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METABOLIC METHANOL: MOLECULAR PATHWAYS AND PHYSIOLOGICAL ROLES

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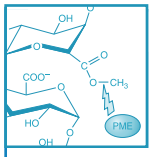

Dorokhov YL, Shindyapina AV, Sheshukova EV, Komarova TV. Metabolic Methanol: Molecular Pathways and Physiological Roles. *Physiol Rev* 95: 603–644, 2015; doi:10.1152/physrev.00034.2014.—Methanol has been historically considered an exogenous product that leads only to pathological changes in the human body when consumed. However, in normal, healthy individuals, methanol and its short-lived oxidized product, formaldehyde, are naturally occurring compounds whose functions and origins have received limited attention. There are several sources of human physiological methanol. Fruits, vegetables, and alcoholic beverages are likely the main sources of exogenous methanol in the healthy human body. Metabolic methanol may occur as a result of fermentation by gut bacteria and metabolic processes involving *S*-adenosyl methionine. Regardless of its source, low levels of methanol in the body are maintained by physiological and metabolic clearance mechanisms. Although human blood contains small amounts of methanol and formaldehyde, the content of these molecules increases sharply after receiving even methanol-free ethanol, indicating an endogenous source of the metabolic methanol present at low levels in the blood regulated by a cluster of genes. Recent studies of the pathogenesis of neurological disorders indicate metabolic formaldehyde as a putative causative agent. The detection of increased formaldehyde content in the blood of both neurological patients and the elderly indicates the important role of genetic and biochemical mechanisms of maintaining low levels of methanol and formaldehyde.

I. INTRODUCTION

Robert Boyle first described wood spirits, or methanol, as the "sowrish spirit" of boxwood pyrolysis in 1661 (44), and the function of methanol in plant and animal life has since been unclear. In higher plants, cell wall (CW) pectin methylesterase (PME) produces methanol by pectin demethylation (248). Terrestrial atmospheric methanol emission comes from volcanoes, H_2 and CO_2 generation within seafloor hydrothermal systems, and biomass combustion, but PME-mediated emission from plants is most likely the largest source of methanol in the atmosphere (512). Methanol accumulates in the intercellular air space or the liquid pool when the stomata close at night, and a large quantity of methanol is released when the stomata open in the morning (193). Gaseous methanol was traditionally considered to be a biochemical "waste product." However, the effects of PME-generated plant methanol ("emitters") on plant defensive reactions ("receivers") and plant-animal communication have recently been shown (111, 112).

In humans, methanol is considered to be a poison because alcohol dehydrogenase 1b (ADH1b) mainly metabolizes methanol into toxic formaldehyde (63). Methanol itself is not toxic to animal cells; however, formaldehyde is responsible for carcinogenesis and age-related damage to neurons in the brain (472).

Until recently, it was believed that the trace amounts of methanol and formaldehyde in the blood of healthy people came only from the consumption of fake or low-quality alcoholic beverages. However, recent data have indicated that methanol and short-lived formaldehyde are actually naturally occurring compounds in normal, healthy human individuals (413). There are several sources of physiological methanol in humans **(FIGURE 1)**. Fruits, vegetables, and alcoholic beverages are likely to be the main sources of exogenous methanol in the healthy humans. More than 50 years ago (126), two other sources were suggested: anaerobic

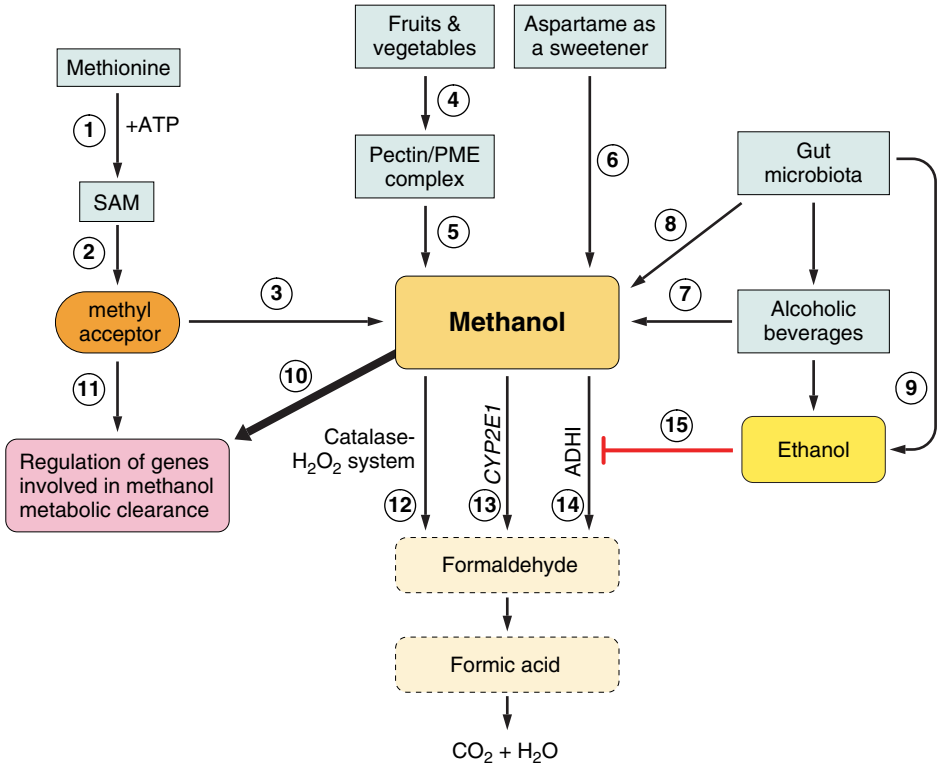

FIGURE 1. Overview of physiological methanol biogenesis. This figure summarizes the data on methanol. The methyl group donor SAM is synthesized via the catalytic activity of methionine adenosyltransferase, which transfers the adenosyl group of ATP to methionine (step 1). *S*-adenosyl homocysteine is formed after SAM transfers a methyl group to a methyl acceptor (step 2) such as DNA; thus methanol is involved in gene regulation (step 11). Methyl esters such as carboxyl methyl esters are unstable and are readily hydrolyzed in neutral and basic pH conditions or by methylesterase to produce methanol (step 3). Other sources of methanol include the human diet, which supplies the methanol-generating pectin/PME complex via fruits and vegetables (steps 4 and 5), aspartame as a synthetic nonnutritive sweetener (step 6) and alcoholic beverages (step 7). The human gut microbiota is a putative methanol source (step 8) and takes part in the generation of human endogenous ethanol (step 9). We suggest that endogenous and dietary methanol may be involved in the regulation of genes involved in the metabolic clearance of methanol (step 10). The first stage of the oxidative metabolism of methanol is executed by the catalase-H_2O_2 system (step 12), cytochrome *P*450 (CYP2E1)-mediated oxidation (step 13) and, mainly, the alcohol dehydrogenase I (ADH1) class of enzymes (step 14). Although ADH1 converts methanol into toxic formaldehyde, physiological ethanol in the bloodstream substantively prevents all formaldehyde production from endogenous and dietary methanol in humans (step 15).

fermentation by gut bacteria (38, 208, 209, 418) and the transformation of *S*-adenosyl methionine (SAM) to methanol by certain metabolic processes (16). Although human blood contains small amounts of methanol and formaldehyde, their contents are sharply increased after receiving even methanol-free ethanol (413). This indicates the existence of an endogenous source of low levels of metabolic methanol in the blood, the regulation of which is controlled by a cluster of genes (246, 413).

Interest in the literature is mainly focused on the mechanism of ethanol metabolism (63, 347). Ethanol consumption, in small amounts, can have a beneficial effect (147), but ethanol abuse leads to pathological changes in the human body (355). In the past, methanol was mainly considered an exogenous product; the consumption of methanol was believed only to lead to pathological changes in the human body (453). Researchers did not pay attention to either the role or the origin of endogenous methanol and formaldehyde in humans. Recent studies of the pathogenesis of neurological disorders indicate that metabolic formaldehyde is a putative causative agent of human pathology (464, 466). The detection of increased formaldehyde content in the blood of not only neurological patients but also elderly people (463) indicates the important role of genetic and biochemical mechanisms in maintaining low levels of methanol and formaldehyde.

This review will cover the wide spectrum of metabolic methanol phenomena, both physiological and pathological, including the origin of human metabolic methanol, the processes of methanol conversion and clearance, and the roles of methanol and formaldehyde in human pathology. Ultimately, an imbalance between the inflow and outflow of formaldehyde may result in the onset of pathologies.

II. TERRESTRIAL METHANOL

A. Methanol in the Earth's Atmosphere

Methanol is an ubiquitous, biogeochemically active compound and a significant component of the volatile organic carbon in the atmosphere (177, 199, 239, 356, 390, 519). The atmosphere contains ~4 Tg (teragrams, 10^{12} g) of methanol (177). Terrestrial atmospheric methanol emissions come from different sources, including volcanoes and H_2 and CO_2 generation within seafloor hydrothermal systems (512). Another source of atmospheric methanol is biomass combustion (131, 315), in which the wood pyrolysis of plant fibers (i.e., cellulose and lignin) induces methanol emission (149, 300, 515). Wood pyrolysis, i.e., the decomposition of wood at elevated temperatures in the absence of oxygen, was the method that allowed Robert Boyle to obtain the "sowrish spirit" (44) from boxwood; much later, this became known mainly as methanol and acetone. However, volatile organic compound emissions from plants are most likely the largest sources of methanol in the atmosphere (193, 407). After CO_2 and isoprene, gaseous methanol is one of the most abundant carbon-containing volatile organic compounds in the atmosphere (156, 407).

B. Plant-Made Methanol

Plant-emitted gaseous methanol is an abundant, volatile organic compound that was considered for a long time to be a waste product of plant metabolism. Now, the diverse biological effects of methanol have been discovered and demonstrated. The main source of plant methanol release is the above-ground parts of the plant (353). Plant methanol accumulates in the leaf intercellular spaces in either a dissolved or a gaseous state when the stomata are closed at night. When the leaf stomata open in the morning, a significant amount of methanol is released (193, 343). Plant methanol release occurs with varying intensity depending on the stage of plant development (343), time of day (193), and other conditions. The quantity of the leaf-emitted methanol varies from a minimum of 0.38 μg·g fresh weight $(FW)^{-1}\cdot h^{-1}$ at night to a maximum of 7 μg·g $FW^{-1}\cdot h^{-1}$ in the morning, while the average value is ~3 μg·g $FW^{-1}\cdot h^{-1}$. It should be noted that under certain types of stress, methanol emission is dramatically increased to up to 100 μg·g $FW^{-1}\cdot h^{-1}$. In addition, damaged plants emit significant amounts of methanol (48, 230). For example, in alfalfa fields, high levels of gaseous methanol were detected after mowing, and methanol emission levels continued to rise over the next 3 days (505).

Plant tissues have been shown to metabolize methanol (115). The majority of endogenous methanol reaches the leaf surface and evaporates, and a minor amount is nonenzymatically oxidized to formaldehyde, which could later be involved in the synthesis of serine, methionine, and phosphatidylcholine. In addition, methanol could be enzymatically oxidized to CO_2 and then directed to the Calvin cycle (151). Methanol metabolism in plants can be accompanied by significant increases in biomass; in some C3 plants, this is often accompanied by an increased photosynthetic efficiency and developmental rate (151, 346). Moreover, plant-generated methanol could be involved in leaf growth during plant development (247). Small amounts of methanol emitted through the stomata are oxidized to carbon dioxide by methylotrophic bacteria either directly on the leaves or later in the soil (244). In general, methanol is a rather stable substance under normal conditions with a half-life of ~10 days (199).

Plants produce methanol in the CW pectin de-methyl-esterification reaction **(FIGURE 2)**. Pectin is a matrix-forming component of the plant CW. Cellulose and xyloglucan fibrils are immersed in the pectin matrix (90). Pectin is mainly formed from homogalacturonan blocks of α-1,4-linked galacturonic acids, but rhamnogalacturonan I, rhamnogalacturonan II, and xylogalacturonan are the other major components of the pectin matrix (165, 369). Pectin homogalacturonan is a compound that is synthesized by plant cells and secreted into the CW in a highly methyl-esterified form **(FIGURE 2)**.

During the growth and development of cells or CW-mechanical damage pectin in the CW can be de-methyl-esterified, resulting in the production of methanol and the accumulation of negatively charged galacturonic acid residues **(FIGURE 2)**. Due to the interaction of Ca^{2+} with the charged carboxyl groups, the rigidity of the CW can be increased if the de-methylated residues are arranged in blocks. On the other hand, in the case of the random de-methyl-esterification of pectin, CW softening can occur, i.e., homogalacturonan becomes available for hydrolysis by pectin-degrading enzymes of the CW, resulting in the initiation of pectin cleavage (511). Both PME-associated modulations of the CW and the degree of pectin methylation play essential roles in mediating the plasticity of the CW, cellular adhesion, ionic composition, and pH (371). All of these aspects are very important in the growth and development of plants, pollination, and fruit ripening, as well as in the responses of plant cells to stress factors.

While they are conserved proteins, plant PMEs comprise a multigene family (371). Some PME genes are ubiquitously expressed, while others are specifically expressed during fruit ripening, microsporogenesis, and germination of the pollen grain, or stem elongation (304). Most higher plant PMEs are synthesized as a precursor protein that contains an NH_2 terminus pre-pro-sequence, which is necessary for the correct processing to form a mature enzyme and deliver it to the CW. The pre-sequence, which may be presented with a signal peptide and/or a transmembrane domain, is

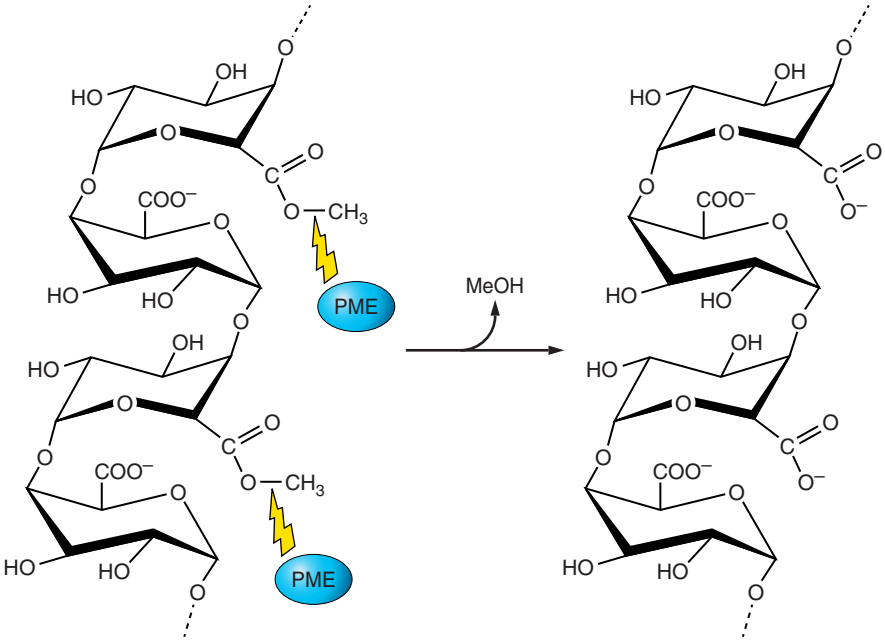

FIGURE 2. Demethylesterification of pectin homogalacturonan by PME with methanol formation.

cleaved at the stage of precursor delivery through the endoplasmic reticulum to the CW (290, 303), and the pro-sequence is removed at the next step of maturation (114, 513). Only group 2 PMEs possess the pro-sequence, which is necessary for the correct folding of the enzyme and is believed to function as an intramolecular chaperone (318, 371). In addition to the importance of PMEs during processes of plant growth and development, PMEs play a significant role in the protection of plants against external stresses. PMEs are involved in modifications of the CW, which is a natural barrier that protects and separates the cell from the environment. Aside from the direct effects of PMEs on the CW, the enzymes also act indirectly through different compounds, including the methanol released via pectin de-esterification (488a, 112, 371). Pectin with a higher degree of methylation provides better resistance to fungal and bacterial pectolytic enzymes. Thus plants that are transgenic for the *PME inhibitor (PMEI)* gene exhibit increased resistance to pathogenic fungi and bacteria compared with wild-type plants (282, 283, 488). On the other hand, PME is activated in response to the invasion of pathogenic fungi and bacteria, and increased PME activity occurs as a part of pattern-triggered immunity (31). CW integrity damage by pathogenic pectolytic enzymes results in the accumulation of oligogalacturonide fragments, which play a role in damage-associated molecular patterns (140). Oligogalacturonide de-methylation by PMEs leads to the enhancement of their activity as elicitors and, thus, the improvement of plant defense responses to the invasion of pathogenic fungi and bacteria (31, 383). With regard to viral pathogens, PMEs have been shown to participate in the virus cell-to-cell movement (74), which is necessary for successful viral infection. PME is known to interact with the movement protein of tobacco mosaic virus (TMV) (75, 113), and this interaction is required for the intercellular transport of TMV. The important role of PME in the spreading of viruses through the plant was confirmed by the fact that *Arabidopsis* transgenic for the *PMEI* gene exhibits decreased intercellular transport of TMV and lower susceptibility to the virus (284). On the other hand, PMEs were also shown to enhance virus-induced gene silencing (113) and to indirectly interfere with nucleocytoplasmic transport (245).

