CASARETT AND DOULL'S
TOXICOLOGY
THE BASIC SCIENCE OF POISONS

Sixth Edition

EDITOR

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ing CYP2E1, are targets for free radical attack. Albano and coworkers (1999) have demonstrated a marked increase in covalent binding of hydroxyethyl radicals to hepatic microsomal proteins from rats following chronic ethanol administration. There is not yet direct evidence that hydroxyethyl radicals contribute to lipid peroxidation, but they do readily react with α-tocopherol, GSH, and ascorbic acid, thereby potentially lowering liver antioxidant levels in vivo. Albano et al. (1999) also describe evidence that hydroxyethyl radical-protein adducts in hepatocytes induce immune responses, which may contribute to chronic ethanol hepatotoxicity.

Alcohol-induced hepatotoxicity is postulated to be caused by elevation of endotoxin in the bloodstream. Endotoxin, released by the action of ethanol on gram-negative bacteria in the gut, is believed to be taken up by Kupffer cells, causing the release of mediators which are cytotoxic to hepatocytes and chemoattractants for neutrophils (Bautista and Spitzer, 1999). These mediators include interleukins, prostaglandins, free radicals, and tumor necrosis factor α. Kupffer cells of female rats are more sensitive to endotoxin than cells of males. Estrogen increases the sensitivity of Kupffer cells of rats to endotoxin. This phenomenon is believed to account in part for the more severe hepatitis and cirrhosis commonly seen in female alcoholics (Thurman, 1998).

Alcohol-induced tissue damage is believed to result from nutritional disturbances as well as direct toxic effects (Lieber, 1997b). Lack of money, poor judgment, prolonged inebriation, and appetite loss contribute to poor nutrition and weight loss in alcoholics. A high percentage of calories in the alcoholic's diet are furnished by alcohol. Malabsorption of thiamine, diminished enterohepatic circulation of folate, degradation of pyridoxal phosphate, and disturbances in the metabolism of vitamins A and D can occur (Mezey, 1985). Prostaglandins released from endotoxin-activated Kupffer cells may be responsible for a hypermetabolic state in the liver. With the increase in oxygen demand, the viability of centrilobular hepatocytes would be most compromised due to their relatively poor oxygen supply (Thurman, 1998). Metabolism of ethanol via ADH and ALDH results in a shift in the redox state of the cell. The metabolites and the more reduced state can result in hyperlactacidemia, hyperuricemia, and hyperglycemia (Lieber, 1997b).

Alcoholism can result in damage of extrahepatic tissues. Cardiomyopathy is one of the more serious consequences. Alcoholic cardiomyopathy is a complex process that is believed to result from decreased synthesis of cardiac contractile proteins, attack of oxygen radicals, and antibody response to acetaldehyde-protein adducts (Richardson et al., 1998). Interestingly, light to moderate drinking is reported to protect against atherosclerosis in the carotid artery, a major cause of ischemic stroke (Hillbom, 1999). It is hypothesized that ethanol metabolism in the vascular wall may inhibit oxidation of low density lipoproteins (LDL), a requisite for atherogenesis. Phenolic antioxidants in wines may also inhibit LDL oxidation as well as reduce platelet aggregation. Conversely, heavy drinking appears to deplete antioxidants and have the opposite effects (Camargo, 1996; Hillbom, 1999). Recent heavy drinking increases the risk of both hemorrhagic and ischemic strokes. Other organ systems can be adversely affected in alcoholics including the brain and pancreas (Preedy et al., 1997; Kril et al., 1997).

There is concern about the role of ethyl alcohol in carcinogenesis, due to the frequent consumption of alcoholic beverages by millions of people. IARC (1988) concluded that there was "sufficient evidence" for causation of tumors of the oral cavity, pharynx and larynx, esophagus, and liver of humans. The associations between alcohol and cancers came primarily from epidemiologic case-control and cohort studies. One such cohort study of 276,000 American men showed increase in total cancer risk with increasing ethanol consumption (Boffeta and Garfinkel, 1990). Ethanol and smoking act synergistically to cause oral, pharyngeal, and laryngeal cancers. It is generally believed that alcohol induces liver cancer by causing cirrhosis or other liver damage and/or by enhancing the bioactivation of carcinogens. Hypotheses of Ahmed (1995) for possible mechanisms of causation of cancers are included in Table 24-3.

