.
169. 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.
170. LEREBOULLET, J., G. BARRES, AND J. P. BRIARD. La courbe d'alcoolèmie de Widmark est-elle toujours fiable? *Bull. Acad. Natl. Med.* 160: 312–315, 1976.
171. LEVIN, W., D. RYAN, S. WEST, AND A. Y. H. LU. Preparation of partially purified lipid depleted cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase from rat liver. *J. Biol. Chem.* 249: 1747–1754, 1974.
172. LEVIN, W., P. E. THOMAS, N. OLDFIELD, AND D. E. RYAN. N-demethylation of n-nitrosodimethylamine catalyzed by purified rat hepatic microsomal cytochrome P-450: isozyme specificity and role of cytochrome b_5. *Arch. Biochem. Biophys.* 248: 158–165, 1986.
173. LIEBER, C. S. Pathogenesis and early diagnosis of alcoholic liver injury. *N. Engl. J. Med.* 298: 888–893, 1978.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

174. LIEBER, C. S. Microsomal ethanol oxidizing system (MEOS) *Enzyme* 37: 45–56, 1987.
175. LIEBER, C. S. Biochemical and molecular basis for alcohol-induced injury to liver and other tissues. *N. Engl. J. Med.* 319: 1639–1650, 1988.
176. LIEBER, C. S. Perspectives: do alcohol calories count? *Am. J. Clin. Nutr.* 54: 976–982, 1991.
177. LIEBER, C. S. *Medical and Nutritional Complications of Alcoholism: Mechanisms and Management.* New York: Plenum, 1992.
178. LIEBER, C. S. Alcohol and the liver: 1994 update. *Gastroenterology* 106: 1085–1105, 1994.
179. LIEBER, C. S. Medical disorders of alcoholism. *N. Engl. J. Med.* 333: 1058–1965, 1995.
180. LIEBER, C. S. Role of oxidative stress and antioxidant therapy in alcoholic and non-alcoholic liver diseases. In: *Advances in Pharmacology,* edited by H. Sies. San Diego, CA: Academic, 1996, vol. 38, p. 601–628.
181. LIEBER, C. S. Pathogenesis and treatment of liver fibrosis: 1997 update. *Dig. Dis.* 15: 42–66, 1997.
182. LIEBER, C. S., E. BARAONA, R. HERNANDEZ-MUNOZ, S. KUBOTA, N. SATO, S. KAWANO, T. MATSUMURA, AND N. INATOMI. Impaired oxygen utilization: a new mechanism for the hepatotoxicity of ethanol in sub-human primates. *J. Clin. Invest.* 83: 1682–1690, 1989.
183. LIEBER, C. S., AND L. M. DECARLI. Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science Wash. DC* 162: 917–918, 1968.
184. LIEBER, C. S., AND L. M. DECARLI. Hepatic microsomal ethanol oxidizing system. In vivo characteristics and adaptive properties in vivo. *J. Biol. Chem.* 245: 2505–2512, 1970.
185. LIEBER, C. S., AND L. M. DECARLI. Reduced nicotinamide-adenine dinucleotide phosphate oxidase: activity enhanced by ethanol consumption. *Science Wash. DC* 170: 78–80, 1970.
186. 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.
187. 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.
188. LIEBER, C. S., AND L. M. DECARLI. Oxidation of ethanol by hepatic microsomes of acatalasemic mice. *Biochem. Biophys. Res. Commun.* 60: 1187–1192, 1974.
189. LIEBER, C. S., L. M. DECARLI, K. M. MAK, C. I. KIM, AND M. A. LEO. Attenuation of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. *Hepatology* 12: 1390–1398, 1990.
190. LIEBER, C. S., A. GARRO, M. A. LEO, K. M. MAK, AND T. M. WORNER. Alcohol and cancer. *Hepatology* 6: 1005–1019, 1986.
191. LIEBER, C. S., J. M. LASKER, L. M. DECARLI, J. SAELI, AND T. WOJTOWICZ. Role of acetone, dietary fat and total energy intake in induction of hepatic microsomal ethanol oxidizing system. *J. Pharmacol. Exp. Ther.* 247: 791–795, 1988.
192. LIEBER, C. S., M. A. LEO, S. I. ALEYNIK, M. K. ALEYNIK, AND L. M. DECARLI. Polyenylphosphatidylcholine decreases alcohol-induced oxidative stress in the baboon. *Alcohol. Clin. Exp. Res.* In press.
193. LIEBER, C. S., S. J. ROBINS, J. LI, L. M. DECARLI, K. M. MAK, J. M. FASULO, AND M. A. LEO. Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology* 106: 152–159, 1994.
194. 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.
195. LIN, R. C., R. S. SMITH, AND L. LUMENG. Detection of a protein-acetaldehyde adduct in the liver of rats fed alcohol chronically. *J. Clin. Invest.* 81: 615–619, 1988.
196. LOIZOU, G. D., G. URBAN, W. DEKANT, AND M. W. ANDERS. Gas-uptake pharmacokinetics of 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123). *Drug Metab. Dispos.* 22: 511–517, 1994.
197. LUCAS, D., F. BERTHOU, Y. DREANO, P. LOZACH, A. VOLANT, AND J. F. MENEZ. Comparison of levels of cytochromes *P*-450, CYP1A2, CYP2E1, and their related monooxygenase activities in human surgical liver samples. *Alcohol. Clin. Exp. Res.* 17: 900–905, 1993.
198. LUCAS, D., C. MENEZ, C. GIRRE, P. BODENEZ, E. HISPARD, AND J. F. MENEZ. Decrease in cytochrome *P*-4502E1 as assessed by the rate of chlorzoxazone hydroxylation in alcoholics during the withdrawal phase. *Alcohol. Clin. Exp. Res.* 19: 362–366, 1995.