One of the indirect ways by which PME affects host-pathogen relationships is via gaseous methanol that is formed during the pectin demethylation reaction in response to injury. The effects of PME-generated methanol from plant emitters on the defensive response of neighboring plant receivers were recently studied (111). It was shown that an increase in methanol emission from PME-transgenic or nontransgenic wounded plants restrains the growth of the pathogenic bacteria *Ralstonia solanacearum* in neighboring receiver plants. However, at the same time, plants exposed to gaseous methanol became more sensitive to the viral infection, likely due to the general activation of intercellular transport. Such antibacterial resistance and virus susceptibility are accompanied by increased activities of the genes responsible for stress control and intercellular communication in receiver plants. These results led to the conclusion that methanol is a signaling molecule that is involved in the intra- and interplant communication (111, 247, 248). The first portion of methanol emitted by damaged leaves is likely produced by PME that is preexisting in the CW; this allows for rapid methanol release into the atmosphere (250, 488a). However, simultaneous de novo PME synthesis is induced, which allows a high level of methanol emission to be maintained (111). Based on the available data, we can conclude that methanol, which is released into the air by damaged plants or plants attacked by herbivorous insects, serves as

an alarm to help neighboring plants or adjacent leaves prepare for defense.

C. Roles of Methanol and Ethanol in Plant-Animal Communication

The role of methanol in plant-herbivore relationships remains rather controversial. As was mentioned above, plants release methanol during their growth and development, as well as in response to wounding. Gaseous methanol plays a role in signaling in the plant community and is also likely to play a role in plant-animal communication. Methanol is toxic to some insects (106, 288) and mediates defense reactions against some herbivores (22). On the other hand, gaseous methanol attracts certain insects such as bark beetles (*Hylurgops palliatus, Tomicus piniperda, and Trypodendron domesticum*), while long-chain alcohols do not act as attractants (56). Methanol emission occurs in response to *Manduca sexta* larvae feeding on *Nicotiana* plants and appears to play a beneficial role for that insect: the treatment of plants with the same amount of methanol as is released during larvae feeding led to decreased plant defense reactions and increased larvae attacking performance (488a). Thus methanol release may induce metabolic changes that influence the susceptibility of plants to herbivores. Furthermore, laboratory mice prefer the odor of methanol to that of other plant-emitted volatiles or ethanol. Methanol emitted by wounded plants was shown to be an attractant: mice in a two-choice Y-maze chose methanol over other plant volatiles. Moreover, inhalation of vapors released by wounded plants led to increased blood methanol levels in mice and changes in the expression profiles of the so-called methanol-responsive genes in the brain (112, 246). This discovery led to the conclusion that methanol emitted from damaged plants should be regarded not only as a signal of plant-to-plant communication but also as a plant-to-animal cross-kingdom signaling molecule that modulates both behavior and mammalian gene expression (112).

As for ethanol, another alcohol, its increased emission mainly accompanies fruit ripening due to the fermentation of sugars by yeasts. Ethanol, among other volatiles, is an important component of the scent of ripe fruit. Moreover, an optimal ethanol concentration is a significant indicator of the quality of fresh citrus fruit (331, 351). However, it seems that increased ethanol levels in fruit adversely affected the taste (80). Dominy (108) analyzed the ethanol content of some Asian fruits and showed that small amounts of ethanol (from 0.005 to 0.48%) were detected in fruits of all developmental stages; the ethanol levels positively correlated with the soluble sugar levels. In this case, ethanol is regarded as an olfactory cue for frugivores to locate food of good quality with high carbohydrate content. Frugivores such as Egyptian fruit bats (*Rousettus aegyptiacus*) were shown to locate fruits by assessing the degree of ripeness and quality based on the intensity of the scent of alcohol (methanol and ethanol). However, the Egyptian fruit bats avoided overripe fruits, or fruits with ethanol contents exceeding 1% (401). Moreover, yellow-vented bulbuls (*Pycnonotus xanthopygus*) were shown to decrease their food intake by 36% if the ethanol concentrations in fruits exceeded 3% (309). The frugivorous tropical butterfly *Bicyclus anynana* locates fruits by using ethanol odor cues as long range signals that guide the butterflies in the direction of food sources containing soluble sugars (101). An interesting relationship has been observed between the bertam palm (*Eugeissona tristis*) and its pollinator, the pentailed tree shrew (*Ptilocercus lowii*). The palm exudes nectar from its flowers when the petals are still closed. This nectar contains a high ethanol concentration and is produced by the plant for periods of up to 46 days. This facilitates the development of yeasts living in the nectar. Thus these flowers release a strong ethanol odor that attracts tree shrews, which then act as pollinators (509).

Thus plant-associated methanol and ethanol both play roles in plant-animal communication as either attractants or repellents, depending on the particular case.

III. EXOGENOUS SOURCES OF PHYSIOLOGICAL METHANOL IN HUMANS

A. Methanol Content in Healthy Human Blood and Breathing Air

Methanol and ethanol are integral components of life for humans and mammals (299). Healthy human blood contains small amounts of metabolic methanol (112, 246, 413) and ethanol (269, 362). Gaseous methanol and ethanol are also detected in the air exhaled by healthy people (126, 212, 213, 474). The methanol concentration in the blood is 400–1,000 times less than the toxic concentration (214, 251) and has been estimated by different researchers to range from 0.20 ± 0.035 to 5.37 ± 0.08 mg/l (24, 25, 78, 84, 112, 385, 413). Methanol is also detected in the urine, saliva (263), and breast milk (370). The methanol concentration in venous blood is, on average, 3 times higher than that in saliva (413) and 1.3 times lower than that in urine (263).

B. Plant Food as a Source of Exogenous Methanol

Fruits and vegetables are the main sources of methanol in the human body. Methanol is known to accumulate in fruit during ripening as a result of PME activity, which among other CW degrading enzymes plays a significant role in fruit softening and ripening (367, 384). Methanol is detected among other volatiles in the headspace of cut fruits (133). Freshly squeezed citrus juices contain an average of 20–40 mg/l methanol, whereas methanol is detected in a lower

concentration of 6.2 mg/l in processed juices or juice concentrates (293).

The consumption of vegetables such as leafy salads results in increased blood methanol contents, by ~30% (112). The ingestion of pectin, fruits with different degrees of ripeness, or fruit juices leads to the rise of the methanol content of exhaled air (126, 451, 474) and blood (278). This occurs mainly due to the demethylation of pectin contained in vegetables by PME, which is also present in the plant CW. When citrus pectin in the presence of PME activity was administered to mice, their blood methanol levels increased by more than 15-fold compared with those administered pectin without PME activity just 10 min after ingestion (112). Pectin demethylation is also believed to occur in the gut by the gut microbiota (418), as some strains of gut-inhabiting bacteria possess pectolytic enzymes. However, the administration of pectin containing no active PME in the gastrointestinal tract of mice did not lead to significant differences in the concentrations of methanol in their blood compared with the control; thus it can be concluded that the contribution of the intestinal microflora in methanol generation from pectin is rather low compared with the methanol production from ingested plant food by PME (112).

Pectin is a water-soluble fiber that is resistant to hydrolysis by gastrointestinal tract enzymes but can be fermented by the microflora in the large intestine. This fermentation results in the formation of short-chain fatty acids, which are then absorbed and metabolized (232). The positive effect of apple pectin on the human gastrointestinal microflora was demonstrated: the daily intake of two apples increases the amount of *Bifidobacterium* and *Lactobacillus*, most strains of which are capable of utilizing pectin (414). Moreover, the daily consumption of whole apples, pomace, or unclarified juice, i.e., products containing water-soluble fibers such as pectin, was shown to lead to lower levels of low-density lipoproteins in the blood (389, 456). This positive effect of dietary pectin can be explained by the ability of pectin to inhibit the reabsorption of cholesterol and bile acids in the lower gastrointestinal tract due to the good gel-forming properties of pectin, as well as by the ability of short-chain fatty acids to decrease cholesterol biosynthesis by the liver (207).

The beneficial effects of plant food are generally recognized, and according to the World Health Organization's (WHO) recommendations, the minimal daily intake of plant food should not be less than 400 g (250 g vegetables and 150 g fruits). Fresh fruits and vegetables are particularly rich in pectin complexed with enzymatically active PME. Despite that PME is a rather thermostable enzyme, its activity significantly decreases after boiling (112). On the other hand, more intense pectin deesterification and methanol formation occurs after the moderate heating of plant food to 45–65°C (blanching) (5). Pectin, mainly from apples and citrus fruits, is used as a dietary supplement to improve digestion and also as a therapeutic and prophylactic agent in several diseases, including atherosclerosis (52, 468) and cardiovascular diseases (458), due to its role in slightly decreasing blood cholesterol levels (51, 160). Pectin is also believed to lower the risk of cancer by decreasing tumor cell proliferation (30, 477), inducing apoptosis (198), and suppressing the metastatic ability of tumors (146). Notably, citrus pectin consumption led to significantly increased blood methanol levels in volunteers; this resulted in changes in the expression profiles of genes in human white blood cells, as demonstrated by microarray analysis (413). Pectin can modulate detoxifying enzymes, stimulate the immune system, modulate cholesterol synthesis, and act as an antibacterial, antioxidant, or neuroprotective agent (261).

With consideration of the recommendations of nutritionists to include a large proportion of plant food and vegetable fiber in the diet, as well as the popularity of vegetarianism, it can be concluded that fresh fruits and vegetables are two of the main sources of exogenous methanol in human blood.

C. Plant Food as a Source of Ethanol

Plant food, mainly ripe fruit, can also be a source of ethanol. It is known that plants are able to synthesize ethanol mainly via anaerobic fermentation by alcohol dehydrogenases (ADHs) (67, 141, 237, 329). Transcription from ADH promoters is induced in response to a lack of oxygen and to cold stress in maize and *Arabidopsis* (107). When respiration is impeded or inhibited, pyruvate is formed as a result of glycolysis. Then, pyruvate is converted to lactate by lactate dehydrogenase. Pyruvate decarboxylase is a key enzyme in the alternative pyruvate metabolic pathway, which results in acetaldehyde formation followed by its reduction to ethanol by ADH. Ethanol is much less toxic to plant cells than lactate or acetaldehyde. This metabolic switch allows plant cells to continuously regenerate ATP and NAD^+, but is less effective than normal conditions (437). Usually, the Michaelis constant (K_m) of ADH for acetaldehyde is less than or equal to 1 mM, while the K_m for ethanol is more than 10-fold higher (33). Thus one of the main functions of ADH in plant cells is the disposal of toxic acetaldehyde. Another function of ADH is the synthesis of C6 and C9 volatiles that define fruit flavor (406), which attracts fruit-eating, seed-spreading animals (frugivores). Thus the roles of ADH in plants include its participation in ethanolic fermentation in hypoxic or anoxic conditions and, in normal aerobic conditions, in fruit and seed ripening to allow for the production and emission of the characteristic scent cues used by pollinators or frugivores. Nowadays, to prolong the storage lengths of fruits and vegetables, they are kept in a low-oxygen atmosphere (for example, with an increased CO_2 content) and are covered with special waxes that also induce hypoxia. These treatments lead to increased ethanol

contents in the stored fruits and vegetables (11, 12, 80). Notably, the methanol concentrations in such fruits and vegetables also rise (11, 12).

Still, the main source of ethanol from fruits is the fermentation of fruit sugars by yeast. The increased ethanol content in fruits during ripening is likely to be an evolutionary adaptation because raised ethanol concentrations restrain active bacterial propagation (118). Thus ethanol is ubiquitous in ripe fruits, and its concentration ranges from 0.04 to 0.72% (7–125 mM) (117, 270). Thus frugivorous animals consume significant amounts of ethanol, as they consume ripe fruits.

D. Alcoholic Beverages as Sources of Ethanol and Methanol

The amount of ethanol obtained by humans from fruits and vegetables is at least two orders of magnitude less than that from alcoholic beverages (beer, wine, distilled spirits), which appear to be the main sources of exogenous ethanol for people. The first evidence of artificial fermented-beverage production comes from China, nearly nine millennia ago (312). Later, fermented beverages started to appear in different cultures; today, they are a part of human life.

At present, the production of different alcoholic beverages is under strict governmental control, and there are regulations that define the maximum acceptable concentrations (MAC) of congeners (methanol, formaldehyde, acetaldehyde, etc.) in produced alcoholic beverages. Methanol has been detected in beer (6–27 mg/l of product) (153), wine, and distilled spirits. On the basis of data available since 1949 on the methanol content in different wines, reviewed by Gnekow and Ough (148), the highest concentrations of methanol, up to 635 mg/l, were found in Spanish and Italian red wines. The average methanol content of the analyzed wines was ~100 mg/l of the final product. The maximum limits for the methanol content in wines allowed by International Organization of Vine and Wine are 400 mg/l for red wines and 250 mg/l for white and rosé wines (354).

Higher methanol concentrations are allowed in distilled fruit spirits due to the nature of the raw material, which is derived from pectin-rich fruits. As was mentioned above, PME activity increases during fruit ripening; thus the methanol content is elevated. Methanol released from pectin after demethylation accumulates in ripe fruits and ends up in the final product, distilled fruit spirits (34) Under laboratory conditions, it was shown that fruit spirits made from cherries and plums could contain from 18.3 to 25 g methanol/l of the 100% vol ethanol (405). Undoubtedly, such high methanol contents exceed the MACs. In the United States, the allowed methanol concentration for distilled fruit spirits is 6–7 g/l of 100% vol ethanol (34, 477a). Since 2008, there have been EU limits on methanol (per liter of 100% vol ethanol) in different distilled spirits, as follows: 0.1 g for vodka; up to 1.35 g for some fruit spirits; 1.5 g for fruit marc spirits; and 2 g, the highest allowed methanol content, for wine spirits and brandy (130a).

Thus the methanol concentration in alcoholic beverages is strictly controlled by legislation, and manufacturers are eager to reduce this concentration in their beverages. Despite this, shockingly, when an individual drinks wine or distilled spirits even with very low or undetectable methanol contents, the endogenous methanol level increases significantly and is comparable to the increased ethanol concentration that results from ingesting ethanol (413). Earlier, it was shown that the ingestion of methanol-free whisky or grain alcohol by a volunteer led to increased methanol content in his breath (451). Healthy volunteers who drank a glass of red wine (150 ml) containing 13.7% vol of ethanol and 33 mg/l of methanol showed nearly twofold increases in their blood methanol content. Moreover, the orders of magnitude for methanol and ethanol concentrations were the same or at least comparable 60–120 min after ingestion. Furthermore, when 50–90 ml per person (1 ml of 40% vol ethanol per 1 kg of weight) of methanol-free 40% vol ethanol was ingested by the same volunteers in another experiment, the methanol contents in their blood were also of the same order of magnitude as ethanol and were even comparable 90 min after ingestion, ~350–400 μM (413). Thus the ingestion of alcoholic beverages always leads to increased blood methanol levels, regardless of how low the methanol content is in the beverage. This likely happens due to the partial depletion of liver ADH by ethanol, while a methanol level continues to rise via endogenous sources. From these data, the level of endogenous methanol production can be estimated to be at least 1.7 $mg \cdot kg^{-1} \cdot h^{-1}$ (413). The above-mentioned methanol concentrations observed after red wine or 40% vol ethanol consumption or even 5-fold higher methanol contents could be tolerated without negative consequences; only a nearly 20-fold higher concentration is regarded as dangerous (364). Despite the fact that alcoholic beverages contain ethanol, which "distracts" ADH from metabolizing methanol, blood formaldehyde levels also rise significantly after ethanol ingestion, but with slight retardation; they start to increase 60 min after ingestion and reach concentrations comparable to methanol and ethanol 60–90 min after red wine or 90–120 min after ethanol consumption (413). Thus the ingestion of alcoholic beverages, even those of high quality, always leads to increased ethanol, methanol, and formaldehyde contents in the blood. Moreover, plant food, which is undoubtedly believed to be healthy, also results in increased methanol and formaldehyde levels in the blood. Taking into account that plant food was the main food source during human evolution, such oscillations of methanol and formaldehyde concentrations might have some beneficial effects in humans.