Methanol

Methanol (methyl alcohol, wood alcohol) is found in a host of consumer products including windshield washer fluid, and is used in the manufacture of formaldehyde and methyl tert-butyl ether (MTBE). Methanol is being promoted as a gasoline additive and alternative automotive fuel. Risks of low-level, chronic exposures will be at issue if the latter uses become commonplace.

Like most hydrocarbon solvents, methanol can produce reversible sensory irritation and narcosis at airborne concentrations below those producing organ system pathology. Serious methanol toxicity is most commonly associated with ingestion. Left untreated, acute methanol poisoning in humans is characterized by an asymptomatic latent period of 12 to 24 h followed by formic acidemia, ocular toxicity, coma, and in extreme cases death. Visual disturbances generally develop between 18 and 48 h after ingestion and range from mild photophobia and misty or blurred vision to markedly reduced visual acuity and complete blindness (Eells et al., 1996). Although there is considerable variability among individuals in susceptibility to methanol toxicity, a frequently cited lethal oral dosage is 1 mL/kg. Blindness and death have been reported with dosages as low as 0.1 mL/kg (ATSDR, 1993).

The target of methanol within the eye is the retina, specifically the optic disk and optic nerve. Optic disk edema and hyperemia are seen, along with morphological alterations in the optic nerve head and the intraorbital portion of the optic nerve. Both axons and glial cells exhibit altered morphologies (Kavet and Nauss, 1990). Evidence is accumulating that Müller cells, neuroglia that function in the maintenance of retinal structure and in intra- and intercellular transport, are early targets of methanol (Garner et al., 1995a). Rods and cones, the photoreceptors of the retina, are also altered functionally and structurally (Seme et al., 1999). There are indications of mitochondrial disruption in Müller and photoreceptor cells. This is consistent with the long-held view that cytochrome

Table 24-3
Possible Mechanisms of Ethanol Carcinogenicity

Congeners, additives and contaminants in alcoholic beverages influence carcinogenicity.

CYP2E1 induction by ethanol increases metabolic activation of procarcinogens.

Ethanol acts as a solvent for carcinogens, enhancing their absorption into tissues of the upper GI tract.

Ethanol affects the actions of certain hormones on hormone-sensitive tissues.

Immune function is suppressed by alcohol.

Absorption and bioavailability of nutrients are reduced by alcohol.

SOURCE: Adapted with permission of Ahmed (1995).

oxidase activity in mitochondria is inhibited, resulting in a reduction in ATP. This mechanism would explain, at least in part, the selective toxicity to photoreceptors and other highly metabolically active cells (Eells et al. 1996).

Elucidation of the mechanism of acute toxicity of methanol was hampered for years by species differences in susceptibility and the lack of appropriate animal models. Largely based on the work of Gilger and Potts (1955), it became apparent that only nonhuman primates respond to methanol similarly to humans. The severe metabolic acidosis frequently seen in humans does not occur in rodents. In all mammalian species studied, methanol is metabolized in the liver to formaldehyde (HCOH), which in turn is very rapidly converted to formate. The conversion of formate to CO_2 then occurs via a two-step, tetrahydrofolate (THF)-dependent pathway. First, formate is converted to 10-formyl-THF by formyl-THF synthetase, after which 10-formyl-THF is oxidized to CO_2 by formyl-THF dehydrogenase (F-THF-DH). The species differences in susceptibility to methanol are thought to result primarily from differences in the rate of THF-dependent oxidation of formate to CO_2. Since rodents have higher hepatic THF levels than primates, formate does not accumulate as it does in humans and monkeys (Medinsky and Dorman, 1995; Martinasevic et al., 1996). Another possible explanation is the lower F-THF-DH activity in primate liver (Johlin et al., 1989). Thus, susceptibility to methanol toxicity is dependent upon the relative rate of formate clearance. Dietary and chemical depletion of endogenous folate cofactors in rats have been shown to increase formate accumulation following methanol, resulting in the development of metabolic acidosis and ocular toxicity similar to that observed in humans (Eells et al., 1996). A simplified scheme of methanol metabolism is presented in Fig. 24-10.