199. MA, X., E. BARAONA, AND C. S. LIEBER. Alcohol consumption enhances fatty acid ω-oxidation, with a greater increase in males than in females. *Hepatology* 18: 1247–1253, 1993.
200. MA, X., J. ZHAO, AND C. S. LIEBER. Polyenylphosphatidylcholine attenuates nonalcoholic hepatic fibrosis and accelerates its regression. *J. Hepatol.* 24: 604–613, 1996.
201. MAEZAWA, Y., M. YAMAUCHI, AND G. TODA. Association between restriction fragment length polymorphism of the human cytochrome *P*-450IIE1 gene and susceptibility to alcoholic liver cirrhosis. *Am. J. Gastroenterol.* 89: 561–565, 1994.
202. MAEZAWA, Y., M. YAMAUCHI, G. TODA, H. SUZUKI, AND S. SUKURAI. Alcohol metabolizing enzyme polymorphisms and alcoholism in Japan. *Alcohol. Clin. Exp. Res.* 19: 951–954, 1995.
202a. MATSUZAKI, S., AND C. S. LIEBER. Increased susceptibility of hepatic mitochondria to the toxicity of acetaldehyde after ethanol consumption. *Biochem. Biophys. Res. Commun.* 75: 1059–1065, 1977.
203. MCCOY, G. D., AND D. R. KOOP. Reconstitution of rabbit liver microsomal *N*-nitrosopyrrolidine α-hydroxylase activity. *Cancer Res.* 48: 3987–3992, 1988.
204. MCGEHEE, R. E., M. J. J. RONIS, R. M. COWHERD, M. INGELMAN-SUNDBERG, AND T. M. BADGER. Characterization of cytochrome *P*-450 2E1 induction in a rat hepatoma FGC-4 cell model by ethanol. *Biochem. Pharmacol.* 48: 1823–1833, 1994.
206. MELDOLESI, J. On the significance of the hypertrophy of the smooth endoplasmic reticulum in liver cells after administration of drugs. *Biochem. Pharmacol.* 16: 125–131, 1967.
207. 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.
208. MIKALSEN, A., J. ALEXANDER, H. WALLIN, M. INGELMAN-SUNDBERG, AND R. A. ANDERSEN. Reductive metabolism and protein binding of chromium (VI) by *P*-450 protein enzymes. *Carcinogenesis* 12: 825–831, 1991.
209. MILLER, K. W., AND C. S. YANG. Studies on the mechanisms of induction of *N*-nitrosodimethylamine demethylase by fasting, acetone, and ethanol. *Arch. Biochem. Biophys.* 229: 483–491, 1984.
210. MISHIN, V. M., T. KOIVISTO, AND C. S. LIEBER. The determination of cytochrome *P*-450 2E1 dependent *p*-nitrophenol hydroxylation by high performance liquid chromatography with electrochemical detection. *Anal. Biochem.* 233: 212–215, 1996.
211. MISRA, P. S., A. LEFÈVRE, 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.
212. MITCHELL, J. R., C. MACK, E. MEZEY, AND W. C. MADDREY. The effects of variation in dietary protein and ethanol on hepatic microsomal drug metabolism in the rat. *Hepatology* 1: 336–340, 1981.
213. 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.
214. MORGAN, E. T., D. R. KOOP, AND M. J. COON. Catalytic activity of cytochrome *P*-450 isozyme 3a isolated from liver microsomes of ethanol-treated rabbits. Oxidation of alcohols. *J. Biol. Chem.* 257: 13951–13957, 1982.
215. MORGAN, E. T., D. R. KOOP, AND M. J. COON. Comparison of six rabbit liver cytochrome *P*-450 isozymes in formation of a reactive metabolite of acetaminophen. *Biochem. Biophys. Res. Commun.* 112: 8–13, 1983.
216. MORIMOTO, M., R. C. REITZ, R. J. MORIN, K. NGUYEN, M. INGELMAN-SUNDBERG, AND S. W. FRENCH. CYP2E1 inhibitors partially ameliorate the changes in hepatic fatty acid composition induced in rats by chronic administration of ethanol and a high fat diet. *J. Nutr.* 125: 2953–2964, 1995.
217. NAKAJIMA, T., E. ELOVAARA, T. OKINO, H. V. GELBOIN, M. KLOCKARS, V. RIIHIMAKI, T. AOYAMA, AND H. VAINIO. Different contributions of cytochromes *P*-450 2E1 and *P*-450 2B1/2 to chloro-

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

form hepatotoxicity in rat. *Toxicol. Appl. Pharmacol.* 133: 215–222, 1995.
218. NAKAJIMA, T., T. OKINO, AND A. SATO. Kinetic studies on benzene metabolism in rat liver: possible presence of three forms of benzene metabolizing enzymes in the liver. *Biochem. Pharmacol.* 36: 2799–2804, 1987.
219. NAKURA, H., S. ITOH, H. KUSANO, H. ISHIZONE, S. IMAKOKA, Y. FUNAE, T. YOKOI, AND T. KAMATAKI. Decrease in the content of cytochrome *P*-450IIE by fasting in liver microsomes of house musk shrew (*Suncus murinus*). *Biochem. Pharmacol.* 43: 1907–1910, 1992.
220. NANJI, A. A., S. ZHAO, R. G. LAMB, A. J. DENNENBERG, S. M. HOSSEIN, H. SADRZADEH, AND D. J. WAXMAN. Changes in cytochromes *P*-450, 2E1, 2B1, and 4A and phospholipases A and C in the intragastric feeding rat model for alcoholic liver disease: relationship to dietary fats and pathologic liver injury. *Alcohol. Clin. Exp. Res.* 18: 902–908, 1994.
221. NANJI, A. A., S. ZHAO, R. G. LAMB, H. SADRZADEH, A. J. DANNENBERG, AND D. J. WAXMAN. Changes in microsomal phospholipases and arachidonic acid in experimental alcoholic liver injury: relationship to cytochrome *P*-450 2E1 induction and conjugated diene formation. *Alcohol. Clin. Exp. Res.* 17: 598–603, 1993.