E. Aspartame

Aspartame, a synthetic nonnutritive sweetener, is a dipeptide composed of aspartic acid and phenylalanine methyl ester. Compared with sucrose, aspartame is 200-fold sweeter, which allows it to be consumed in much lower doses to make foods or beverages sweet; for this reason, aspartame is regarded as a low-calorie sweetener (298). As aspartame is a methyl ester, its degradation could result in methanol formation. The stability of aspartame depends on the storage conditions: it becomes unstable after heating in aqueous solutions and at neutral or alkaline pH. None of the degradation products of aspartame is sweet. Thus aspartame is usually used in dry form or in carbonated beverages and is not recommended for heat-treated foods. Furthermore, the appropriate storage conditions and the expiration dates of foods and beverages with aspartame are very important. Despite the fact that aspartame was approved as a safe sweetener by the United States Food and Drug Administration (FDA) in 1996 (136, 477b) and in Europe in 1994 (130), the debates around this compound have not subsided, and research into the effects of aspartame on human health are in progress; the question of its safety remains acute (423). The main safety concern regarding aspartame is the formation of methanol as a result of the inevitable degradation of phenylalanine methyl ester. As methanol is essentially converted to formaldehyde in mammalian organisms, excessive aspartame consumption may be hazardous because of its contribution to the formation of formaldehyde (467).

Currently, the acceptable daily intake (ADI) of aspartame in Europe is 40 mg/kg body weight (bw) and in the United States is 50 mg/kg bw. The highest aspartame consumption is observed in teenagers and diabetics; the maximum intake was estimated at ~13.6 mg·kg bw^{-1}·day^{-1} for teenage girls consuming high amounts of soft drinks (9), which, nevertheless, is at least two times lower than the ADI. For children and adults with diabetes, the daily consumption of aspartame in the worst-case scenario also did not exceed the ADI (298). When volunteers consumed 600 mg aspartame eight times a day at 1-h intervals, there were no detectable differences in their blood methanol levels (434). It is worth mentioning that the average body weight of the volunteers was 70.8 ± 13.5 kg; thus the doses of aspartame used in the experiment exceeded the present ADI. According to Stegink's (433) data, the ingestion of 34 mg/kg bw of aspartame (which is very close to the ADI) did not lead to a detectable increase of blood methanol levels, likely due to a lack of sensitivity of the detection method. Only doses from 100 mg/kg bw (twice as high as the ADI) resulted in detectable rises of blood methanol levels up to a mean value of 12.7 mg/l (433). Notably, a comparable methanol concentration of ~11 mg/l in the blood is obtained from endogenous sources after methanol-free 40% vol ethanol ingestion (413). In another study, the ingestion of 7.5–8.5 mg/kg aspartame (the FDA estimation for the average daily consumption) resulted in a mean rise over endogenous values of 1.06 mg/l serum (which is ~0.5 mg/l blood), but this increase is of the same order of magnitude as the variations in endogenous methanol levels and between-individuals differences (95). Thus it can be concluded that moderate aspartame consumption within the recommended doses leads to a rise in the blood methanol content, similar to the consequence of plant food, juice, or alcoholic beverage ingestion.

F. Food as an Exogenous Source of Acetaldehyde and Formaldehyde

Aside from being a source of ethanol and methanol, food may also be an exogenous source of acetaldehyde and formaldehyde. For example, the acetaldehyde content in apples ranged from 0.4 to 2.3 mg/kg, in bananas ranged from 1.88 to 18.27 mg/kg, in fresh orange juice was 5.89 mg/kg, and in fermented dairy products, such as fruit-flavored yogurt, was up to 17 mg/kg. Daily acetaldehyde consumption with food (excluding ethanol) is estimated to be ~40 μg/kg bw, which is considerably lower than the exposure from ethanol consumption or tobacco smoking (476). The average daily exposure to acetaldehyde from alcoholic beverages was estimated at 0.112 mg/kg bw (257). Significant amounts of acetaldehyde (up to 37 mg/l) were detected in different samples of beer, whiskey, and other distilled spirits (up to 10 mg/l) (440).

Some food could be also a source of exogenous formaldehyde. The formaldehyde contents in numerous products were analyzed with chromatographic methods. In different fruits and vegetables, the formaldehyde concentrations ranged from 3 to 26 mg/kg (469). High levels of formaldehyde were detected in fish of the *Gadidae* family, ranging from 6.4 to 293 mg/kg (32); in milk (0.027 mg/kg in fresh milk and 0.164 mg/kg in processed milk) (222) and milk products [in Italy, formaldehyde is used as a bacteriostatic agent in cheese production (391)]; and in coffee [3.4–4.5 mg/kg in commercially brewed and 10–16.3 mg/kg in instant coffees (171)]. Moreover, natural antioxidants that are *O*- and *N*-demethylated by cytochrome P450 in humans (125) could be regarded as a source of formaldehyde. The same applies to some drugs such as those containing codeine (98, 490). According to various assessments, the daily intake of direct formaldehyde is estimated to be in the range of 1.5–14 mg/day for the average adult (98).

One significant non-food source of exogenous methanol and formaldehyde for humans that cannot be ignored is cigarette smoke, which has an estimated methanol content of 180 μg/cigarette and formaldehyde content of 45–73 mg/cigarette (157, 302).

G. Food Modulating Activity of Alcohol-Metabolizing Enzymes

Aside from the food- and beverage-related intake of alcohols (methanol and ethanol) and aldehydes, changes in the contents of these compounds can also result from ADH and aldehyde dehydrogenase (ALDH) activity modulation. Agents that affect these enzymes could also be food components. Plants that contain inhibitors or activators of ADH and ALDH are used in traditional Chinese medicine to relieve hangovers or to treat alcoholism. Recently, several of these phytotherapy-based products also appeared in Western societies (313). The most popular herb for treating alcoholism is Kudzu (*Pueraria lobata*). It contains daidzein (4′,7-dihydroxyisoflavone), puerarin (8-C-glucoside of daidzein), and daidzin (7-glucoside of daidzein), which reversibly inhibits mitochondrial ALDH2 (235). The structure of the daidzin/ALDH2 complex was resolved at 2.4 Å and showed that the daidzin isoflavone moiety is placed very closely to the substrate-binding site and glycosyl to a hydrophobic patch immediately outside the isoflavone-binding pocket (291). Daidzein is a dietary phytoestrogen that is also found in soy products. Soy products that are popular in Asia contain another isoflavone, genistein, which together with daidzin lowers ethanol and acetaldehyde concentrations in the blood due to the enhancement of ethanol metabolism; this was shown in rats that ingested soy milk and fermented soy milk together with ethanol (225). In humans, daidzein is metabolized to daidzin by bowel bacteria (313). High levels of serum daidzein, 240–280 μM, are characteristic for the Japanese population and are ~20 times higher than those in the United Kingdom population (330); this difference can be explained by dietary preferences.

Thus the risks of acetaldehyde and formaldehyde intoxication as a result of ethanol consumption or smoking are higher for the Asian population.

Another plant, *Hovenia dulcis*, also known as the Japanese raisin tree, is used in traditional Asian medicine for detoxification after ethanol consumption. Extracts from the fruits of this plant significantly decreased the blood ethanol concentration by stimulating the activity of liver ADH, ALDH, and glutathione *S*-transferase in mice and rats. Hovenodulinol is believed to be the compound responsible for this effect (194), but the exact mechanism of its action remains unknown.

Other foods have also been shown to affect ethanol metabolism. In experiments in which mango flesh or mango peel was administered to mice, followed by ethanol ingestion, blood ethanol levels were lower by more than 50% compared with the control group. This difference in ethanol concentration likely occurred due to the activation of ADH and cytosolic ALDH in the liver (238). The authors assumed that the effect observed could be mediated by fructose and aspartate from the mango, which produce NAD^+ and thus stimulate ADH and ALDH.

Some phytophenols were also shown to modulate ethanol metabolism. For example, vanillin, syringaldehyde, caffeic acid, and ellagic acid appeared to strongly inhibit liver ADH1 in mice. When these phytophenols were administered to mice together with ethanol, they prevented the elimination of blood ethanol through the ADH metabolic pathway (170). This effect led to reduced acetaldehyde accumulation after ethanol ingestion. Interestingly, all of these compounds were found in mature whisky, and their concentrations rose with the time of maturation. These compounds are also present in different foods: vanillin is found in vanilla beans and as a flavoring agent in confections; caffeic acid is found in coffee beans, soy beans, argan oil, and barley; and ellagic acid is present in blackberries, cranberries, pecans, pomegranates, raspberries, strawberries, walnuts, grapes, and peaches.

Thus methanol, ethanol, formaldehyde, and acetaldehyde are natural components found in the human body. People come into daily contact with all of these compounds via food and inhaled air.

IV. HUMAN GUT MICROBIOTA AS A SOURCE OF METHANOL

A. Overview of Methanol Metabolism in Bacteria

In mammals, the intestinal flora is the most relevant candidate for the production of methanol followed by its oxidation to formaldehyde, formic acid, and carbon dioxide. To prove this, it was recently tested whether the intestinal microbes in rats generate methanol (246). Removal of the bowel in rats resulted in a lessened methanol increase in the blood following the administration of 4-methylpyrazole into the liver compared with the control group (246). Currently, a large number of bacteria and several types of yeast are known to be capable of growing on medium wherein methanol is the sole carbon source **(TABLE 1)**. Bacteria utilize three classic mechanisms of include methanol metabolism: the Calvin cycle, the ribulose monophosphate cycle, and the serine cycle (387). First, the most energetically costly pathway of methanol metabolism begins with the oxidation of methanol to carbon dioxide, which is then incorporated into the Calvin cycle as a carbon source. The process of methanol utilization through the Calvin cycle was detected for the first time in *Micrococcus denitrificans* (91). Later, this process was observed in the bacteria *Xanthobacter autotrophicus* (21), *Methylacidiphilum infernorum* (358), as well as in bacteria belonging to the *NC10* phylum (129). The classical method of methanol oxidation to carbon dioxide takes place in three stages: the oxidation

Table 1. *Summary of the most abundant bacteria that carry genes encoding methanol-producing (PME, BioH, PMG) and methanol-oxidizing enzymes (katG, katE) in stool samples*

Bacteria Name	Number of Samples Containing Bacteria (Out of 5)	Average Number of Clones per Sample	*PME*	*BioH*	*PGM*	*katG*	*katE*
Eubacterium rectale	5	3,412			+		
Escherichia coli	5	3,183	+	+	+	+	
Bacteroides vulgatus	5	2,293	+				+
Alistipes finegoldii	5	589					+
Bacteroides thetaiotaomicron	5	538	+				+
Parabacteroides distasonis	5	527					+
Bifidobacterium breve	5	402					+
Butyrivibrio fibrisolvens	5	156			+		
Eubacterium eligens	5	143	+		+		
Bacteroides fragilis	5	119					+
Butyrate-producing bacterium SS3/4	4	72			+		
Ruminococcus albus	3	59	+		+		
Clostridium phytofermentans	5	28	+				
Geobacillus thermoglucosidasius	5	23			+		+
Alistipes shahii	5	18	+				+
Xanthomonas campestris	4	18	+		+	+	+
Klebsiella pneumoniae	3	15	+	+		+	+
Enterobacter cloacae	2	17	+	+	+		+
Bacillus cereus	3	4		+			+
Geobacillus thermodenitrificans	3	3			+	+	+

For the analyses, we took the 16S RNA sequencing data from citizens of New Zealand, United States, Hungary, Norway, and Hong Kong. The data were obtained from the MG-RAST database, loaded and performed by the American Gut Project (http://metagenomics.anl.gov/linkin.cgi?project=6594).

of methanol to formaldehyde, formaldehyde to formic acid, and formic acid to carbon dioxide (491). In some facultative methylotrophs, instead of three reactions for converting methanol to carbon dioxide, formaldehyde is oxidized to carbon dioxide via the dissimilatory hexulose phosphate cycle (82). In this section, we focus on bacterial enzymes that either use methanol as substrate or produce methanol as a product.

Bacteria oxidize alcohols via a few different groups of enzymes. The first group includes mammalian-like ADHs with different electron acceptors (i.e., NAD, NADP, heme). ADHs from the aerobic bacterial intestinal flora actively participate in ethanol metabolism. The intestinal flora in piglets produces great amounts of acetaldehyde from ethanol in vivo (211). Bacteria contain a branch of enzymes that metabolize ethanol, but ADHs oxidize most of it. Although ADHs play an important role in methanol metabolism in mammals, bacteria utilize methanol in alternative ways. Bacterial NADP-dependent ADH (493), NAD-dependent ADH (124, 348), quinohemoprotein ADH (70), and membrane-associated quinohemoprotein ADH (412) have little or no activity toward methanol and mostly oxidize C2–C6 alcohols. An exception is NAD-dependent ADH (EC 1.1.1.1) from *Bacillus stearothermophilus*, DSM 2334, which is able to oxidize methanol with an efficacy comparable to that of horse liver ADH (411).

Enzymes that use hydrogen peroxide to metabolize alcohols (methanol and ethanol) comprise a second group of enzymes that catalyze the first step of alcohol oxidation in bacteria. The first catalase enzyme (EC 1.11.1.6) is likely the most ancient alcohol-oxidizing enzyme and can be found in many organisms, from archaea to human. In bacteria, catalase is encoded by the *katE* gene, which is an ortholog of the human *CAT* gene. The second catalase enzyme (EC 1.11.1.21) is encoded by the *katG* gene, which is specifically found in bacteria and fungi. According to the KEGG database, both genes are present in all phyla of bacteria and in more than 1,000 species, highlighting their crucial role in bacterial alcohol metabolism. Along with ADHs, bacterial catalases from the intestinal flora participate in ethanol oxidation and metabolize ~26–32% of the total ethanol in vitro (459). Although the data on methanol oxidation by catalase in microorganisms are very limited, it is known that methanol can act as a hydrogen donor in catalase reaction in some bacteria and yeast (166, 395, 482b). Based on the number of intestinal bacteria that carry the *katG* and *katE* genes **(TABLE 1)** and the experimental evidence of methanol utilization by the catalase enzymes,

we hypothesize that catalases may participate in methanol oxidation by the intestinal microflora.

Bacteria mainly oxidize methanol to formaldehyde by the following methanol dehydrogenases (MDH): cytochrome *c*-dependent methanol dehydrogenase, which contains a pyrroloquinoline quinine cofactor (PQQ-MDH); NAD-dependent methanol dehydrogenase (NAD-MDH) and NADPH-dependent methanol dehydrogenase (NADPH-MDH). On the basis of the analysis of bacteria from human stool samples, three types of bacteria that oxidize methanol by MDHs were revealed: *Methylobacterium extorquens* and *Methylobacterium nodulans*, using PQQ-MDH, and *Bacillus methanolicus*, using NAD-MDH (10). However, the MDH-containing bacteria are not typical residents of the human microflora, and few clones of these bacteria were detected in analyzed stool samples.

Formaldehyde that is produced in the methanol and methane oxidation reaction is either assimilated in the ribulose monophosphate (RuMP) or the serine cycle or further oxidized into carbon dioxide, which is then incorporated into the Calvin cycle **(FIGURE 3)**. In the RuMP cycle, formaldehyde and D-ribulose 5-phosphate are condensed by hexulose-6-phosphate synthase to form hexulose 6-phosphate. *Bacillus subtilis* (521) and *Brevibacillus brevis* (528) are two examples of intestinal flora bacteria that assimilate formaldehyde via the RuMP cycle. In the serine cycle, serine hydroxymethyltransferase uses formaldehyde and tetrahydrofolate as cofactors to form serine from glycine. The serine pathway of formaldehyde assimilation was discovered in *Methylobacterium extorquens* AM1 (76), a gram-negative *Alphaproteobacteria* that can be found in human intestines.