For years there was considerable debate about whether HCOH or formate was responsible for methanol's ocular toxicity. The finding that HCOH does not accumulate following methanol treatment, even in folate-deficient monkeys profoundly sensitive to methanol, argues against a role for HCOH. Also, species of differing susceptibility exhibit comparable blood HCOH half-lives (~1 to 2 min). In contrast, formate has been shown to accumulate in the human and monkey following methanol treatment, and to induce ocular toxicity in monkeys after an infusion where normal blood pH was maintained (Martin-Amat et al., 1978). Eells et al. (1996), using a rat model in which formate oxidation had been selectively

inhibited with nitrous oxide, have also shown that methanol-induced retinal dysfunction can be produced in the absence of metabolic acidosis. In this same publication, methanol-derived formate was quantified in the vitreous humor, retina, and to a lesser extent in the optic nerve. Moreover, these authors demonstrated that methanol-induced retinal dysfunction, as indicated by diminution of the amplitude of electroretinogram (ERG) *a* and *b* waves, was negatively correlated in a linear fashion with blood formate concentrations. Others have reported similar relationships between blood formate and ERG responses indicative of photoreceptor dysfunction (Seme et al., 1999). Thus, formate appears to act as a direct ocular toxin and not indirectly through the induction of an acidotic state, though acidosis may potentiate formate toxicity since the inhibition of cytochrome oxidase increases as pH decreases.

The question has been raised as to whether ocular toxicity is simply a function of circulating formate reaching the visual tract, or whether metabolism in retinal or optic nerve tissues generates toxic metabolites locally. This is a legitimate question considering that metabolism of methanol to formaldehyde via peroxisomal enzymes (catalase) has been demonstrated in rat retina in vitro (Garner et al., 1995a), and the presence of cytoplasmic aldehyde dehydrogenase activity has been demonstrated in several regions of the rat and mouse eye, including the retina (Messiha and Price, 1983; McCaffery et al., 1991). By use of a folate-deficient rat model that mimics human methanol toxicity, Garner et al. (1995b) showed that a level of blood formate generated by IV infusion of pH-buffered formate did not diminish the ERG *b*-wave amplitude generated by Müller cells of the retina, as did a comparable level of blood formate derived from methanol. This suggests that the intraretinal metabolism of methanol is necessary for the initiation of retinal toxicity by formate. Not only are the enzymes necessary to produce formate present in the retina, but so too are folate and formyl-THF dehydrogenase, both necessary for formate oxidation. Formyl-THF dehydrogenase was found to be localized in the mitochondria of Müller cells, prompting the suggestion that formyl-THF dehydrogenase may serve a dual role, one protective of the Müller cell and the other toxic. Protection would come in the form of formate oxidation; toxicity from the overconsumption of ATP required for formate metabolism via the folate pathway (Martinasevic et al., 1996). These findings raise new questions about the safety of methanol exposure. For example, chamber studies of human volunteers exposed to 200 ppm methanol for 4 or 6 h showed no blood formate accumulation above background (Lee et al., 1992; d'Alessandro et al., 1994). While this might be considered as evidence that exposure at the current ACGIH TLV and OSHA PEL of 200 ppm poses no risk for ocular toxicity, such an interpretation may not be valid given that ocular toxicity may be a function of intraretinal methanol metabolism rather than circulating formate levels.

While there is still much to be learned about mechanisms of methanol toxicity, what is known allows for effective therapies, if they are applied in a timely manner. Sodium bicarbonate is usually given iv to correct severe acidosis. Metabolic blockade is usually achieved with ethanol or 4-methylpyrazole, both acting as effective competitive inhibitors of ADH by virtue of their greater affinities than methanol for ADH. Folate therapy is also indicated to increase the efficiency of formate oxidation. Methanol exemplifies the benefits of knowing a chemical's mode of action when treating the poisoned patient. This knowledge also aids in identifying potentially sensitive subpopulations, such as those suffering from dietary folate deficiency.
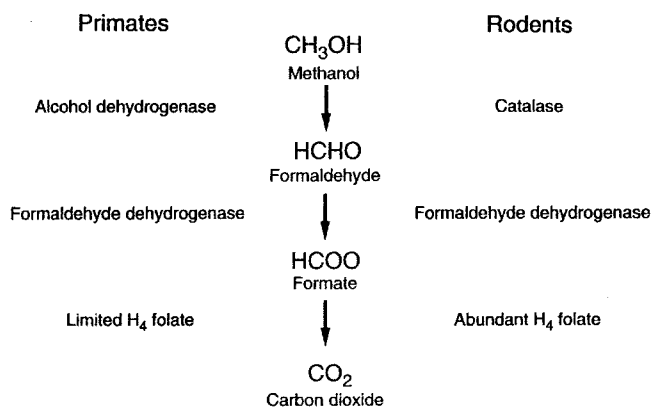

Figure 24-10. Scheme for the metabolism of methanol.