222. NEBERT, D. W., M. ADESNIK, M. J. COON, R. W. ESTABROOK, F. J. GONZALEZ, P. GUENGERICH, I. C. GUNSALUS, E. F. JOHNSON, B. KEMPER, W. LEVIN, I. R. PHILLIPS, R. SATO, AND M. R. WATERMAN. The *P*-450 gene superfamily: recommended nomenclature. *DNA* 6: 1–11, 1987.
223. NEBERT, D. W., D. R. NELSON, M. ADESNIK, M. J. COON, R. W. ESTABROOK, F. J. GONZALEZ, F. P. GUENGERICH, I. C. GUNSALUS, E. F. JOHNSON, B. KEMPER, W. LEVIN, I. R. PHILLIPS, R. SATO, AND M. R. WATERMAN. The *P*-450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* 8: 1–13, 1989.
224. NELSON, D. R., T. KAMATAKI, D. J. WAXMAN, F. P. GUENGERICH, R. W. ESTABROOK, R. FETEREISEN, F. J. GONZALEZ, M. J. COON, I. C. GUNSALUS, O. GOTOH, K. OKUDA, AND D. W. NEBERT. The *P*-450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol.* 12: 1–51, 1993.
225. NOMURA, F., AND C. S. LIEBER. Binding of acetaldehyde to rat liver microsomes: enhancement after chronic alcohol consumption. *Biochem. Biophys. Res. Commun.* 100: 131–137, 1981.
226. NORDMANN, R., C. RIBIERE, AND H. ROUACH. Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radical Biol. Med.* 12: 219–240, 1992.
227. 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.
228. 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.
229. OLSON, M. J., S. G. KIM, C. A. REIDY, J. T. JOHNSON, AND R. F. NOVAK. Oxidation of 1,1,1,2-tetrafluoroethane in rat liver microsomes is catalyzed primarily by cytochrome *P*-450IIE1. *Drug Metab. Dispos.* 19: 298–303, 1990.
230. ONO, S., T. HATANAKA, H. HOTTA, M. TSUSUI, T. SATOH, AND F. Y. GONZALEZ. Chlorzoxazone is metabolized by human CYP1A2 as well as by human CYP2E1. *Pharmacogenetics* 5: 143–150, 1995.
231. 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.
232. OWEN, O. E., V. E. TRAPP, C. L. SKUTCHES, M. A. MOZZOLI, R. D. HOIELDTKE, G. BODEN, AND G. A. REICHARD, JR. Acetone metabolism during diabetic ketoacidosis. *Diabetes* 31: 242–248, 1982.
233. PAN, J., J.-Y. HONG, AND C. S. YANG. Posttranscriptional regulation of mouse renal cytochrome *P*-450 2E1 by testosterone. *Arch. Biochem. Biophys.* 299: 110–115, 1992.
234. PATTEN, C. J., H. ISHIZAKI, T. AOYAMA, M. LEE, S. M. NING, W. HUANG, F. J. GONZALEZ, AND C. S. YANG. Catalytic properties of human cytochromes *P*-450 2E1 produced by cDNA expression in mammalian cells. *Arch. Biochem. Biophys.* 299: 163–171, 1992.
235. PENG, H. M., G. M. RANER, A. D. VAZ, AND M. J. COON. Oxidative cleavage of esters and amides to carbonyl products by cytochrome *P*-450. *Arch. Biochem. Biophys.* 318: 333–339, 1995.
236. PERSSON, I., I. JOHANSSON, H. BERGLING, M. L. DAHL, J. SEIDEGARD, R. RYLANDER, A. RANNUG, J. HABERG, AND H. SUNDBERG. Genetic polymorphism of cytochrome *P*-4502E1 in a Swedish population: relationship to incidence of lung cancer. *FEBS Lett.* 319: 207–211, 1993.
237. PERSSON, J. O., Y. TERELIUS, AND M. INGELMAN-SUNDBERG. Cytochrome *P*-450 dependent formation of reactive oxygen radicals: isozyme specific inhibition of *P*-450 mediated reduction of oxygen and carbon tetrachloride. *Xenobiotica* 20: 887–900, 1990.
238. PETER, R., R. BÖKER, P. H. BEAUNE, M. IWASAKI, F. P. GUENGERICH, AND C. S. YANG. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome *P*-450IIE1. *Chem. Res. Toxicol.* 3: 566–573, 1990.
239. PIKKARAINEN, P. H., AND C. S. LIEBER. Concentration dependency of ethanol elimination rates in baboons: effects of chronic alcohol consumption. *Alcohol. Clin. Exp. Res.* 4: 40–43, 1980.
240. PINKSTON, J. N., AND K. F. A. SONIMAN. Effect of light and fasting on the circadian variation of ethanol metabolism in the rat. *J. Interdiscip. Cycle Res.* 10: 185–193, 1979.
241. PIRMOHAMED, M,, N. R. KITTERINGHAM, L. J. QUEST, R. L. ALLOTT, V. J. GREEN, I. T. GILMORE, AND B. K. PARK. Genetic polymorphism of cytochrome *P*-4502E1 and risk of alcoholic liver disease in Caucasians. *Pharmacogenetics* 5: 351–357, 1995.
242. PIROLA, R. C., AND C. S. LIEBER. The energy cost of the metabolics of drugs, including ethanol. *Pharmacology* 7: 185–196, 1972.
243. PORTER, T. D., S. C. KHANI, AND M. J. COON. Induction and tissue specific expression of rabbit cytochrome *P*-450IIE1 and IIE2 genes. *Mol. Pharmacol.* 36: 61–65, 1989.