There are two classes of reactions that result in methanol production: methyl ester hydrolysis and redox reactions **(TABLE 2)**. Despite the versatility of methanol-producing reactions, only a few are typical for bacterial metabolism under normal conditions. On the other hand, some of the methanol-producing reactions can be found only in a few species of bacteria. For example, methane monooxygenases (soluble, sMMO; membrane-bound form, pMMO) are enzymes specific to methanotrophs, a small group of bacteria

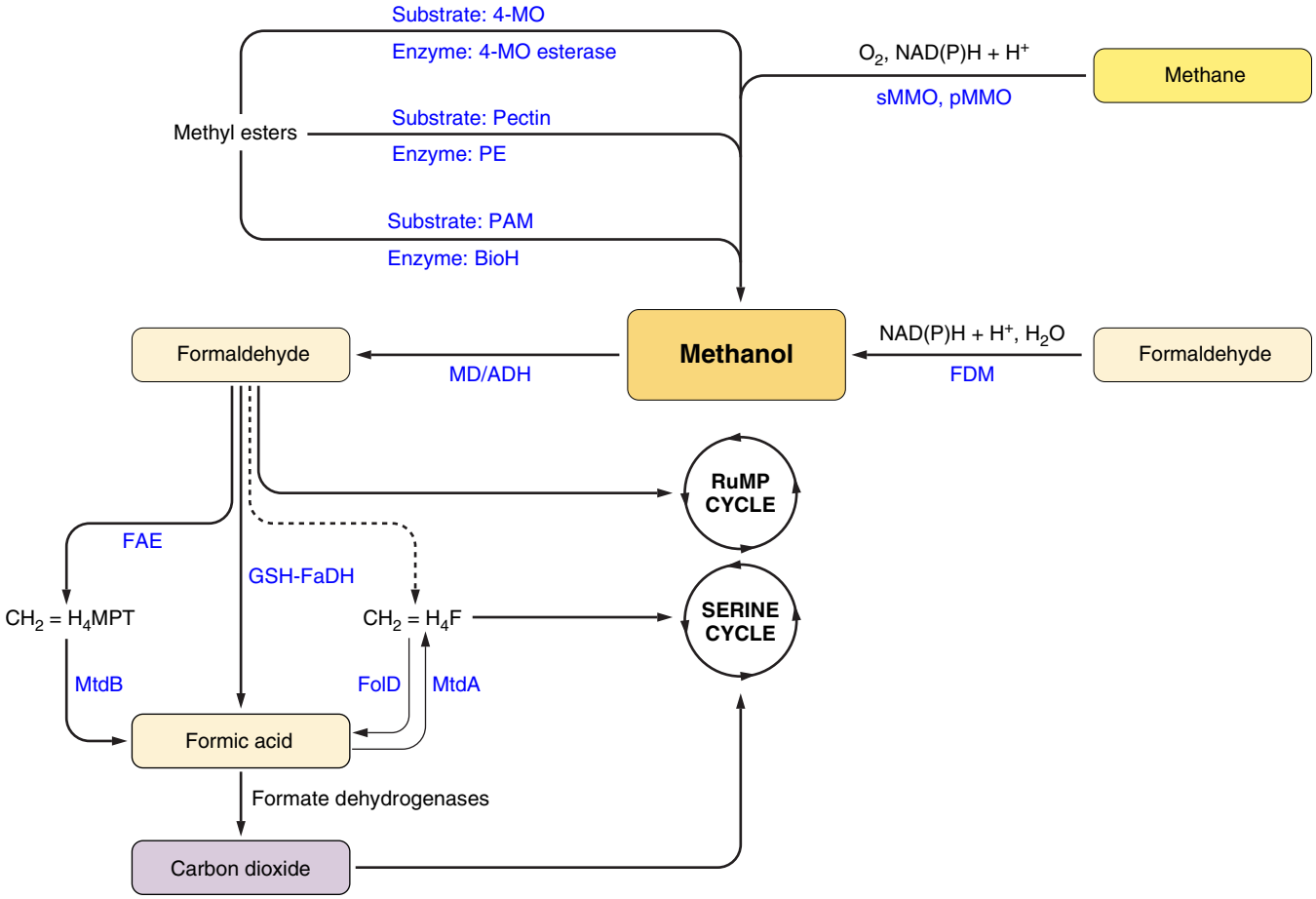

FIGURE 3. Methanol metabolism and production by bacteria. 4-MO, 4-methyl oxaloacetate; PAM, pimelyl-[acp] methyl ester; FDM, formaldehyde dismutase; MDs, methanol dehydrogenases; ADHs, alcohol dehydrogenases; sMMO/pMMO, soluble/membrane-bound methane monooxygenase; GSH-FaDH, glutathione-linked formaldehyde oxidation; FAE, formaldehyde activating enzyme; FolD, 5,10-methylene-H_4folate dehydrogenase/5,10-methenylH_4folate cyclohydrolase.

Table 2. *List of methanol-producing enzymes, their substrate, and the type of reaction they catalyze in bacteria*

Ferment Name [EC Number]	Substrate	Reaction Class
Pectinesterase [3.1.1.11]	Methyl esters of pectin	Hydrolysis
sMMO/pMMO [1.14.13.25]	Methane, oxygen	Redox reaction
Formaldehyde dismutase [1.2.99.4]	Formaldehyde	Redox reaction
Protocatechuate 4,5-dioxygenase [1.13.11.8]	3-*O*-Methylgallate	Redox reaction
4-Methyloxaloacetate esterase [3.1.1.44]	4-Methyloxaloacetate	Hydrolysis
Pimeloyl-[acyl-carrier protein]-methyl-ester hydrolase (BioH) [3.1.1.85]	Pimeloyl-[acyl-carrier protein]-methyl ester	Hydrolysis
Protein-L-glutamate-O4-methyl-ester acylhydrolase [3.1.1.61]	Protein glutamate methyl ester	Hydrolysis

sMMO, soluble methane monooxygenase; pMMO, particulate methane monooxygenase.

able to grow on methane as the sole carbon and energy source (163).

Methanol concentrations in the blood of volunteers of different ages, genders, diets, as well as smoking and alcohol habits are very similar. Additionally, methanol accumulates with similar kinetics in humans and mice after the administration of ADH inhibitors. Both parameters indicate that endogenous sources of methanol work constitutively under normal conditions. Here, we will review the reactions that result in methanol production and *1*) occur under normal conditions, *2*) are common among intestinal bacteria, and *3*) work constitutively.

B. Bacterial Pectin Methylesterase

Pectin methylesterase (PME; EC 3.1.1.11) is generally associated with plant physiology and CW structure. PME from bacteria is of particular interest due to its role in the degradation of the CW in plants (260) and its various industrial applications (187). Additionally, bacterial PME is an essential part of pectin fermentation in humans because our enzymes cannot break down pectin (514). PME is one of the most abundant enzymes participating in methanol production by intestinal bacteria. Approximately 30 species of intestinal bacteria carry the *PME* gene, and some of them are commonly present in the microflora **(TABLE 1)**. However, little is known about characteristics of the intestinal bacteria that produce methanol via PME, and few works have demonstrated the ability of the mammalian intestinal flora to produce methanol as the result of pectin breakdown by PME under aerobic and anaerobic conditions (38, 208, 209, 418). As a result of the digestion of dietary pectin (from vegetables and fruit) and pectin capsules by the microflora and/or by pectin-associated PME, methanol levels in human blood increased up to 1.5 times than that before exposure (112). However, PME only catalyzes methanol production in the presence of exogenous pectin; thus PME activity alone cannot explain the basic, similar methanol concentration found in the diverse group of volunteers and the constitutive production of methanol in mammals.

C. Protein-Glutamate Methylesterase

Protein-glutamate methylesterase (PGM) is another common enzyme in the microflora **(TABLE 1)** that hydrolyzes methyl esters of glutamate that bind to proteins. Methylation and demethylation are well-known mechanisms of controlling expression, translation, and protein activities. Depending on the type of bind between methyl radicals and biopolymers, active demethylation can produce either formaldehyde or methanol. The product of carbonic acid (amino acids, glutamate, oxaloacetate, etc.) or methyl ester demethylation is methanol, while DNA demethylation generates formaldehyde. PGM is of particular interest due to its ability to produce detectable levels of methanol in vivo (461), and, in contrast to PME, only endogenous molecules are substrates of PGM for methanol production. The *CheB* gene, which encodes PMG, is present in more than 50 species of intestinal bacteria from stool samples, including abundant species **(TABLE 1)**. However, *CheB* expression correlates with decreased chemotactic stimuli (43), and PMG does not maintain permanent methanol concentrations over time. PMG seems to participate in methanol formation by intestinal bacteria, but it is unlikely that PMG plays a crucial role in this process.

D. Pimeloyl-[Acyl-Carrier Protein]-Methyl-Ester Hydrolase

Pimeloyl-[acyl-carrier protein]-methyl-ester hydrolase (BioH) is an enzyme with hydrolysis activity that might also participate in methanol production by microflora. BioH participates in biotin (vitamin H) production in bacteria and other organisms. The demethylation reaction terminates the part of the biotin biosynthesis pathway that is catalyzed by fatty acid synthesis enzymes (281) and results in methanol and biotin precursor production. Vitamin H serves in all living organisms as a covalently bound enzyme cofactor that is essential for the introduction of carboxylic acid groups to substrates via carboxylases. Mammals have no enzymes capable of biotin synthesis and thus use microflora- and food-derived biotin. Hence, bacteria containing genes that encode components of the biotin biosynthesis pathway (including *BioH*, **TABLE 1**) are present in the intes-

tinal flora. BioH activity was originally demonstrated in *Escherichia coli*, which is one of the major microflora bacteria. Thus BioH participates in an essential biosynthetic pathway and is widespread in the microflora; hence, BioH is the best candidate for methanol production by the intestinal flora.

To summarize, there are a few strong candidates among bacterial enzymes that might participate in methanol metabolism and serve as the source of methanol metabolism in mammals. Catalase seems to oxidize most of the methanol in bacteria, while ADH and MDH are less important. PME and PGM require exogenous sources to produce methanol, so they cannot be an efficient endogenous source. Because BioH participates in an essential biosynthetic pathway and is widespread in the microflora, it is the best candidate for methanol production by the intestinal flora. This analysis is in agreement with the experimental data on the microflora of rats (246) and gives new clues for further investigation of methanol metabolism by intestinal bacteria in mammals.

V. METHANOL METABOLISM IN HUMANS

Along with dietary methanol and methanol produced by the intestinal microflora, processes that involve SAM participation also contribute to the pool of physiological methanol (265). SAM is a universal, endogenous methyl donor for several reactions, including the methylation of proteins, phospholipids, DNA, RNA, and other molecules involved in basic epigenetic mechanisms (196). Genome-wide methylation analysis has identified DNA methylation profiles specific to aging and longevity. Moreover, analysis of the DNA methylation landscape revealed that DNA obtained from a 103-yr-old donor was more unmethylated overall than DNA from the same cell type isolated from a neonate (181). Differentially methylated genes are strikingly enriched in loci associated with neurological disorders, psychological disorders, and cancers (531). Protein carboxymethylation involves the methylation of amino acid COOH groups; this reaction is catalyzed by methyltransferases, which produce carboxyl methyl esters that are readily hydrolyzed under neutral and basic pH conditions, or by methyl esterase, which produces methanol (104). Protein carboxymethylase is highly localized to the brain and pituitary gland in several mammalian species (105).

In a healthy person, no matter from what sources methanol is derived in the body, it is eventually displayed and kept at a low physiological level via physiological and metabolic clearance mechanisms.

A. Nonmetabolic (Physiological) Clearance of Methanol

Because methanol metabolism involves the same enzymes as ethanol metabolism, information about the nonmetabolic clearance of ethanol may be relevant to our consideration of methanol metabolism. Approximately 90% of ethanol is removed by oxidation, and <10% of ethanol is excreted in breath, sweat, and urine (63, 347). Animal studies have reported that inhaled methanol is eliminated mainly by metabolism (70–97% of absorbed dose), and only a small fraction is eliminated as unchanged methanol in the urine and expired air (>3–4%) (42, 110, 188). The determination of methanol and ethanol contents in the human breath after the consumption of alcoholic beverages and various amounts of fruits revealed that methanol concentrations increased from a natural (physiological) level of ~0.4 to ~2 ppm a few hours after eating ~0.5 kg fruit (451). The same concentration was reached after drinking of 100 ml brandy containing 24% ethanol and 0.19% methanol. We estimated the levels of endogenous methanol generation by considering the probable removal of methanol via pulmonary and renal excretion (413). Assuming renal methanol clearance in the average volunteer of 1.0 ml/min (231, 483), we believe that a clearance of 60 ml of blood in 1 h occurs and has little effect on the total blood methanol content. For the estimation of the pulmonary excretion of methanol, we used estimates (200) indicating that each minute, 5.6 ml of blood is hypothetically completely cleared of methanol, i.e., the effect of the pulmonary clearance of plasma methanol is also negligible. Thus, following exogenous uptake or endogenous production, unchanged methanol either is excreted (direct excretion) in the urine or exhaled breath or enters a metabolic pathway.

B. First Phase of Methanol Catabolism

The main way methanol is eliminated from the body is via its oxidation to formaldehyde and then to formic acid, which can then be either excreted in the urine or further oxidized to carbon dioxide. The first phase of the oxidative metabolism of methanol and ethanol is similar in insects (162, 503, 504), animals, and humans (63, 168, 453). Ethanol and methanol are converted into formaldehyde and acetaldehyde, respectively, via one of at least three separate pathways **(FIGURE 4)**. The first involves cytochrome *P*450 monooxygenases (CYP) such as CYP2E1 in humans, which catabolizes ~9% of exogenous alcohol, especially at high concentrations (57, 87, 495). The second process, which oxidizes up to 1% of exogenous alcohol, is greatly dependent on catalase (64, 97). The third pathway, which oxidizes up to 90% of alcohol, is catalyzed by cytosolic alcohol dehydrogenase I (ADH I) via NAD^+-dependent oxidation (63, 168, 297). The contribution of each of these mechanisms in the catabolism of alcohol varies by human organs. In the liver, most alcohol catabolism occurs via ADH1b (122), while in the brain, the first phase of methanol and ethanol oxidative metabolism is mainly carried out by two other mechanisms involving CYP2E1 and catalase-H_2O_2 compound I (89, 143, 272). Although there are species differences in methanol and formic acid pharmacokinetics in

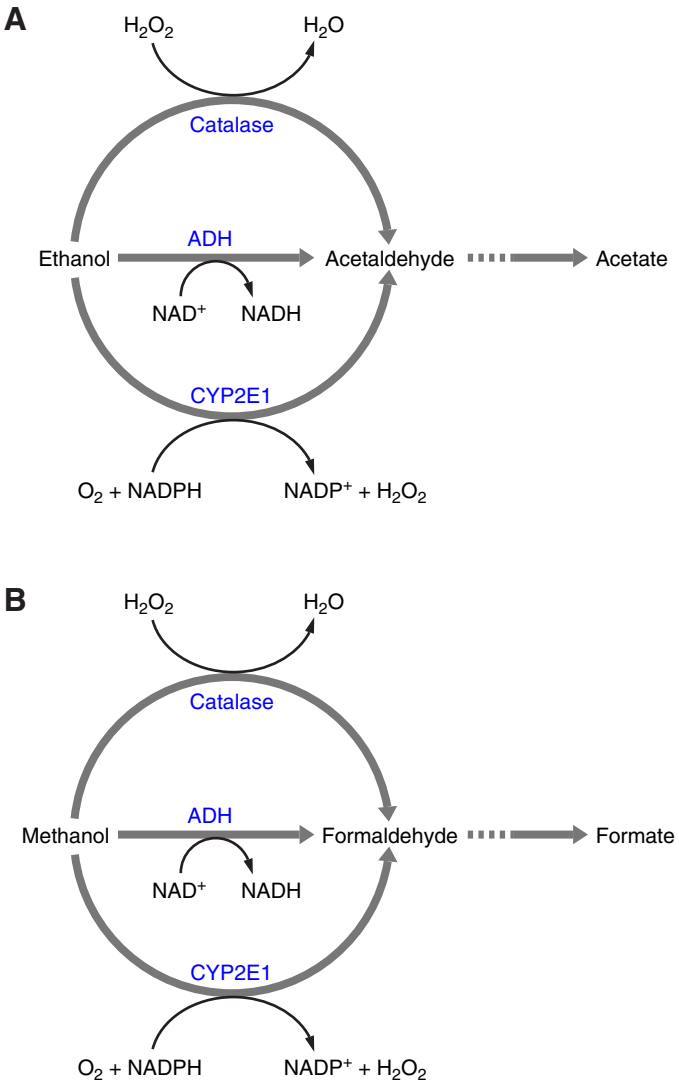

FIGURE 4. The first phase of ethanol (*A*) and methanol (*B*) catabolism to acetaldehyde and formaldehyde, respectively, by three enzymatic systems.

mice, rabbits, and primates (442, 443), methanol is metabolized to formaldehyde primarily by ADH in humans. Rodents are reported to rely on the peroxidative activity of catalase (45, 64, 229).