Major enzymes are listed for primates (*left*) and rodents (*right*). Conversion of formate to CO_2 is rapid in rodents, but relatively slow in primates. (Used with permission of Dorman DC, Welsch F: *CIIT Activities* 16:1–7, 1996.)

ing site of the mitochondrial permeability transition pore (Szabo et al., 1992) and subsequently open it, which initiates the cytochrome *c*–caspase cascade of apoptosis in rods (He et al., 2000).

In vitro extracellular and intracellular electrophysiologic recordings in isolated whole retinas or photoreceptors reveal that nanomolar to micromolar concentrations of lead chloride selectively depress the amplitude and absolute sensitivity of the rod but not cone photoreceptor potential (Fox and Sillman, 1979; Sillman et al., 1982; Tessier-Lavigne et al., 1985; Frumkes and Eysteinsson, 1988). These electrophysiologic results are similar to the ERG alterations observed in occupationally lead-exposed workers (Cavelleri et al., 1982; Betta et al., 1983; Signorino et al., 1983; Campara et al., 1984; Jeyaratnam et al., 1986) and in adult rats exposed to low and moderate levels of lead only during development (Fox and Farber, 1988; Fox and Rubinstein, 1989; Fox et al., 1991a; Fox and Katz, 1992). In addition, these developmentally lead-exposed rats exhibit rod-mediated increases in dark and light adaptation time, decreases in critical flicker fusion frequency (i.e., temporal resolution), decreases in relative sensitivity, and increases in a- and b-wave latencies (Fox and Farber, 1988; Fox and Rubinstein, 1989; Fox et al., 1991a; Fox and Katz, 1992) and decreases in the temporal response properties of both sustained (X-type) and transient (Y-type) RGCs, such as decreased optimal temporal frequency and temporal resolution (Ruan et al., 1994). By extension, these results suggest that there is a common underlying biochemical mechanism responsible for these rod-mediated deficits. In vivo and in vitro data suggest that lead-induced inhibition of cGMP PDE and resultant elevation of rod Ca^{2+} underlies the ERG deficits (Fox and Katz, 1992; Medrano and Fox, 1994; Fox et al., 1997; He et al., 2000). Finally, rod-mediated alterations in dark adaptation and b-wave amplitude are also observed in adult rats and monkeys with prenatal and lifetime moderate- and high-level lead exposure (Hennekes et al., 1987; Lilienthal et al., 1988; Lilienthal et al., 1994). In the lead-exposed monkeys, the amplitude of the scotopic b-wave was increased—an effect hypothesized to result from the loss of dopaminergic amacrine cells or their processes (Lilienthal et al., 1994; Kohler et al., 1997). If rods and blue-sensitive cones in humans exhibit the same sensitivity to a lead-induced inhibition of cGMP-PDE as they do to the drug-induced inhibition of cGMP-PDE (Zrenner and Gouras, 1979; Zrenner et al., 1982). Fox and Farber (1998) predicted that blue-cone color vision deficits as well as scotopic deficits may be found in adults and children exposed to lead. Recently, S- (or blue-) cone deficits were observed in an occupationally lead-exposed worker (Scholl and Zrenner, 2000).