244. RASHBA-STEP, J., N. J. TURRO, AND A. I. CEDERBAUM. Increased NADPH- and NADH-dependent production of superoxide and hydroxyl radical by microsomes after chronic ethanol consumption. *Arch. Biochem. Biophys.* 300: 401–408, 1993.
245. RAUCY, J. L., G. CURLEY, AND S. P. CARPENTER. Use of lymphocytes for assessing ethanol-mediated alterations in the expression of hepatic cytochrome *P*-4502E1. *Alcohol. Clin. Exp. Res.* 19: 1369–1375, 1995.
246. RAUCY, J. L., P. FERNANDES, M. BLACK, S. L. YANG, AND D. R. KOOP. Identification of a human liver cytochrome *P*-450 exhibiting catalytic and immunochemical similarities to cytochrome *P*-450 3a of rabbit liver. *Biochem. Pharmacol.* 36: 2921–2926, 1987.
247. RAUCY, J. L., J. M. LASKER, J. C. KRAMER, D. E. SALAZER, C. S. LIEBER, AND G. B. CORCORAN. Induction of *P*-450IIE1 in the obese rat. *Mol. Pharmacol.* 39: 275–280, 1991.
248. REICHARD, G. A., JR., A. C. HAFF, C. L. SKUTCHES, P. PAUL, C. P. HOLROYDE, AND O. E. OWEN. Plasma acetone metabolism in the fasting human. *J. Clin. Invest.* 63: 619–626, 1979.
249. REICHARD, G. A., JR., C. L. SKUTCHES, R. D. HOELDKE, AND O. W. OWEN. Acetone metabolism in humans during diabetic ketoacidosis. *Diabetes* 35: 668–674, 1986.
250. REINKE, L. A., E. K. LAI, AND P. B. MCCAY. Ethanol feeding stimulates trichlormethyl radical formation from carbon tetrachloride in liver. *Xenobiotica* 18: 1311–1318, 1988.
251. REINKE, L. A., J. M. RAU, AND P. B. MCCAY. Possible roles of free radicals in alcoholic tissue damage. *Free Radical Res. Commun.* 9: 205–211, 1990.
252. RIFKIND, A. B., C. LEE, T. K. CHANG, AND D. J. WAXMAN. Arachidonic acid metabolism by human cytochrome *P*-450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch. Biochem. Biophys.* 320: 380–389, 1995.
253. ROBERTS, B. J., B.-J. SONG, Y. SOH, S. S. PARK, AND S. E. SHOAF. Ethanol induces CYP2E1 by protein stabilization. *J. Biol. Chem.* 270: 29632–29635, 1995.
254. RONIS, M. J., J. HUANG, J. CROUCH, C. MERCADO, D. IRBY, C. R. VALENTINE, C. K. LUMPKIN, M. INGELMAN-SUNDBERG, AND T. M. BADGER. Cytochrome *P*-450 CYP 2E1 induction during chronic alcohol exposure occurs by a two-step mechanism associated with blood alcohol concentration in rats. *J. Pharmacol. Exp. Ther.* 264: 944–950, 1993.
255. RYAN, D. E., D. R. KOOP, P. E. THOMAS, M. J. COON, AND W.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

LEVIN. Evidence that isoniazid and ethanol induce the same microsomal cytochrome *P*-450 isozyme 3a. *Arch. Biochem. Biophys.* 246: 633–644, 1986.
256. RYAN, D. E., AND W. LEVIN. Purification and characterization of hepatic microsomal cytochrome *P*-450. *Pharmacol. Ther.* 45: 153–239, 1990.
257. RYAN, D. E., L. RAMANTHAN, S. IIDA, P. E. THOMAS, M. HANIU, J. E. SHIVELY, C. S. LIEBER, AND W. LEVIN. Characterization of a major form of rat hepatic microsomal cytochrome *P*-450 induced by isoniazid. *J. Biol. Chem.* 260: 6385–6393, 1985.
258. 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 (Abstract). *Gastroenterology* 73: A47, 1977.
259. SALASPURO, M. P., AND C. S. LIEBER. Non-uniformity of blood ethanol elimination: its exaggeration after chronic consumption. *Ann. Clin. Res.* 10: 294–297, 1978.
260. SALAZAR, D. E., C. L. SORGE, AND G. B. CORCORAN. Obesity as a risk factor for drug-induced organ injury. VI. Increased hepatic *P*-450 concentration and microsomal ethanol oxidizing activity in the obese overfed rat. *Biochem. Biophys. Res. Commun.* 157: 315–320, 1988.
261. SALMELA, K. S., I. G. KESSOVA, I. B. TSYRLOV, AND C. S. LIEBER. Ethanol is oxidized by human cytochromes *P*-4502E1, *P*-4501A2 and *P*-4503A4 (Abstract). *Alcohol. Clin. Exp. Res.* 20: 36, 1996.
262. SATO, C., AND C. S. LIEBER. Mechanism of the preventive effect of ethanol on acetaminophen-induced hepatotoxicity. *J. Pharmacol. Exp. Ther.* 218: 811–815, 1981.
263. SATO, M., AND C. S. LIEBER. Hepatic vitamin A depletion after chronic ethanol consumption in baboons and rats. *J. Nutr.* 111: 2015–2023, 1981.
264. SAVOLAINEN, M. J., E. BARAONA, P. PIKKARAINEN, AND C. S. LIEBER. Hepatic triacylglycerol synthesizing activity during progression of alcoholic liver injury in the baboon. *J. Lipid Res.* 25: 813–820, 1984.
265. SCHNIER, G. G., C. L. LAETHAM, AND D. R. KOOP. Identification and induction of cytochromes *P*-450, *P*-450IIE1, and *P*-4501A1 in rabbit bone marrow. *J. Pharmacol. Exp. Ther.* 251: 790–796, 1989.