1. ADH-mediated methanol catabolism

ADH is a zinc-containing enzyme family that consists of two subunits of ~40 kDa (345). The function of ADH in the human body consists mainly of the oxidation of ethanol and methanol produced in the body by microorganisms of the gastrointestinal tract and taken in via fruit, vegetable, and alcoholic beverage consumption. The enzyme is located in the cytosolic fraction of cells, primarily in the liver, gastrointestinal tract, kidney, nasal mucosa, testes, and uterus, and is absent in brain cells (143). Approximately 90% of ingested ethanol and methanol is metabolized via hepatic ADH1b **(TABLE 3)**, which catalyzes an oxidative pathway (65) according to the general scheme presented in **FIGURE 4**. The control of ADH activity is complex and includes *1*) dissociation of the product NADH, which is the rate-limiting step; *2*) product inhibition by NADH and acetaldehyde or formaldehyde; and *3*) substrate inhibition by high concentrations of ethanol and methanol. Mammalian ADHs **(TABLE 3)** catalyze the reversible oxidation of alcohols to aldehydes, acting on a wide variety of substrates ranging from methanol to long-chain alcohols and sterols (120). Mammalian liver ADH1b is a well-characterized enzyme that uses ethanol as a substrate. Relatively little is known about the interaction between ADH1b and methanol, but there is extensive structural homology between methanol and ethanol. ADHs were isolated from the human liver by Vallee et al. (489) 50 years ago; this resulted in partially purified preparations. Subsequent efforts improved upon the purity, which allowed the study of human liver ADH enzymology (286). Later, its primary and tertiary structures, catalytic mechanisms, and enzymatic properties were well studied (301, 376, 381). In vitro, ADH1b catalyzes the transfer of a hydride ion from an alcohol substrate to the NAD^+ cofactor, yielding the corresponding aldehyde and the reduced cofactor NADH. ADH1b is also an excellent catalyst of the reverse reaction. In enzymatic tests carried out in vitro, methanol is oxidized to formaldehyde by NAD^+ in the presence of ADH; then, formaldehyde can be oxidized to formate by NAD^+ or reduced to methanol by NADH. These reactions follow a rapid equilibrium random mechanism. Among these three reactions, the reduction of formaldehyde is the most rapid. The rate of formaldehyde oxidation is faster than that of methanol oxidation (380).

Each of the two liver ADH1b subunits contains one catalytic and one structural zinc ion (83). The catalytic zinc ion is situated at the bottom of the cleft between the coenzyme binding domain and the catalytic domain. It is accessible through two channels, one that accommodates the coenzyme and another through which the substrate approaches the catalytic site (382). When the ternary complex forms, the catalytic domain rotates ~10° to allow the residues in the catalytic domain to move closer to the coenzyme domain, and the cleft between domains closes (83). There are significant differences in the enzymatic behavior for methanol and ethanol. In vitro, methanol is oxidized by live ADH1b at a much slower rate. The rate of methanol oxidation catalyzed by purified ADH1b is only ~3% of the rate of ethanol oxidation. Within the alcohol binding site of liver ADH1b, there are two binding regions: a hydroxyl binding region and a hydrophobic binding region (99, 100). Methanol binds to a similar site as ethanol in ADH1b. Pyrazole, an efficient liver ADH1b inhibitor, binds to the enzyme-NAD^+ complex and blocks the substrate binding site (378). The inhibition patterns and inhibition constants of pyrazole are similar for both methanol and ethanol (380). This confirms that the binding of methanol to the catalytic zinc is similar to that of ethanol. On the other

Table 3. *ADH genes and proteins*

Gene Name	Class	Amino Acid Differences Between Alleles	Protein Name	K_m (Ethanol), mM	K_m (Methanol), mM
ADH1A	I		A	4.0[a] to 4.2[d]	150.0[b,c]
*ADHB*1*		Arg48, Arg370	β_1	0.05[a,d] to 1.2[b]	6.0[d] to 7.0[e]
*ADH1B*2*		His48, Arg370	β_2	0.9[d]	ND
*ADH1B*3*		Arg48, Cys370	β_3	34.0[d]	ND
*ADH1C*1*		Arg272, Ile350	γ_1	1.0[d]	ND
*ADH1C*2*		Gln272, Val350	γ_2	0.6[d] to 1.0[b]	30.0[b]
*ADH1C*352Thr*		Thr352	ND	ND	ND
ADH4	II		Π	34[d]	No activity[b]
ADH5	III		χ or FDH	>1,000.0[f]	No activity[b]
ADH6	V		ADH6[g]	ND	ND
ADH7	IV		Σ	30.0[f]	ND

K_m indicates the concentration of alcohol (ethanol or methanol) at which the enzyme works at 50% capacity. ND, not determined; FDH, formaldehyde dehydrogenase. [a]Bosron et al., 1983 (41); [b]Wagner et al., 1983 (492); [c]For $\alpha\gamma 1$ heterodimer; [d]Crabb et al., 1987 (92); [e]Pietruszko, 1975 (378); [f]Edenberg, 2007 (122); [g]Ostberg et al., 2013 (361).

hand, the second step, hydride transfer, which is the conversion of the ternary complex, is the rate-limiting step for methanol oxidation. Because of the lack of an appreciable hydrophobic chain, methanol exhibits different kinetics than ethanol (50, 122, 380). The hydrophobic interaction in the active site plays a fundamental role in substrate binding. When a substrate binds to the enzyme, the interaction between the hydrophobic binding region and the hydrophobic chain on the substrate stabilizes substrate binding. Methanol, with a chain of only one carbon atom, cannot bind to the hydrophobic region as ethanol can. Because the methyl group is not held in the proper orientation for hydride transfer, methanol exhibits both a weak binding constant and a slow turnover rate. The binding of NAD^+ to the enzyme does not exhibit a significant effect on the binding of methanol, nor does methanol affect the binding of NAD^+ (380). Pyrazole and its derivatives such as 4-methylpyrazole are potent inhibitors of ADH1b and block the interactions of all class I ADH isozymes with ethanol (523) and methanol (378). These compounds function as ligands of zinc, which is essential for the catalytic activity of the native forms of ADH. They form slowly dissociating ternary complexes with ADH and NAD^+ and act as competitive inhibitors with respect to ethanol and methanol. 4-Methylpyrazole, the most potent inhibitor, binds to the enzyme-NAD^+ complex as an inner sphere ligand of the catalytic zinc (416), thus blocking the substrate binding site and the formation of a dead-end ternary complex with the enzyme and NAD^+ (455). The strong sensitivity of class I ADHs to pyrazole inhibition explains the powerful inhibition of ethanol metabolism in humans by these agents. The K_i (4-methylpyrazole) value of class I ADHs is ~0.1 μM (63) with ethanol as a substrate, and the K_i value for methanol is similar, equal to 0.09 μM (378). The $\pi\pi$ ADH isoform **(TABLE 3)**, named for its pyrazole insensitivity, is inhibited much less effectively by 4-methylpyrazole (K_i = 2 mM), although the pyrazole derivatives 4-bromo-, 4-nitro-, or 4-pentylpyrazole are more efficient with ethanol (K_i values between 4 and 27 μM) (40).

In addition to ethanol and methanol, ADH isoforms also oxidize several metabolic alcohols with high catalytic efficiency, including retinol, ω'-hydroxy fatty acids, hydroxysteroids, and hydroxyl derivatives of dopamine and epinephrine metabolites (37, 382). Vitamin A and its derivatives (retinoids) are essential components in vision. Animals are, in general, unable to synthesize vitamin A de novo. Retinol, retinal, and retinoic acid are C20 isoprenoids that are metabolically derived from the oxidative cleavage of C40 carotenoids. In the presence of NAD^+, the enzyme oxidizes the alcohol retinol to the aldehyde retinal, which is necessary for vision. ADH utilizes a mechanism for retinol-retinal interconversion that is similar to that used for ethanol/methanol oxidation and acetaldehyde/formaldehyde reduction (37, 382). In contrast to the methanol-formaldehyde, ethanol-acetaldehyde, and other short-chain alcohol-aldehyde systems, the equilibrium constant for the retinol-retinal interconversion is ~200 times higher. The K_m value (60 μM) indicates that the interactions between the long carbon chain of the retinoid and the hydrophobic pocket of the enzyme provide a major driving force for the binding process. The oxidation of these alcohols can be inhibited by ethanol and methanol, and therefore, the role of primary alcohol substrate competition is an important issue in the effects of alcohol on humans.

2. Human ADH genes

The human genome contains seven genes encoding ADH (122) **(TABLE 3) (FIGURE 5)**. They form a cluster of genes located in chromosome 4 (4q21–23), spanning ~370 kb (359, 360) and encoding multiple forms of ADH with dif-

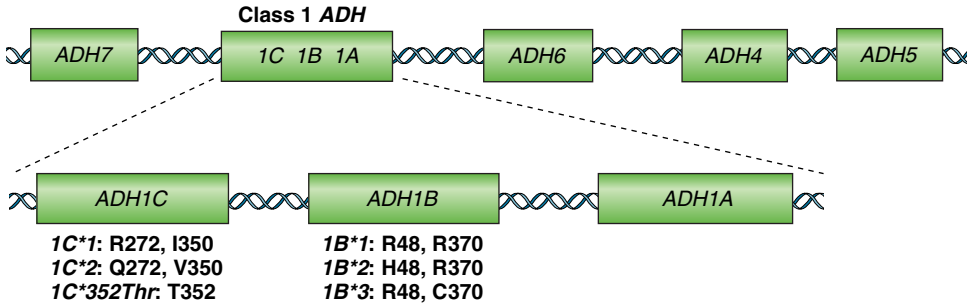

FIGURE 5. Relative map of the SNP sites and *ADH* alleles. *Top panel* represents the whole *ADH* gene cluster, and *bottom panel* shows the genes of the class 1 *ADH* cluster with the different alleles and amino acid substitutions indicated. [Modified from Osier et al. (360) and Edenberg (122), both with permission from Elsevier.]

ferent substrate specificities (63, 92). The human liver contains seven ADH isoforms, which are divided into five classes **(TABLE 3)**.

Class I ADHs are encoded by three genes, *ADH1*, *ADH2*, and *ADH3*, which encode these subunits: α (ADHIA), β1, β2, β3 (ADHIB), and γ1 and γ2 (ADHC). The ADH1 subunits share ~94% sequence identity. These different units and polymorphs can form homodimers or various heterodimers (e.g., $\alpha\alpha$, $\beta1\beta1$, $\alpha\beta2$, $\beta1\gamma2$) (41, 393, 492). The class I ADH isoforms play the most important role in alcohol oxidation. The study of ADH1-null mice showed that ADH1 enzymes together are responsible for ~70% of the total ethanol-oxidizing capacity of liver, indicating that the non-ADH1 pathway accounts for the remaining 30% (169). The relative contributions of each of the ADH isozymes to ethanol oxidation change with the hepatic ethanol concentration. The K_m of human class I ADH for ethanol is very low **(TABLE 3)**. Therefore, the enzyme becomes saturated after the intake of just 28–30 ml of ethanol, and ethanol is removed from the human body at a constant rate, independent of the concentration (347). Hence, the rate of disappearance of ethanol from the blood is virtually independent of the prevailing blood-ethanol concentration (zero-order kinetics) (92). All isozymes of class I ADH show substrate inhibition ([ethanol] >20 mM) because an excess of ethanol decreases the speed of NADH dissociation from the enzyme (223).

The ADH α-subunits, encoded by the gene *ADH1A*, are monomorphic, whereas the other two subunits were identified as isoforms that differ in ADH catalytic activity levels. The *ADH1B* gene was shown to comprise two functionally important polymorphisms **(TABLE 3)**: *1*) one encoding ADH1B*2, which occurs in human populations of North Africa, Eurasia, and Oceania, and *2*) one encoding ADH1B*3, which is found only in African populations. Experiments on isolated preparations of human liver ADH showed that the arginine amino acid at position 48 participates in the binding of the pyrophosphate group NAD^+, but the replacement of arginine for histidine leads to a lower optimal pH and a 100-fold increase in the β2-ADH turnover (215, 308). The study of the role of this change on the metabolism of ethanol in the human body did not provide such an unambiguous picture. The first-pass study showed that the hepatic ethanol clearance of *ADH1B*2* individuals was higher than that of the *ADH1B*1* individuals (267). Evidence of the high enzymatic activity of β2-ADH was also obtained in the study of another *ADH1B*2/*2* group (224); the blood acetaldehyde concentrations of *ADH1B*2/*2* individuals were higher than those of *ADH1B*1/*2* individuals only in the *ALDH2*1/*2* group. However, the blood ethanol concentrations of the *ADH1B*2/*2* group were higher than those of the *ADH1B*1/*2* group regardless of the *ALDH2* genotype. These findings are unexpected and are difficult to explain from the viewpoint of ADH enzyme activity alone.

The Arg370Cys amino acid replacement in the *ADH1B*3* allele, which almost never occurs in populations of European and Asiatic origins, also leads to an increased reaction speed due to a changed efficiency of the interaction between the $\beta_3\beta_3$ dimer and NAD^+ (55).

ADH1C also contains single nucleotide polymorphisms, of which *ADH1C1* and *ADH1C*2* are the most studied. The enzyme encoded by *ADH1C*1 (γ1-ADH)* contains Arg at position 272 and isoleucine (Ile) at position 350, whereas that encoded by *ADH1C*2 (γ2-ADH)* contains glutamine (Gln) at position 272 and valine (Val) at position 350 (359, 360). The kinetic differences between γ1-ADH and γ2-ADH are small (185) **(TABLE 3)**.

Classes II, III, and IV enzymes are homodimeric forms of the π, χ, and σ subunits, respectively. The *ADH4* gene encodes class II ADHs, including the $\pi\pi$ ADH isoform, named for its pyrazole insensitivity. The $\pi\pi$ ADH isoform is more labile than the other ADH isoforms and was first detected in liver biopsies and fresh autopsy samples. Due to its rather high K_m for ethanol **(TABLE 3)**, this enzyme may be more important in the metabolism of high concentrations of ethanol (92). $\pi\pi$ ADH accounts for nearly 30% of the total ethanol-oxidizing capacity of the liver (192) and exhibits a more limited substrate specificity than do the other molecular forms of the human liver. Methanol, glycerol, and ethylene glycol, even at concentrations up to 100 mM, cannot be oxidized by $\pi\pi$ ADH (40).

The *ADH5* gene encodes class III ADHs, including the $\chi\chi$ ADH isoform, which is widely distributed in the tissues

(63). χχ ADH is virtually not inhibited at all by 4-methylpyrazole (492), has a very high K_m for ethanol, and likely plays almost no role in ethanol and methanol elimination (63).

3. Cytochrome P450s-mediated oxidation of methanol and ethanol

The CYP enzymes are named CYP for cytochrome *P*450, followed by an Arabic number denoting the family, a letter designating the subfamily and, finally, an Arabic numeral representing the individual gene in the subfamily (236, 341, 530). The *P*450s are arranged in families based on sequence homologies (340, 342) and are very versatile catalysts that activate dioxygen and insert a single oxygen atom into almost any organic compound imaginable (255). Cytochrome *P*450s are a family of heme-containing monooxygenases that are involved in the oxidation of ethanol, methanol, steroids, fatty acids, and numerous xenobiotics (acetone, benzene, and other alcohols) ingested from the environment (62, 249, 252, 295, 530). The heme iron of the CYP enzymes binds two oxygen atoms. However, in contrast to the iron found in hemoglobin, this iron atom is bound rather tightly to the anionic thiolate sulfur of cysteine. This binding mode gives heme the properties necessary for splitting the dioxygen molecule into two atoms. Because one oxygen atom forms a water molecule, the CYP enzymes were also named mixed function oxidases. The second oxygen atom is activated for introduction into the substrate molecule; therefore, CYP enzymes have been included in the monooxygenase enzyme class. Hence, the splitting of the dioxygen molecule to two atoms results in the formation of a hydroxylated product (6). Most *P*450s are considered to operate according to a general scheme **(FIGURE 6)** (26, 27, 86, 508). There are many *P*450 isoforms encoded by more than 100 gene families (249, 252, 340, 342, 530, 538). *P*450 functions in conjunction with microsomal enzymes such as NADPH-cytochrome *P*450 reductase and cytochrome b_5 (342) **(FIGURE 6)**.