Methanol   Methanol is a low-molecular-weight (32), colorless and volatile liquid that is widely used as an industrial solvent; a chemical intermediate; a fuel source for picnic stoves, racing cars, and soldering torches; an antifreeze agent; and an octane booster for gasoline. The basic toxicology and references can be found in two thorough reviews (Tephly and McMartin, 1984; Eells, 1992). Briefly, methanol is readily and rapidly absorbed from all routes of exposure (dermal, inhalation, and oral), easily crosses all membranes, and thus is uniformly distributed to organs and tissues in direct relation to their water content. Following different routes of exposures, the highest concentrations of methanol are found in the blood, aqueous and vitreous humor, and bile as well as the brain, kidneys, lungs, and spleen. In the liver, methanol is oxidized sequentially to formaldehyde by alcohol dehydrogenase in human and nonhuman primates or by catalase in rodents and then to formic acid. It is excreted as formic acid in the urine or oxidized further

to carbon dioxide and then excreted by the lungs. Formic acid is the toxic metabolite that mediates the metabolic acidosis as well as the retinal and ON toxicity observed in humans, monkeys, and rats with a decreased capacity for folate metabolism (Tephly and McMartin, 1984; Murray et al., 1991; Eells, 1992; Lee et al., 1994a; Lee et al., 1994b; Garner et al., 1995a; Garner et al., 1995b; Eells et al., 1996; Seme et al., 1999).

Human and nonhuman primates are highly sensitive to methanol-induced neurotoxicity due to their limited capacity to oxidize formic acid. The toxicity occurs in several stages. It first occurs as a mild CNS depression, followed by an asymptomatic 12- to 24-h latent period, followed by a syndrome consisting of formic acidemia, uncompensated metabolic acidosis, ocular and visual toxicity, coma, and possibly death (Tephly and McMartin, 1984; Eells, 1992). The treatment of methanol poisoning involves both combating acidosis and preventing methanol oxidation, but it is not discussed further here. Experimental rats have been made as sensitive to acute methanol exposure as primates by using two different, but related, procedures that effectively reduce the levels of hepatic tetrahydrofolate. One study used a brief (4 h) conditioning and then continuous exposure (60 h) to subanesthetic concentrations of nitrous oxide to inhibit methionine synthase and reduce the level of hepatic tetrahydrofolate (Murray et al., 1991; Eells et al., 1996; Seme et al., 1999). The other fed rats a folate-deficient diet for 18 weeks (Lee et al., 1994a; Lee et al., 1994b). Administration of methanol to these rats with a decreased capacity for folate metabolism resulted in toxic blood formate concentrations of 8 to 16 mM (Murray et al., 1991; Lee et al., 1994a; Lee et al., 1994b; Garner et al., 1995a; Garner et al., 1995b; Eells et al., 1996; Seme et al., 1999). Permanent visual damage occurs in humans and monkeys when the blood folate levels exceed 7 mM (Tephly and McMartin, 1984; Eells, 1992).

Acute methanol poisoning in humans, monkeys, and experimental rats results in profound and permanent structural alterations in the retina and ON and visual impairments ranging from blurred vision to decreased visual acuity and sensitivity to blindness. Ophthalmologic studies of exposed humans and monkeys reveal varying degrees of edema of the papillomacular bundle, ON head, and entire optic disk (Benton and Calhoun, 1952; Potts, 1955; Baumbach et al., 1977; Hayreh et al., 1980). Histopathologic and ultrastructural investigations in methanol-exposed monkeys and folate-modified rats show retinal edema, swollen and degenerated photoreceptors, degenerated RGCs, swollen retinal pigment epithelial cells, axonal (ON) swelling, and mitochondrial swelling and disintegration in each of these cells but especially in the photoreceptors and ON (Baumbach et al., 1977; Hayreh et al., 1980; Murray et al., 1991; Seme et al., 1999). Considering the differences in species, methanol exposures, time course of analysis, and procedures utilized, the overall data for the acute effects of methanol on the ERG are remarkably consistent. Following methanol exposure, the ERG b-wave amplitude in humans, monkeys, and folate-modified rats starts to decrease significantly when the blood formate concentration exceeded 7 mM (Potts, 1955; Ruedeman, 1961; Ingemansson, 1983; Murray et al., 1991; Lee et al., 1994b). These ERG b-wave alterations, as well as flicker-evoked ERG alterations (Seme et al., 1999), occur at lower formate concentrations than those associated with structural changes in the retina and ON, as discussed above. Decreases in the a-wave amplitude are delayed, relative to the b-wave and occur when blood formate concentrations further increase (Ruedeman, 1961; Ingemansson, 1983; Murray et al., 1991; Eells et al., 1996). In addi-