266. SEATON, M. J., M. H. FOLLANSBEE, AND J. A. BOND. Oxidation of 1,2-epoxy-3-butene to 1.2:3,4-diepoxybutane by cDNA-expressed human cytochromes *P*-450 2E1 and 3A4 and human, mouse and rat liver microsomes. *Carcinogenesis* 16: 2287–2293, 1995.
267. SEEFF, L. B., B. A. CUCCHERINI, H. J. ZIMMERMAN, E. ADLER, AND S. B. BENJAMIN. Acetaminophen hepatotoxicity in alcoholics. *Ann. Intern. Med.* 104: 399–404, 1986.
268. SEITZ, H. K., P. CZYGAN, K. WALDHERR, S. VEITH, AND B. KOMMERELL. Ethanol and intestinal carcinogenesis in the rat. *Alcohol* 2: 491–494, 1985.
269. 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.
270. SHAW, S., E. JAYATILLEKE, W. A. ROSS, E. R. GORDON, AND C. S. LIEBER. Ethanol induced lipid peroxidation: potentiation by long-term alcohol feeding and attenuation by methionine. *J. Lab. Clin. Med.* 98: 417–425, 1981.
271. SHIGETA, C. P., F. NOMURA, S. IIDA, M. A. LEO, M. R. FELDER, AND C. S. LIEBER. Ethanol metabolism in vivo by the microsomal ethanol oxidizing system in deermice lacking alcohol dehydrogenase (ADH). *Biochem. Pharmacol.* 33: 807–814, 1984.
272. SHIMADA, T., H. YAMAZAKI, M. MIMURA, Y. INUI, AND F. P. GUENGERICH. Interindividual variations in human liver cytochrome *P*-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* 270: 414–423, 1994.
273. SHIMIZU, M., J. M. LASKER, M. TSUTSUMI, AND C. S. LIEBER. Immunohistochemical localization of ethanol inducible *P*-450IIE1 in the rat alimentary tract. *Gastroenterology* 99: 1044–1053, 1990.
274. SHIMOJO, N., T. ISHIZAKI, S. IMAOKA, Y. FUNAE, S. FUJII, AND K. OKUDA. Changes in amounts of cytochrome *P*-450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozocin induced diabetes. *Biochem. Pharmacol.* 46: 621–627, 1993.
275. SIEGERS, C. P., K. HEIDBUCHEL, AND M. YOUNES. Influence of alcohol, dithiocarb and (+) catechin on the hepatotoxicity and metabolism of vinylidene chloride in rats. *J. Appl. Toxicol.* 3: 90–95, 1983.
276. SILVIA, M., L. VINCENZO, M. ARTURO, AND G. P. GIOVANNI. Microsomal metabolism of *N,N*-diethylacetamide and *N,N*-dimethylacetamide and their effects on drug metabolizing enzymes of rat liver. *Biochem. Pharmacol.* 48: 717–726, 1994.
277. SLATER, T. F. Free-radical mechanisms in tissue injury. *Biochem. J.* 222: 1–15, 1984.
278. SOHDA, T., M. SHIMIZU, S. KAMIMURA, AND M. OKUMURA. Immunohistochemical demonstration of ethanol inducible *P*-450 2E1 in rat brain. *Alcohol Alcohol.* 1B, *Suppl.*: 69–75, 1993.
279. SOHN, O. S., H. ISHIZAKI, C. S. YANG, AND E. S. FIALA. Metabolism of azoxymethane, methyl azoxymethanol and *N*-nitrosodimethylamine by cytochrome *P*-450IIE1. *Carcinogenesis* 12: 127–131, 1991.
280. SOLOMON, L. R. Evidence for the generation of transaminase inhibitor(s) during ethanol metabolism by rat liver homogenates a potential mechanism for alcohol toxicity. *Biochem. Med. Metab. Biol.* 38: 9–18, 1987.
281. SONG, B. J., H. V. GELBOIN, S. S. PARK, C. S. YANG, AND F. J. GONZALEZ. Complementary DNA and protein sequences of ethanol inducible rat and human cytochrome *P*-450s: transcriptional and posttranscriptional regulation of the rat enzyme. *J. Biol. Chem.* 261: 16689–16697, 1986.
282. SONG, B. J., T. MATSUNAGA, J. HARDWICK, S. S. PARK, R. I. VEECH, C. S. YANG, H. V. GELBOIN, AND F. J. GONZALEZ. Stabilization of cytochrome *P*-450J messenger ribonucleic acid in the diabetic rat. *Mol. Endocrinol.* 1: 542–547, 1987.
283. SONG, B. J., R. I. VEECH, S. S. PARK, H. V. GELBOIN, AND F. J. GONZALEZ. Induction of rat hepatic *N*-nitrosodimethylamine demethylase by acetone is due to protein stabilization. *J. Biol. Chem.* 264: 3568–3572, 1989.
284. SONG, B. J., R. L. VEECH, AND P. SAENGER. Cytochrome *P*-450IIE1 is elevated in lymphocytes from poorly controlled insulin-dependent diabetics. *J. Clin. Endocrinol. Metab.* 71: 1036–1040, 1990.
285. SPEERSCHNEIDER, P., AND W. DEKANT. Renal tumorigenesis of 1,1-dichloroethane in mice: the role of male-specific expression of cytochrome *P*-450 2E1 in the renal bioactivation of 1,1-dichloroethene. *Toxicol. Appl. Pharmacol.* 130: 48–56, 1995.
286. STEPHENS, E. A., J. A. TAYLOR, N. KAPLAN, C.-H. YANG, L. L. HSIEH, G. W. LUCIER, AND D. A. BELL. Ethnic variation in the *CYP2E1* gene: polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. *Pharmacogenetics* 4: 185–192, 1994.
287. STURTEVANT, F. M., R. P. STURTEVANT, L. E. SCHEVING, AND J. E. PAULY. Chronopharmacokinetics of ethanol. II. Circadian rhythm in rate of blood level decline in a single subject. *Naunyn-Schniedebergs Arch. Pharmacol.* 293: 203–208, 1976.