CYP2E1 is the *P*450 enzyme with the highest activity for oxidizing ethanol and methanol to acetaldehyde and formaldehyde, respectively. CYP2E1 accounts for ~6% of the total *P*450 content in the human liver and catalyzes the metabolism of 2% of commercially available drugs (150, 538). The human gene encoding CYP2E1 is the only gene of the *CYP2E* subfamily located at chromosome 10q26.3; it contains 9 exons and comprises several polymorphisms (236). Several different CYP2E1 polymorphisms have been identified (266). Human *CYP2E1* expression is undetectable in the fetal liver but is the highest expressed cytochrome *P*450 in the adult liver, where it is present mainly in the endoplasmic reticulum (microsomal fraction) (271, 272, 274, 275) but are also found in mitochondria (15, 18, 19, 242, 273). Aside from the liver, *CYP2E1* is also expressed in the brain (137, 138, 164, 178, 216, 246, 349, 482) and lungs (227). *CYP2E1* expression has been found also in the nasal mucosa, kidney cortex, testes, ovaries, and gastrointestinal tract at lower levels (273, 454) and in cardiac tissue at somewhat higher levels (317, 532, 533). *CYP2E1* is regulated by several mechanisms, including transcriptional and posttranscriptional processes (350, 530). *CYP2E1* induction appears to occur via two steps: a posttranslational mechanism at low ethanol concentrations and an additional transcriptional mechanism at high ethanol concentrations (17, 397).

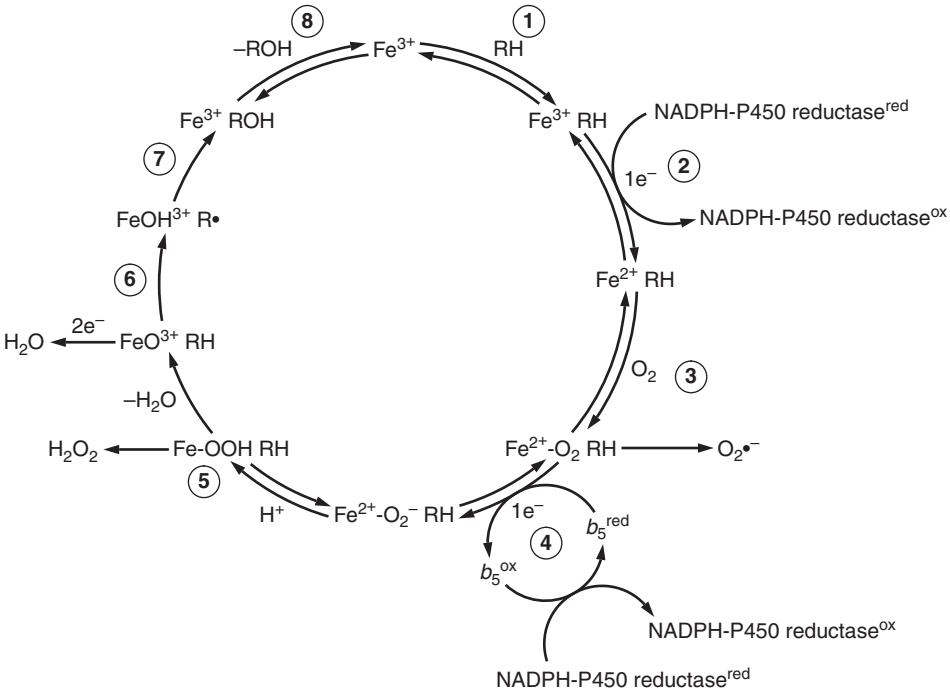

FIGURE 6. The general scheme for *P*450-catalyzed oxidation reactions (26, 27). RH, substrate; ROH, product. The reversibility of some of the latter steps is unknown. The outlets for the uncoupled reduced oxygen products O_2, H_2O_2, and H_2O are shown. Following substrate binding (step 1), ferric *P*450 receives 1 electron via NADPH-P450 reductase (step 2). The ferrous form of hem binds O_2 (step 3) before undergoing a second 1-electron reduction to begin O_2 activation (step 4). Although this second electron originates from NADPH-*P*450 reductase, the accessory protein cytochrome b_5 takes part in the delivery of the electron to *P*450. Insertion of the activated oxygen into the substrate occurs via C-H bond cleavage (step 6) followed by rapid oxygen rebinding to form the product (step 7). Step 8 is the release of the product from the active site of the enzyme.

Ethanol and methanol are both inducers and substrates of CYP2E1 (61, 63, 226, 357, 398). Acetaminophen, caffeine, chlorzoxazone, and an array of carcinogens such as benzene, styrene, acrylonitrile, and nitrosamines are also metabolized by CYP2E1. The K_m of CYP2E1 for ethanol is 10 mM, which is 10-fold higher than the K_m of ADH for ethanol. Therefore, liver ADH has a much higher capacity for ethanol oxidation than the CYP2E1 system at low ethanol concentrations, but CYP2E1 may be the basis of the metabolic adaptation to high concentrations of ethanol that develops upon chronic ethanol consumption (272). CYP2E1 is likely to provide ~10% of the total ethanol-oxidizing capacity of the liver during modest ethanol intake (62). Alcohol oxidation increases at higher ethanol concentrations, and much of this increase is due to the metabolism of ethanol by CYP2E1. CYP2E1 levels are increased by chronic ethanol administration by a mechanism that largely involves protection of the enzyme against proteolysis. *CYP2E1* expression is also induced in diabetics, in the fasted nutritional state, and in nonalcoholic steatohepatitis (NASH). NASH is a progressive liver disorder that occurs in patients with high hepatic CYP2E1 levels but without significant ethanol consumption (506).

CYP2E1 is highly conserved within the human population (271), suggesting significant physiological functions, including nutritional support via the catalysis of fatty acid ω'-1 and ω'-2 hydroxylation reactions (2, 4, 259). Thus CYP2E1 plays dual physiological roles, one in the conversion of ketones into glucose and one in detoxification (271). An important feature of ethanol and methanol oxidation via CYP2E1 is the generation of reactive oxygen species (ROS), which contribute to the damage of liver cells (57, 272, 516). CYP2E1 is an effective generator of ROS such as the superoxide anion radical and hydrogen peroxide as a result of the uncoupling of oxygen consumption and NADPH oxidation. The significant levels of CYP2E1 within the mitochondria could further contribute to ROS deleterious effects (18). Moreover, metabolism by CYP2E1 results in a significant release of free radicals that, in turn, diminish reduced glutathione levels (18) and other oxidative stress defense systems that play major pathogenic roles in alcoholic (60, 242, 272) and nonalcoholic liver disease (14, 66). CYP2E1 is a causative player in alcoholic liver disease, as well as in NASH, likely through the enhancement of hepatic lipid peroxidation (14, 60, 242).

4. Catalase-H_2O_2 system

The ability of peroxisomal catalase to oxidize short-chain alcohols such as methanol and ethanol was discovered long ago (64, 81, 234, 274). With the use of hydrogen peroxide (H_2O_2) as a cosubstrate, the heme-containing enzyme catalase forms the catalase-H_2O_2 system (also known as compound I), which is also the major pathway of formaldehyde oxidation. The role of this system in the oxidation of alcohols is small in the liver (63) but significant in the brain (499, 536, 537), which lacks ADH activity (143). Ethanol in the brain is oxidized into acetaldehyde by the action of the catalase-H_2O_2 system (7, 8, 81, 145, 203, 228) and cytochrome *P*4502E1 (CYP2E1) (537). It is estimated that the catalase-H_2O_2 system in the brain is responsible for 60–70% of the acetaldehyde generated in this organ, while CYP2E1 accounts for 10–20% (537). Inhibitors of catalase, aminotriazole, and sodium azide depress the oxidation of ethanol and methanol to acetaldehyde and formaldehyde, respectively, in the brain (64, 537). Acetaldehyde derived from the catalase-dependent oxidation of ethanol in the brain has been suggested to play a role in the mediation of many effects of ethanol in the brain, including behavioral, neurochemical, and neurotoxic actions, and plays a crucial role in the development of alcoholism (89, 334). Participation of the catalase-H_2O_2 system in the oxidation of ethanol and methanol in the brain was confirmed by experiments with alpha lipoic acid (ALA, 1,2-dithiolane-3-pentanoic acid). ALA is considered to be an H_2O_2 scavenging agent and thus an inhibitor of the catalase-H_2O_2 complex; treatment with ALA prevents the formation of acetaldehyde in the brain and, therefore, prevents its neurochemical and neurotoxic actions (264). Indeed, on the one hand, ALA reduced ethanol self-administration in rats (368) and mice (264), and on the other hand, ALA treatment prevented methanol exposure-induced oxidative damage of tissues of the rat nervous system (388). However, these results can also be explained by another property of ALA, namely, the ability of ALA to activate ALDH2 (116, 171a, 285, 310). The ALA-mediated decreases of formaldehyde and acetaldehyde in animal tissues can be induced by both interfering with their formation and accelerating their oxidation by ALDH2. It could be suggested that the beneficial effects of ALA on ethanol metabolism are related to the capacity of ALA to control both reactions.

C. Second Phase of Methanol Catabolism

Formaldehyde is a naturally occurring biological compound that is present in all tissues, cells, and biological fluids (174). The concentration of endogenous formaldehyde in the blood of rats (176), monkeys (58), and humans (176, 413, 463) does not exceed 0.1 mM, and that in the rat and human brain is 0.2–0.4 mM (464, 466). Most formaldehyde is produced as the oxidation product of methanol. However, another endogenous source of formaldehyde is the oxidative deamination of methylamine derived primarily from creatine (526) by semicarbazide-sensitive amine oxidases (SSAOs) (201, 436, 527), which generate formaldehyde together with ammonia and hydrogen peroxide, as follows (233, 363, 525, 527): $CH_3NH_2 + O_2 + H_2O \xrightarrow{SSAO} HCHO + H_2O_2 + NH_3$.

In mammals, among the SSAOs, vascular adhesion protein 1 is one of the most extensively studied members of this group of enzymes (119, 202, 524). It is interesting that some

monoamine oxidase inhibitors such as phenelzine with antidepressant properties (20) are able to protect neurons and astrocytes against formaldehyde (424).

Formaldehyde is also part of the one-carbon pool, which is utilized for the biosynthesis purines, thymidine, and several amino acids, which are incorporated into DNA, RNA, and proteins during macromolecular synthesis (220). Besides the oxidation of methanol, formaldehyde is formed by the conversion of serine and glycine in the presence of tetrahydrofolate (35). Formaldehyde is also generated during chromatin structure remodeling in the reactions of removing methyl groups from lysine residues in histones that are catalyzed by lysine-specific demethylase 1 and JmjC domain-containing histone demethylases (79, 189, 287, 470, 497). Aside from endogenous sources, formaldehyde can also be produced and released from different exogenous products that naturally contain formaldehyde, including food products such as coffee, codfish, meat, poultry, and maple syrup (95b, 98, 298, 391). Drugs also may be sources of formaldehyde. For example, Selegiline (Anipryl, L-deprenyl, Eldepryl, Emsam, Zelapar), which is used to treat Parkinson's disease, gives off formaldehyde as a byproduct as a result of the metabolic transformation circuit (219).

Efficient systems of formaldehyde oxidation exist in mammals. Despite the multiple endogenous and exogenous sources of formaldehyde, a low physiological level of formaldehyde in bodily fluids and tissues is maintained by the continuous action of cellular formaldehyde-metabolizing enzymes **(FIGURE 7)**.

The oxidation of formaldehyde to formate occurs by at least three separate pathways with the participation of *P*450 monooxygenases, mitochondrial aldehyde dehydrogenase 2 (ALDH2), and the gene encoding ADH5, $\chi\chi$ ADH, **(TABLE 3)**, also called ADH3 or formaldehyde dehydrogenase (FDH) (473) **(FIGURE 7)**. Liver ADH1b plays the main role in the first phase of methanol oxidation but has a negligible effect on the reduction of formaldehyde to methanol because of its higher K_m value for formaldehyde (~30 mM) (419).

Similarly to other aldehydes, formaldehyde can be oxidized by *P*450s (27, 183, 255, 289, 452), as shown in **FIGURE 6**. Interestingly, transgenic *Petunia hybrida* plants harboring the *CYP2E1* gene have improved resistance to formaldehyde (533).

In humans, two members of the divergent ALDH superfamily, a cytosolic (ALDH1A1) and a mitochondrial (ALDH2) enzyme (204, 420, 427, 435, 484, 502), can directly metabolize formaldehyde (142, 297, 452, 453, 473, 485). Mitochondrial ALDH2, which is especially important for the oxidation of high concentrations of formaldehyde, has a high K_m for formaldehyde (0.2–0.5 mM) compared with that of FDH (see below) (<0.01 mM) (59, 175, 336, 432). The intracellular concentration of free formaldehyde is likely too low to result in significant oxidation by ALDH2 (175).

Human *FDH* or *ADH5* gene encoding **(FIGURE 6)** $\chi\chi$ ADH belongs to the family of medium-chain zinc-containing

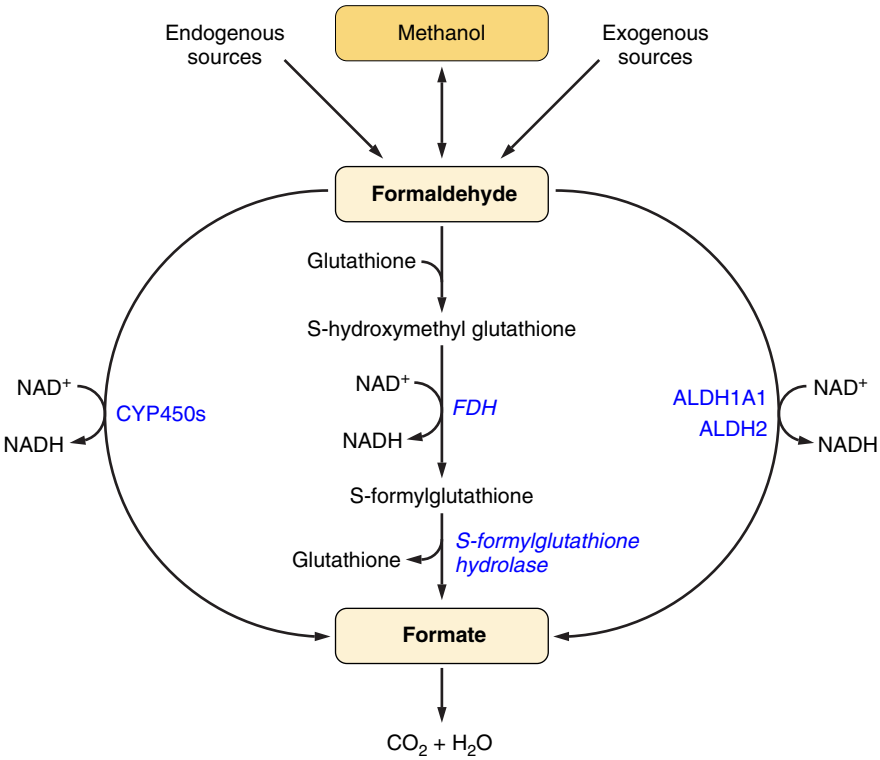

FIGURE 7. Formaldehyde metabolism. Overview of the pathways of the conversion of formaldehyde into water and CO_2. For details, see text.

ADHs (TABLE 3). In the literature, different ADH nomenclatures have been used in early reports, in which ADH3 was referred to as glutathione-dependent FDH, χχ ADH or class III ADH (218, 432). We will use glutathione-dependent FDH in our review because this name more precisely reflects its formaldehyde oxidizing function.

In contrast to other mammalian ADH isoenzymes that are active toward ethanol and methanol, FDH shows very poor activity toward ethanol and methanol and prefers long-chain alcohol substrates such as ω′-hydroxy fatty acids, including 12-hydroxydodecanoic acid and retinol (vitamin A) (243, 268, 320, 332, 432). Compared with the other ADH classes, FDH has two additional activities: a glutathione-dependent formaldehyde oxidizing activity and an *S*-nitrosoglutathione (GSNO) reductase activity (186).