288. STYLES, J. A., A. DAVIES, C. K. LIM, F. DEMATTEIS, L. A. STANLEY, I. N. WHITE, Z. X. YUAN, AND L. L. SMITH. Genotoxicity of tamoxifen, tamoxifen epoxide, and toremifene in human lymphoblastoid cells containing human cytochromes *P*-450S. *Carcinogenesis* 15: 5–9, 1994.
289. SUBRAMANIAN, U., AND A. E. AHMED. Intestinal toxicity of acrylonitrile: in vitro metabolism by intestinal cytochrome *P*-450 2E1. *Toxicol. Appl. Pharmacol.* 135: 1–8, 1995.
290. SUGIMURA, H., G. S. HAMADA, I. SUZUKI, T. IWASE, E. KIYOKAWA, I. KINO, AND S. TSUGANE. CYP1A1 and CYP2E1 polymorphism and lung cancer, case control study in Rio de Janeiro, Brazil. *Pharmacogenetics* 5, *Suppl.*: S145–S148, 1995.
291. SURBROOK, S. E., JR., AND M. J. OLSON. Dominant role of cytochrome *P*-450 2E1 in human hepatic microsomal oxidation of the CFC-substitute 1,1,1,2-tetrafluoroethane. *Drug Metab. Dispos.* 20: 518–524, 1992.
292. SURH, Y. J., AND S. S. LEE. Capsaicin, a double-edged sword: toxicity, metabolism, and chemopreventive potential. *Life Sci.* 56: 1845–1855, 1995.
293. SVEGLIATI-BARONI, G., E. BARAONA, A. S. ROSMAN, AND C. S.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

LIEBER. Collagen-acetaldehyde adducts in alcoholic and nonalcoholic liver diseases. *Hepatology* 20: 111–118, 1994.
294. TAKAGI, T., H. ISHII, H. TAKAHASHI, S. KATO, F. OKUNO, Y. EBIHARA, H. YAMAUCHI, Y. NAGATA, M. TASHIRO, AND M. TSUCHIYA. Potentiation of halothane hepatotoxicity by chronic ethanol administration in rat: an animal model of halothane hepatitis. *Pharmacol. Biochem. Behav.* 18, *Suppl.* 1: 461–465, 1983.
295. TAKAHASHI, H., I. JOHANSSON, S. W. FRENCH, AND M. INGELMAN-SUNDBERG. Effects of dietary fat composition on activities of the microsomal ethanol oxidizing system and ethanol-inducible cytochrome *P*-450 (CYP2E1) in the liver of rats chronically fed ethanol. *Pharmacol. Toxicol.* 70: 347–352, 1992.
296. TAKAHASHI, T., J. M. LASKER, A. S. ROSMAN, AND C. S. LIEBER. Induction of cytochrome *P*-4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology* 17: 236–245, 1993.
297. TANG, B. K., F. J. GONZALEZ, AND W. KALOW. Caffeine as marker of cytochrome *P*-450 CYP2E1 (Abstract). In: *Int. Symp. Microsomes Drug Oxidations 10th Toronto Canada 1994*, p. 496.
298. TASSANEEYAKUL, W., D. BIRKETT, M. E. McMANUS, W. TASSANEEYAKUL, M. E. VERONESE, T. ANDERSSON, R. H. TUKEY, AND J. O. MINERS. Caffeine metabolism by human hepatic cytochromes *P*-450: contributions of 1A2, 2E1 and 3A isoforms. *Biochem. Pharmacol.* 47: 1767–1776, 1994.
299. TASSANEEYAKUL, W., M. E. VERONESE, D. J. BIRKETT, F. J. GONZALEZ, AND J. O. MINERS. Validation of 4-nitrophenol as an in vitro substrate probe for human liver CYP2E1 using cDNA expression and microsomal kinetic techniques. *Biochem. Pharmacol.* 46: 1975–1981, 1993.
300. TERELIUS, Y., AND M. INGELMAN-SUNDBERG. Metabolism of *n*-pentane by ethanol-inducible cytochrome *P*-450 in liver microsomes and reconstituted membranes. *Eur. J. Biochem.* 161: 303–308, 1986.
301. TERELIUS, Y., AND M. INGELMAN-SUNDBERG. Metabolism of organic solvents by ethanol inducible cytochrome *P*-450 (*P*-450IIEI). In: *Cytochrome P-450. Biochemistry and Biophysics*, edited by I. Schuster. London: Taylor & Francis, 1988, p. 608–611.
302. TERELIUS, Y., C. NORSTEN-HOOG, T. CRONHOLM, AND M. INGELMAN-SUNDBERG. Acetaldehyde as a substrate for ethanol inducible cytochrome *P*-450 (CYP2E1). *Biochem. Biophys. Res. Commun.* 179: 689–694, 1991.
303. 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, 1972.
304. TESCHKE, R., Y. HASUMURA, AND C. S. LIEBER. Hepatic microsomal ethanol oxidizing system: solubilization, isolation and characterization. *Arch. Biochem. Biophys.* 163: 404–415, 1974.
305. TESCHKE, R., Y. HASUMURA, AND C. S. LIEBER. NADPH dependent oxidation of methanol, ethanol, propanol and butanol by hepatic microsomes. *Biochem. Biophys. Res. Commun.* 60: 851–857, 1974.
306. 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. *Mol. Pharmacol.* 11: 841–849, 1975.
307. TESCHKE, R., Y. HASUMURA, AND C. S. LIEBER. Hepatic microsomal alcohol oxidizing system. Affinity for methanol, ethanol and propanol. *J. Biol. Chem.* 250: 7397–7404, 1975.
308. 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. *Alcohol. Clin. Exp. Res.* 1: 7–15, 1977.