Human FDH is the most efficient formaldehyde-metabolizing enzyme among all of the enzymes elucidated so far (432) and was first purified from human liver and partially characterized by Uotila and Koivusalo (479, 480). FDH is the most conserved class of all of the ADHs (186). Like other human ADH isoenzymes, FDH exists as a dimer and contains two covalently bound zinc ions per subunit (520). FDH is considered to be the main scavenger of exogenous formaldehyde and is ubiquitously expressed with relatively little inter-tissue variation in mammals, in contrast to other ADHs. FDH is primarily a cytosolic enzyme, although was revealed in nucleus where it likely protects DNA from formaldehyde-mediated damage (139, 195).

FDH is a glutathione-dependent χ-ADH and oxidizes formaldehyde to formate in a two-step process (FIGURE 7). The polarized carbonyl group formaldehyde is a compound with high reactivity with thiols, spontaneously forming *S*-hydroxymethyl glutathione after the interaction of formaldehyde with glutathione (256). Thus, in the first step of FDH-mediated formaldehyde oxidation, *S*-hydroxymethyl glutathione is formed and is subsequently used as an FDH substrate to generate *S*-formylglutathione (168, 297, 431, 457). The conjugate *S*-formylglutathione is then hydrolyzed by a *S*-formylglutathione transferase to generate formate and glutathione (167, 168, 297, 430, 431, 452, 457, 473).

The formate generated by formaldehyde oxidation can undergo further oxidization to carbon dioxide (FIGURE 7) in metabolic pathways involving catalase (85, 419), 10-formyltetrahydrofolate dehydrogenase, also known as ALDH1L1, or ALDH1L2, its mitochondrial isoform (419, 473).

Formaldehyde interacts with GSNO and functions as a GSNO reductase in NO homeostasis (431, 432, 457). GSNO depletion is associated with various diseases, including asthma. Importantly, FDH-mediated S-(hydroxymethyl)glutathione oxidation is accelerated in the presence of GSNO, which is concurrently reduced by immediate cofactor recycling (430, 432, 457).

D. Features of Methanol Metabolism in the Brain and in Embryos

The metabolism of methanol and formaldehyde in brain cells is important for the function of this vital organ (472). Moreover, the elevation of brain formaldehyde levels is likely to result in neurodegenerative diseases (386, 448, 450, 463, 464, 517, 518). Methanol and the products of its metabolism in brain cells may be synthesized in situ or be introduced from peripheral organs via the bloodstream by overcoming the blood-brain barrier.

1. Production of methanol and formaldehyde by brain cells

Cultured brain cells, including astrocytes and neurons, contain mRNAs encoding SSAO and lysine-specific demethylase 1, as well as for the enzymes involved in formaldehyde metabolism (472, 473). Synthesis of formaldehyde by brain cells can occur via protein carboxymethylation (69, 265), as well from the participation of SSAOs and in the reactions of removing methyl groups from lysine residues in histones, which are catalyzed by lysine-specific demethylase 1 and JmjC domain-containing histone demethylases (79, 189, 287, 470, 497).

SAM might be transformed to methanol and *S*-adenosyl homocysteine in the bovine pituitary gland and other animal brain tissue (16, 422). SAM is a universal endogenous methyl donor and is a limiting factor in various methylation reactions, including protein carboxymethylation (135). Protein carboxymethylase is highly expressed in the brain and pituitary gland of several mammalian species (103, 105). The effects of SAM-induced protein carboxymethylation on the formation of methanol, formaldehyde, and formic acid in rat brain striatal tissues were investigated; this study directly showed that excessive SAM-dependent methylation increased levels of methanol, formaldehyde, and formic acid in rat brain striatal homogenates (265).

Formaldehyde can also be generated in the brain by the reactions catalyzed by lysine-specific demethylase 1 and JmjC domain-containing histone demethylases (79, 189, 288, 470, 472, 497).

In other metabolic pathways, SSAOs located in the outer membranes of vascular smooth muscles and the endothelium of the brain catalyze the deamination of methylamine and generate formaldehyde together with ammonia and hydrogen peroxide (73, 233, 363, 527). It has been shown that the incubation of methylamine in the presence of SSAO-rich tissues, e.g., human brain meninges, results in cross-linked

proteins. This cross-linkage can be completely blocked by a selective SSAO inhibitor (155). Recently (386), the participation of brain SSAOs in formaldehyde generation was shown in a well-established mouse model of Alzheimer's disease, the senescence accelerated mouse-prone 8 (SAMP8) strain (466). Formaldehyde levels were elevated in the brains of 3-mo-old SAMP8 mice (94.43 ± 1.32 μmol/g) compared with those of age-matched senescence accelerated resistant mouse 1 (SAMR1) mice (85.21 ± 1.39 μmol/g). In contrast to serine hydroxymethyl transferase and CYP450 2E1, SSAOs play a major role in the generation of brain formaldehyde in SAMP8 mice. Levels of formaldehyde-producing SSAOs are higher in the brains of SAMP8 mice compared with SAMR1 mice, whereas the FDH mRNA content was reduced in SAMP8 mice. Both the reduced ADH3 activity and SSAO mRNA/protein levels correlate well with the observed formaldehyde accumulation in the brains of SAMP8 mice; these data allowed the authors to suggest a causal relationship between the two phenomena (386).

2. Metabolism of methanol/formaldehyde introduced into the brain through blood-brain barrier

One pertinent question is whether methanol and formaldehyde synthesized in the gastrointestinal tract and liver can enter the blood and ultimately reach the brain by crossing the blood-brain barrier (BBB) (472). The BBB ensures the optimal control of homeostasis of the brain's internal environment. The anatomical structure of the BBB comprises endothelial cells of arterioles, capillaries, veins, and epithelial cell surfaces, with the presence of tight junctions between the endothelial cells of brain capillaries and oligodendrocytes. The endothelial cells enable the very selective transport of substances from the blood to the brain, and vice versa (326, 481). Methanol and ethanol from the blood apparently reach the brain cells without hindrance (112, 159, 197). At the same time, peripherally produced acetaldehyde and formaldehyde penetrate the BBB to enter the brain with difficulty. Although the physical-chemical properties of formaldehyde and acetaldehyde suggest that these molecules would easily penetrate the BBB, the presence of the "metabolic" barrier of ALDH and FDH hinders their crossing of the BBB (89, 334, 410, 535).

3. The modes of brain methanol and formaldehyde catabolism

ADH is responsible for the metabolism of ethanol and methanol in the liver but has not been definitively demonstrated to play a significant role in the brain. The dominating view is that ADH1 does not participate in methanol metabolism in the brain (143, 217, 472). This conclusion is strongly supported by the direct determination of the localization of different classes of ADHs in the brain by Northern blot and enzyme activity analyses (143). This study did not reveal any ADH1 activity in the brain, whereas class III χχ ADH or FDH had high activity in the brains of adult humans, mice, and rats. No ADH1 mRNAs were revealed in the mouse brain following methanol inhalation (246). Although ADH1 activity was not detected in the whole brain homogenate, it was found to be expressed specifically in granular and Purkinje cells of the cerebellum, indicating that ADHs are likely to play a role in particular regions of the brain (143). On the other hand, brain cell malignization results in increased total ADH activity and, specifically, increased class I ADH activity. The other tested classes of ADH and ALDH enzymes did not show statistically significant differences in activity in cancer and normal cells (262).

The literature does not provide conclusive or reliable information about phase I metabolism of methanol in the brain. However, considering that ethanol is converted into acetaldehyde by the system that includes the participation of CYP450s and the catalase-H_2O_2 system, it is safe to assume that these systems are also involved in methanol conversion in the brain (178). Catalase and CYP450s represent the major enzymes in the brain that catalyze ethanol oxidation. CYP450s are abundantly expressed within the microsomes of certain brain cells and are localized to particular brain regions. There is evidence that catalase serves as the primary ethanol-metabolizing enzyme in the brain (486) and accounts for 60% of the ethanol oxidation in brain (537). The studies of ethanol metabolism conducted by perfusing living rat brains confirmed the participation of catalase in acetaldehyde formation. Moreover, the addition of the catalase inhibitor aminotriazole to the perfusing fluid increased the ethanol level in the perfusate, i.e., ethanol elimination was significantly decreased. The presence of CYP450s in the brain has been established (178), but the total level of CYP450s in the brain is much lower than that in the liver, and these enzymes are concentrated in specific regions and cell types, indicating their potentially considerable impact on local metabolism (138). The expression of CYP2E1 and CYP2B6 within the brain can be substantially increased in response to ethanol (137, 400). Brain CYP450s are regulated by transcriptional, posttranscriptional, and posttranslational mechanisms (138). Experiments on animals harboring genetic deficiencies in CYP2E1 indicated that CYP2E1 is responsible for ~20% of the ethanol metabolism in the brain (537), but the mechanism responsible for metabolizing the remaining 20% of ethanol remains unclear (178, 375, 537).

Although no direct studies of the metabolic conversion of formaldehyde in the mammalian brain have been conducted, experiments with exogenous formaldehyde primarily indicated its neurotoxicity (472). The extent of damage depends on the dose of formaldehyde and the duration of the exposure (13, 425, 426, 450). The conversion of formaldehyde into formic acid apparently occurs with the participation of ALDHs (472). Fifteen Aldh isozymes and their

mRNAs were revealed in the mouse brain (3). After methanol inhalation by mice, *Aldh 1* and *2* mRNA contents were dramatically increased in the brain (246). The contribution of ALDHs in the oxidation of formaldehyde in the human brain is unknown. However, the mitochondrial ALDH2 activity toward formaldehyde in the liver was essentially less in human subjects with mutant alleles (*ALDH2*2*) compared with those with wild-type alleles (502).

The enzymatic degradation of formaldehyde in the brain is also regulated by FDH/ADH3. In the human brain, FDH is also likely involved in the oxidation of formaldehyde because its activity was detected in histological preparations (328). The most intense FDH immunostaining was observed in hippocampal pyramidal neurons, cerebellar Purkinje cells, cerebral cortical neurons in layers IV and V, and perivascular and subependymal astrocytes.

Studies conducted in adult rats and mice showed that Adh3 is the only ADH class present in the rodent brain (507). The important role of this enzyme for mammalian life was proven by studies of mice with a mutated *Adh3* gene. *Adh3*-knockout mice have a high sensitivity to formaldehyde and a significantly reduced LD_{50} value (96). Moreover, *Adh3*−/− mice exhibited smaller litter sizes, smaller body weights at 14 wk of age, and lower postnatal survival rates compared with wild-type mice. Less than 20% of these mice survive past postnatal day 6 (320). These features indicate the inability of *Adh3*−/− mice to provide metabolic clearance of endogenous formaldehyde. Reduced expression of the *Adh3* gene was also revealed in SAMP8 mice; along with increased SSAO activity, high formaldehyde contents in the brain and cognitive function disruption were observed in these mice (386).

The metabolism of methanol in embryos is important for mammalian procreation and has a great deal in common with those in the central nervous system. In general, prenatal ethanol exposure has been found to be a risk factor for fetal mortality, stillbirth, and infant and child mortality. The elimination of ethanol from the fetus relies on the metabolic capacity of the mother (54). The main feature of methanol metabolism in embryos is the lack of ADH1 activity (507). Testing for *Adh* class mRNAs by in situ hybridization revealed the complete absence of *Adh1* and *Adh4* mRNAs from all brain structures at all prenatal stages investigated during the embryonic development of mice and rats. Adh3 is the only ADH class investigated that was found to be present in the rodent brain during embryogenesis. These data were in agreement with another in situ hybridization study (143) that demonstrated only the presence of Adh3 in the brains of adult mice, rats, and humans. The ubiquitous expression of Adh3 mRNA found in both humans and mice from the earliest stages examined suggests a housekeeping role for this gene. The important role of Adh3 in the detoxification of formaldehyde also strengthens this suggested housekeeping function (432).

Thus the metabolic clearance of methanol and formaldehyde in the central nervous system and the embryo occurs via two strategies. The first strategy aims to prevent the oxidation of methanol, i.e., preventing in situ formaldehyde formation. In accordance with this strategy, there is no ADH1 activity in the brain and in embryos, thereby preventing the creation of endogenous formaldehyde, i.e., formaldehyde synthesized in situ; furthermore, any methanol introduced through the BBB is made nontoxic by physiological clearance. The second strategy involves the oxidation of any formaldehyde in the brain and in embryos that is synthesized in situ or inserted into bloodstream by enzymes, including FDH and ALDH2.

VI. METHANOL/FORMALDEHYDE AND HUMAN PATHOLOGY

This section discuss the results of studies pointing to the possible involvement of metabolic methanol and formaldehyde in human pathology. We believe that the mechanisms of human poisoning by exogenous methanol, as well as the practical therapies in cases of accidental methanol intake, cannot be ignored.

A. Formaldehyde Neurotoxicity

Formaldehyde easily penetrates cell membranes, possesses neurotoxic potential, and causes deficits in memory and learning (386, 425, 466, 472). High levels of exogenous formaldehyde without a concurrent elevation in the capacity to clear formaldehyde raise the formaldehyde levels in the body and lead to formaldehyde stress (171b, 472). Although low concentrations of metabolic formaldehyde cannot cause acute toxicity in brain cells, experiments on cultured cells have provided some idea of how the metabolism in the brain is changed by the putative impact of formaldehyde. It has been shown that the metabolism of cultured astrocytes is altered after formaldehyde exposure (471). Although the cells efficiently oxidized formaldehyde to formate, likely by using ALDH2 and FDH, enhanced glycolytic flux was observed due to the inhibition of mitochondrial respiration. In cultured cerebellar granule neurons exposed to formaldehyde, a high rate of formaldehyde oxidation accompanied by significant increases in the cellular and extracellular formate concentrations increased glucose consumption, and lactate release strongly accelerated the export of the antioxidant glutathione (473). Hydrogen sulfide (H_2S) is considered the third gaseous neuromediator, along with nitric oxide and carbon monoxide (327, 500, 501). In the mammalian brain, H_2S is formed from the amino acid cysteine by the action of cystathionine β-synthase, the key enzyme in the transsulfuration pathway that

processes homocysteine, which induces neurotoxicity (449) and defects in the learning and memory of rats (277). In Alzheimer's disease brains, H_2S was found to be decreased, but homocysteine was increased (128). Formaldehyde has also been shown to decrease endogenous H_2S generation and to induce neurotoxicity and intracellular ROS accumulation in PC12 cells derived from rat adrenal medulla pheochromocytoma (448). The interaction of formaldehyde with H_2S was the trigger of learning and memory dysfunctions in rats (448).

B. Formaldehyde and Neurodegenerative Diseases

Although the concentration of formaldehyde is low in the blood, it is high in the brain, suggesting that formaldehyde plays roles in brain function and dysfunction (441). There is a direct correlation between elevated formaldehyde levels in the brain and memory impairment (463, 464, 466); it is currently unclear whether formaldehyde or a disruption in gene expression is the cause of the pathogenesis of the mental disease. Notably, brain formaldehyde concentrations were significantly elevated to pathological levels (~0.5 mM) in aged rats. It has been suggested that excess formaldehyde is associated with a global decline DNA methylation (DNA demethylation) in the hippocampus during aging (464). Intrahippocampal injection with excess formaldehyde induces memory deficits in adult rats. Furthermore, intrahippocampal injection with excess formaldehyde (0.5 mM) not only obviously impaired the ability of spatial learning in the adult rats but also damaged the memory abilities of the rats (464, 465). In humans, a correlation was observed between the degree of senile dementia and the levels of urine formaldehyde in different patients (466). Moreover, brain formaldehyde was increased in Alzheimer's disease patients (463, 466) and animal models (365, 386). The cortex and hippocampal formaldehyde levels of Alzheimer's disease patients were significantly higher than those of age-matched controls or young people, reaching ~0.5 mM (463). Interestingly, regularly drinking water can decrease endogenous formaldehyde. Furthermore, this activity can mitigate age-related cognitive impairment (460).

Highly reactive formaldehyde can be both a cause and a consequence of pathological processes in humans. To understand the mechanism of its pathological effects on brain cells, the chemical properties of formaldehyde must be taken into account. The polarized carbonyl group of formaldehyde reacts with the amine group of lysine or arginine, forming Schiff bases and producing irreversibly covalently cross-linked complexes between proteins, as well as between proteins and single-stranded DNA (73, 155, 171b, 307, 432, 534). Formaldehyde, which is found at elevated levels in Alzheimer's disease (386, 439, 448, 450, 463, 464, 517, 518), may play important roles in β-amyloid (Aβ) aggregation and cerebral amyloid angiopathy related to Alzheimer's disease pathology (319, 421, 428, 478, 525). The potential implications of endogenous aldehydes in Aβ misfolding, oligomerization, and fibrillogenesis was confirmed by in vitro experiments in which formaldehyde was capable not only of enhancing the rate of formation of Aβ oligomers and Aβ-APOE protein complexes but also of increasing the size of the aggregates (72, 73, 394). In line with this hypothesis, the overexpression of SSAOs, which were colocalized with Aβ deposits, was detected in the cerebrovascular tissue of patients with Alzheimer's disease (210, 478).