309. TESCHKE, R., F. MORENO, AND A. S. PETRIDES. Hepatic microsomal ethanol oxidizing system (MEOS): respective roles of ethanol and carbohydrates for the enhanced activity after chronic alcohol consumption. *Biochem. Pharmacol.* 30: 1745–1751, 1981.
310. THOMPSON, G. N. *Alcoholism.* Springfield, IL: Thomas, 1956.
311. THURMAN, R. G., H. G. LEY, AND R. SCHOLZ. Hepatic microsomal ethanol oxidation. Hydrogen peroxide formation and the role of catalase. *Eur. J. Biochem.* 25: 420–430, 1972.
312. TSUTSUMI, M., J. M. LASKER, M. SHIMIZU, A. S. ROSMAN, AND C. S. LIEBER. The intralobular distribution of ethanol-inducible *P*-450IIE1 in rat and human liver. *Hepatology* 10: 437–446, 1989.
313. TSUTSUMI, M., J. M. LASKER, T. TAKAHASHI, AND C. S. LIEBER. In vivo induction of hepatic *P*-4502E1 by ethanol: role of increased enzyme synthesis. *Arch. Biochem. Biophys.* 304: 209–218, 1993.
314. TSUTSUMI, R., M. A. LEO, C. KIM, M. TSUTSUMI, J. M. LASKER, N. LOWE, AND C. S. LIEBER. Interaction of ethanol with enflurane metabolism and toxicity: role of *P*-450IIE1. *Alcohol. Clin. Exp. Res.* 14: 174–179, 1990.
315. TSUTSUMI, M., M. SHIMIZU, J. M. LASKER, AND C. S. LIEBER. Intralobular distribution of ethanol-inducible cytochrome *P*-450IIE1 in liver (Abstract). *Hepatology* 8: 1237, 1988.
316. TSUTSUMI, M., A. TAKADA, AND J. S. WANG. Genetic polymorphism of cytochrome *P*-4502E1 related to the development of alcoholic liver disease. *Gastroenterology* 107: 1430–1435, 1994.
317. UEMATSU, F., S. IKAWA, H. KIKUCHI, I. SAGAMI, R. KANAMARA, T. ABE, K. SATOH, M. MOTOMIYA, AND M. WATANABE. Restriction fragment length polymorphism of the human *CYP2E1* (cytochrome *P*-450IIE1) gene and susceptibility to lung cancer: possible relevance to low smoking exposure. *Pharmacogenetics* 4: 58–63, 1994.
318. UEMATSU, F., H. KIKUCHI, M. MOTOMIYA, T. ABE, I. SAGAMI, T. OHMACHI, A. WAKUI, R. KANAMARU, AND M. WATANABE. Association between restriction fragment length polymorphism of the human cytochrome *P*-450IIE1 gene and susceptibility to lung cancer. *Jpn. J. Cancer Res.* 82: 254–256, 1991.
319. UENO, T., AND F. J. GONZALEZ. Transcriptional control of the rat hepatic *CYP2E1* gene. *Mol. Cell. Biol.* 10: 4495–4505, 1990.
320. 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.
321. UMENO, M., O. W. McBRIDE, C. S. YANG, H. V. GELBOIN, AND F. J. GONZALEZ. Human ethanol-inducible *P*-450IIE1: complete gene sequence, promoter characterization, chromosomes mapping, and cDNA directed expression. *Biochemistry* 27: 9006–9013, 1988.
322. UMENO, M., B. J. SONG, C. KOZAK, H. V. GELBOIN, AND F. J. GONZALEZ. The rat *P*-450IIE1 gene: complete intron and exon sequence, chromosome mapping, and correlation of developmental expression with specific 5′ cytosine demethylation. *J. Biol. Chem.* 263: 4956–4962, 1988.
323. URBAN, G., P. SPEERSCHNEIDER, AND W. DEKANT. Metabolism of the chlorofluorocarbon substitute 1,1-dichloro-2,2,2-trifluoroethane by rat and human liver microsomes: the role of cytochrome *P*-450 2E1. *Chem. Res. Toxicol.* 7: 170–176, 1994.
324. VALENZUELA, A., V. FERNANDEZ, AND L. A. VIDELA. Hepatic and biliary levels of flutathione and lipid peroxides following iron overload in the rat: effect of simultaneous ethanol administration. *Toxicol. Appl. Pharmacol.* 70: 87–95, 1983.
325. VATSIS, K. P., AND M. P. SCHULMAN. Absence of ethanol metabolism in "acatalatic" hepatic microsomes that oxidize drugs. *Biochem. Biophys. Res. Commun.* 52: 588–594, 1973.
326. VATSIS, K. P., AND M. P. SCHULMAN. An unidentified constituent of ethanol oxidation in hepatic microsomes (Abstract). *Federation Proc.* 33: 554, 1974.
327. VAZ, A. D. N., E. S. ROBERTS, AND M. J. COON. Reductive β-scission of the hydroperoxides of fatty acids and xenobiotics: role of alcohol inducible cytochrome *P*-450. *Proc. Natl. Acad. Sci. USA* 87: 5499–5503, 1990.
328. WATANABE, J., S. HAYASHI, AND K. KAWAJIRI. Different regulations and expression of the human *CYP2E1* gene due to the *Rsa* I polymorphism in the 5′ flanking region. *J. Biochem.* 116: 321–326, 1994.
329. WATANABE, K., S. NARIMATSU, I. YAMAMOTO, AND H. YOSHIMURA. Oxygenation mechanism in conversion of aldehyde to carboxylic acid catalyzed by a cytochrome *P*-450 isozyme. *J. Biol. Chem.* 226: 2709–2711, 1991.
330. WATANABE, J., J. P. YANG, H. EGUCHI, S. HAYASHI, K. IMAI, K. NAKACHI, AND K. KAWAJIRI. An *Rsa* I polymorphism in the *CYP2E1* gene does not affect lung cancer risk in a Japanese population. *Jpn. J. Cancer Res.* 86: 245–248, 1995.
331. WATKINS, J. B., R. A. SANDERS, AND G. A. BECK. The effect of long-term stretozotocin induced diabetes on the hepatotoxicity of

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017

bromobenzene and carbon tetrachloride and hepatic biotransformation in rats. *Toxicol. Appl. Pharmacol.* 93: 229–238, 1988.
332. WHEELER, C. W., S. A. WRIGHTON, AND T. M. GUENTHNER. Detection of human lung cytochromes *P*-450 that are immunochemically related to cytochrome *P*-450IIE1 and cytochrome *P*-450IIIA. *Biochem. Pharmacol.* 44: 183–186, 1992.
333. WICKRAMASINGHE, S. N., G. BARDEN, AND L. LEVY. The capacity of macrophages from different murine tissues to metabolize ethanol and generate an ethanol dependent nondialyzable cytotoxic activity in vitro. *Alcohol Alcohol.* 22: 31–39, 1987.
334. WILSON, R. H. L., E. J. NEWMAN, AND H. W. NEWMAN. Diurnal variation in rate of alcohol metabolism. *Hepatology* 6: 823–829, 1956.
335. WINTERS, D. K., L. A. CLEJAN, AND A. I. CEDERBAUM. Oxidation of glycerol to formaldehyde by rat liver microsomes. *Biochem. Biophys. Res. Commun.* 153: 612–617, 1988.
336. WRIGHTON, S. A., P. E. THOMAS, D. T. MOLOWA, M. HANIU, J. E. SHIVELY, S. L. MAINES, P. B. WATKINS, G. PARKER, G. MENDEZ-PICON, W. LEVIN, AND P. S. GUZELIAN. Characterization of ethanol inducible human liver *N*-nitrosodimethylamine demethylase. *Biochemistry* 25: 6731–6735, 1986.
337. WRIGHTON, S. A., P. E. THOMAS, D. E. RYAN, AND W. LEVIN. Purification and characterization of ethanol inducible human hepatic cytochrome *P*-450Hlj. *Arch. Biochem. Biophys.* 258: 292–297, 1987.
338. WU, D., AND A. I. CEDERBAUM. Presence of functionally active cytochrome *P*-450IIE1 in the plasma membrane of rat hepatocytes. *Hepatology* 15: 515–524, 1992.
339. WU, D., AND A. I. CEDERBAUM. Induction of liver cytochrome *P*-4502E1 by pyrazole and 4-methylpyrazole in neonatal rats. *J. Pharmacol. Exp. Ther.* 264: 1468–1473, 1993.
340. WU, D., L. A. CLEJAN, B. POTTER, AND A. I. CEDERBAUM. Rapid decrease of cytochrome *P*-450IIE1 in primary hepatocyte culture and its maintenance by added 4-methylpyrazole. *Hepatocyte* 6: 1379–1389, 1990.
341. YAMAZAKI, H., Z. GUO, AND F. P. GUENGERICH. Selectivity of cytochrome *P*-4502E1 in chlorzoxazone 6-hydroxylation. *Drug Metab. Dispos.* 23: 438–440, 1995.
342. YANG, C. S., Y. Y. TU, D. R. KOOP, AND M. J. COON. Metabolism of nitrosamines by purified rabbit liver cytochrome *P*-450 isozymes. *Cancer Res.* 45: 1140–1145, 1985.
343. YANG, C. S., J.-S. YOO, H. ISHIZAKI, AND J. HONG. Cytochrome *P*-450IIE1: roles in nitrosamine metabolism and mechanisms of regulation. *Drug Metab. Rev.* 22: 147–159, 1990.
344. YOO, J. H., J. Y. HONG, S. M. NING, AND C. S. YANG. Role of dietary corn oil in the regulation of cytochromes *P*-450 and glutathione *S*-transferases in rat liver. *J. Nutr.* 120: 1718–1726, 1990.
345. YOO, J. H., S. M. NING, C. B. PANTUCK, E. J. PANTUCK, AND C. S. YANG. Regulation of hepatic microsomal cytochrome *P*-450 IIE1 level by dietary lipids and carbohydrates in rats. *J. Nutr.* 121: 959–965, 1991.
346. YU, M.-W., A. GLADEK-YARBOROUGH, S. CHIAMPRASERT, R. M. SANTELLA, Y.-F. LIAW, AND C.-J. CHEN. Cytochrome *P*-450 2E1 and glutathione *S*-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroenterology* 109: 1266–1273, 1995.
347. YUN, Y.-P., J. P. CASAZZA, D. H. SOHN, R. L. VEECH, AND B. J. SONG. Pretranslational activation of cytochrome *P*-450IIE1 during ketosis induced by a high fat diet. *Mol. Pharmacol.* 41: 474–479, 1992.
348. ZAND, R., S. D. NELSON, J. T. SLATTERY, K. E. THUMMEL, T. F. KALHORN, S. P. ADAMS, AND J. M. WRIGHT. Inhibition and induction of cytochrome *P*-4502E1 catalyzed oxidation by isoniazid in humans. *Clin. Pharmacol. Ther.* 54: 142–149, 1993.
349. ZHANG, H., L. A. LONEY, AND B. J. POTTER. Effect of chronic alcohol feeding on hepatic iron status and ferritin uptake by rat hepatocytes. *Alcohol. Clin. Exp. Res.* 17: 394–400, 1993.
350. ZHANG, Z. Y., AND L. S. KAMINSKY. Characterization of human cytochromes *P*-450 involved in theophylline 8-hydroxylation. *Biochem. Pharmacol.* 50: 205–211, 1995.
351. ZIEGLER, D. M. Discussion in microsomes and drug oxidation. In: *Microsomes and Drug Oxidations*, edited by R. W. Estabrook, R. J. Gillette, and K. C. Liebman. Baltimore, MD: Williams & Wilkins, 1972, p. 458.

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on January 2, 2017