Another biomarker of Alzheimer's disease is neuronal tau, an important protein in the promotion and stabilization of the microtubule system that is involved in cellular transport and neuronal morphogenesis (429). Whereas tau normally contains 2–3 mol of phosphates per mole, tau phosphorylation levels in Alzheimer's disease brains are three- to fourfold higher. Formaldehyde at low concentrations induces tau polymerization into globular amyloid-like aggregates in vitro and in vivo (191, 344). As shown in the study of mouse neuroblastoma (N2a) cells treated with formaldehyde, tau hyperphosphorylation and DNA damage occurred (296). Tau became hyperphosphorylated not only in the cytoplasm but also in the nucleus of mouse brains (292). Chronic methanol feeding led to pathological changes that were related to Alzheimer's disease development, including tau hyperphosphorylation in the brains of mice (517) and rhesus macaques, non-human primates (518).

Indirect evidence of the involvement of formaldehyde in the pathogenesis of Alzheimer's disease was obtained by investigating ALDH2 activity, a key enzyme that oxidizes aldehydes, including formaldehyde. The hypothesis that a decrease in ALDH2 activity contributes to Alzheimer's disease was supported by studies of transgenic mice with low ALDH2 activity; these mice exhibited age-dependent neurodegeneration and memory loss (352).

In humans, ALDH2 dysfunction is likely to be one of the risk factors for Alzheimer's disease. The ALDH2*2 allele is a risk factor for late-onset Alzheimer's disease. Approximately 0.6–1% of the Japanese population are estimated to belong to the group with a combination of ALDH2*2 genotypes, and nearly all of these people are expected to develop Alzheimer's disease (221, 352). A Chinese survey confirmed these data (498).

C. Formaldehyde and Hangover

Methanol and formaldehyde are known to participate in the development of a hangover, known for unpleasant sensations and uncomfortable "morning after" symptoms in people following excessive ethanol intake (373, 377). Hangovers after binge drinking are likely more common in the young than in older aged persons (462). Among hang-

over causes, which include imbalances in the immune system (373), effects of dehydration such as headaches (415) and sleep disturbances, acetaldehyde accumulation, dysregulated cytokine pathways, and hormonal alterations (487, 510), the metabolism of methanol and production of formaldehyde are likely the most important (28, 446). The first support for the contribution of methanol to hangovers comes from data showing that brandies and whiskeys, which are more frequently associated with the development of a hangover, contain the highest methanol concentrations. The first excellent study of methanol metabolism and hangover found that methanol accumulated in the blood of alcoholic subjects during a 10- to 15-day period of chronic ethanol intake (299). The disappearance of blood methanol lagged behind the linear disappearance of ethanol by ~6–8 h, and complete clearance of blood methanol took several days. Importantly, the accumulation and clearance patterns of methanol and ethanol were similar in subjects who consumed either whisky (bourbon) with a high methanol content or grain alcohol with low methanol content. The authors suggested that methanol accumulates in the blood as a result of the well-known competitive inhibition of ADH by ethanol (263) and the presence of endogenous methanol (413), which may contribute to the hangover severity (299). An experimental study with healthy subjects who consumed red wine containing 100 mg/l of methanol also showed that elevated blood methanol levels persisted for several hours after the ethanol was metabolized and that this corresponded to the time course of hangover symptoms (212, 213). The half-life of methanol in healthy men during a hangover was estimated to be 142 min. This indicates that elevated methanol concentrations in the blood persist for ~12 h (213). The author suggested that methanol lingers after ethanol levels drop because ethanol competitively inhibits methanol metabolism. Ethanol readministration ("hair-of-the-dog" drinking) (377) fends off the hangover effects that may be based on the ability of ethanol to block methanol metabolism, thereby slowing the production of formaldehyde and formic acid (213, 413).

D. Effect of Alcoholic Beverages on the Cardiovascular System: U-Shape

The consumption of ethanol has an effect on the cardiovascular system in humans and can cause coronary heart disease (CHD) (333, 355). A plethora of epidemiological evidence has demonstrated a J- or U-shaped association between alcoholic beverages consumption and all-cause mortality, as well as cardiovascular morbidity and mortality. On the other hand, moderate alcoholic beverages consumption was inversely associated with CDH mortality (144, 147, 241, 305, 335, 337, 366, 402). According to the Dietary Guidelines for Americans (314), moderate alcoholic beverages consumption is considered an intake of no more than 1 drink per day for women and no more than 2 drinks per day for men, where 1 drink is equal to ~12 g ethanol. Moderate ethanol consumption also has a beneficial effect in reducing the risk of vascular disorders of the brain. Heavy alcoholic beverage consumption increases the relative risk of any type of stroke, whereas light or moderate ethanol consumption may be protective against ischemic stroke (241, 366). The mechanism of this phenomenon is not completely understood. Numerous hypotheses have been proposed to explain the benefit of light-to-moderate ethanol intake on the heart and brain, including an increase of high-density lipoprotein cholesterol (47) and the promotion of antioxidant effects with the participation of a protein kinase B/nuclear factor (erythroid-derived 2)-like 2-dependent mechanism (77, 241, 494). A recent study also suggested the involvement of the *ADH1b* gene in the U-shaped curve. A survey of over 260,000 individuals showed that carriers of the *rs1229984 A*-allele (*ADH1B*2*, **TABLE 3**) with a 100-fold increase in β2-ADH turnover (215, 308) consumed less ethanol and had a reduced frequency of coronary heart disease compared with noncarriers of this allele (184). In the search for mechanisms to explain the U-shaped relationship between ethanol consumption levels and coronary heart disease, researchers have focused on methanol and its oxidation product, formaldehyde. Recently, formaldehyde was hypothesized to participate in the process (322). The proposed mechanism relies on the fact that the common ADH1b enzyme carries out one-phase catabolism of methanol and ethanol. Moderate ethanol consumption competitively inhibits the conversion of methanol to formaldehyde, thus reducing the endogenous formaldehyde content in the organs. Another possible mechanism for the beneficial effects of moderate ethanol intake was indicated by the results of a study in rats showing that ALDH2 oxidizes aldehydes, including formaldehyde, and may serve as a potential endogenous neuroprotective target and a promising therapeutic strategy for the management of stroke (438). Ethanol administration activated ALDH2 and enhanced the detoxification of aldehydes (161). The study of ALA also indicated that ALDH2 participates in the formaldehyde and acetaldehyde detoxification process (116, 171a, 285, 310).

E. Methanol Fatalities and Antidote Therapy

Many cases of poisoning that result in fatalities are caused by methanol ingestion. When methanol was discovered, nothing was known about the toxicity of its metabolites in humans; thus the use of methanol as a substitute for ethanol became widespread in industry (453). Quickly thereafter, the dangers of methanol intoxication through skin absorption and inhalation became apparent (311), as numerous cases of industrial poisoning were reported (263). Moreover, as the odor and appearance of methanol are almost the same as those of ethanol, these two alcohols were often confused, and methanol was used instead of ethanol as a beverage, thereby increasing the cases of poisonings and fatalities.

Today, methanol is more widely used in industry, but the number of industrial poisonings are reduced due to the existence of the necessary precautions and safety instructions. Nevertheless, methanol poisoning and fatal cases remain a problem. These cases are mainly due to the intended or unintended ingestion of "surrogate alcohols," namely, non-beverage alcohol (e.g., industrial spirits, fire -liquids, windshield washer liquid, antifreeze), moonshine, homemade alcohol, samogon, etc. (258). Cases of homicide and suicide with methanol-containing liquids have also been reported (214). Fewer cases are related to methanol intoxication through percutaneous methanol absorption or vapor inhalation (316a).

The minimal dose of methanol that could lead to a lethal outcome without adequate treatment is currently considered to be ~1 g/kg (396), and 10–15 ml may cause blindness (316). According to the literature, the toxic effects of methanol could be observed after the ingestion of 15 ml of 40% methanol (29) to 600 ml of pure methanol (253). These significantly different results could be explained by insufficient and incorrect data regarding the amount of alcohol consumed; usually, the ingested volume is self-reported by the patients or is indirectly obtained. Moreover, the important role of different factors associated with the ingestion of methanol, including joint ethanol-methanol consumption, for example, should not be underestimated. Furthermore, the variability of toxic or lethal doses is dependent on the general state of health, diet, and the amount of tetrahydrofolate, which is known to take part in methanol metabolism, of the organism (396, 453).

Upon ingestion or inhalation, methanol initially has a narcotic effect as ethyl alcohol, followed by an asymptomatic period of ~10–12 h. After this period, methanol metabolites may cause nausea, vomiting, dizziness, headaches, respiratory difficulty, abdominal pain, visual disturbances, and metabolic acidosis (396). The main consequences of methanol intoxication are metabolic acidosis, hyperosmolality, formic acid, lactic acid, formaldehyde and ketones accumulation in the organism, and retinal damage with blindness (253). The high level of formic acid (formate) is believed to be the main cause of the onset of the clinical signs of methanol poisoning (23). Formate accumulates in bodily fluids and tissues, leading to metabolic acidosis and blood bicarbonate depletion (409). Formate inhibits mitochondrial cytochrome oxidase, resulting in tissue hypoxia; this effect is enhanced when the pH is low. Notably, formate injection per se leads to optic disk damage, independently of acidosis; the ocular effects associated with methanol poisoning appear to be due to hypoxia in areas of the cerebral and distal optic nerves (276). One study directly demonstrated this by intravenously injecting monkeys with formate at concentrations similar to those observed in methanol poisoning. The blood formate levels in these animals were constantly elevated for 24–48 h, whereas the blood pH maintained at physiological levels. All animals developed ocular toxicity within 24 h, and its symptoms were the same as those of methanol poisoning. Thus ocular toxicity is caused by formate but not by methanol or acidosis (306). Further studies showed that acidosis correction did not prevent ocular toxicity.

One of the first massive methanol poisoning outbreaks to be documented was in the 1940s. During the Second World War, more than 100 fatal cases were registered in the German army. Lachenmeier (258) summarized further outbreaks of methanol poisoning from surrogate alcohols that have been reported in the scientific literature since the 1950s.

Modern toxicology handbooks recommend treating the consequences of methanol intoxication when blood methanol concentrations are more than 0.2 g/l in a nonacidotic patient. However, it is likely that this threshold was chosen arbitrarily (251), as this approach does not take into account the time passed since the moment the methanol-containing liquid was ingested. For example, if the blood methanol content is 0.2 g/l 12 h after ingestion, then the peak level 1 h after ingestion could be estimated as more than 1 g/l. The same concentration of 0.2 g/l measured 24 or 48 h after ingestion corresponds to peak levels of 2.15 or 4.2 g/l, respectively (251). According to this approach, the amount of ingested methanol might be significantly underestimated, which could lead to improper treatment. Thus the time and circumstances of the ingestion should be taken into account whenever possible. The levels of blood formate should also be assessed because the relative concentrations of methanol and formate in the blood are functions of the amount of methanol ingested. Different methods, including enzymatic measurements and gas chromatography, for formic acid analysis in both ante-mortem and postmortem blood samples were used to provide additional information to assist with the interpretation of methanol fatalities (190, 495). The best picture of toxic and lethal blood methanol concentrations was obtained from the analysis of postmortem methanol and formate levels. Blood methanol and formate contents estimated ante-mortem and postmortem were shown to be very close. The case report study performed by Jones (214) analyzed postmortem methanol and formate concentrations in 73 cases; blood methanol levels varied significantly, ranging from 0.7 to 7.9 g/l, whereas formate concentrations showed a more narrow range between 0.53 and 1.40 g/l. In 97% of cases, the formate concentrations ranged from 0.60 to 1.10 g/l. Similar ranges were detected by Wallage (495). Another study of 15 cases of methanol poisoning (no fatalities included) reported approximate blood methanol levels ranging from 0.14 to 4.5 g/l and formate levels ranging from 0.01 to 1.48 g/l. The authors also noted that there was no correlation between methanol and formate levels. Nonetheless, the levels of both com-

pounds are strongly recommended to be monitored in hospitals for patients with methanol poisoning (190).

Methods of treatment for methanol poisoning are highly dependent on how quickly treatment is initiated and the stage of poisoning. Gastric lavage, vomiting induction, and the use of activated charcoal can only be conducted within 1 h after methanol ingestion (253); after 1 h, the methanol is fully absorbed by the gastrointestinal tract. For those patients, therapy with inhibitors of subsequent methanol metabolism can be applied. After methanol is rapidly and completely absorbed by the gastrointestinal tract, it penetrates all bodily fluids. With the participation of liver ADH, methanol then is metabolized to formaldehyde and formic acid. The following methods can be applied as therapies for methanol poisoning (399): *1*) intravenous bicarbonate injection to reduce metabolic acidosis; *2*) the use of antidotes such as ethanol and fomepizole (4-methylpyrazole) to terminate the conversion of methanol to formic acid; and *3*) hemodialysis to remove methanol and formic acid from the blood, used for uncomplicated poisoning with methanol (316).

Therapy with the use of ethanol or fomepizole can be used in cases in which the time of methanol intake is known and the methanol levels in the blood rise above 10–20 mg/dl or the osmolal gap is >10 mosmol/kgH_2O. In cases in which the time of methanol consumption is not known, any two of the following criteria should be present: a bicarbonate concentration in the blood serum of <20 meq/l, an arterial hydrogen rate (pH) less than 7.3, and an osmolal gap of >10 mosmol/kgH_2O (22, 87, 227, 230). Treatment with ethanol is the most accessible, due to cost effectiveness and ease of use (peroral or intravenous), but to use ethanol as an antidote, high serum levels of 100 mg/dl must be maintained. Thus it is not recommended for patients with liver disease and stomach ulcers. Additionally, ethanol should be used with caution in patients who use drugs that suppress the central nervous system because ethanol may exacerbate their condition (1, 23, 253).

Fomepizole, on the other hand, has a higher affinity for ADH than ethanol (by close to 1,000 times) and can completely inhibit ADH1b at significantly lower serum concentrations (46, 316). Fomepizole concentrations of more than 0.8 mg/l (10 mM) in the serum provide permanent inhibition of ADH1b (23). For patients not undergoing hemodialysis, the recommended doses of fomepizole are known (392): an initial dose of 15 mg/kg, followed by 10 mg/kg every 12 h, and then an increased dose of 15 mg/kg at 48 h, followed by another 15 mg/kg dose after 12 h.

For patients undergoing hemodialysis, two options have been proposed for fomepizole administration. In the first case, the dosage remains the same as for patients not undergoing hemodialysis but with a reduced time interval between successive doses: the second dose is given 6 h after the first dose, and subsequent doses are given every 4 h. In the second case, continuous intravenous infusion of 1.0–1.5 $mg \cdot kg^{-1} \cdot h^{-1}$ fomepizole is performed after the primary dose. However, the use of fomepizole to treat methanol poisoning is not possible for all patients at every clinic. Most people with methanol poisoning are alcoholics who cannot afford such an expensive drug; thus ethanol is more commonly used in such cases (253, 392).

VII. GENES INVOLVED IN ENDOGENOUS METHANOL CATABOLISM AND HUMAN PATHOLOGY

A. Cluster of Genes Involved in Endogenous Methanol Catabolism

Carbon dioxide and nitrogen oxide are signaling molecules with small molecular weights that are widespread in the environment. Methanol might be another example of a small regulator of gene expression. Recently, a number of genes were shown to undergo expression changes due to increased methanol levels in the plasma of humans and mice. Most of the genes affected by methanol fluctuation are involved in ethanol metabolism (*ADH1, ALDH2, GSTO1, MGST3, GSTP1*, *CYP2E1*), Alzheimer's disease pathogenesis (*PSENEN, APOE, SNCA, MME*), and hemoglobin production (*HBB2, HBA1*) (246, 413). Although ethanol was known to alter the gene expression of alcohol-metabolizing enzymes in mammals, our data reveal the signaling function of methanol or formaldehyde in mammals for the first time. There are many examples, including formaldehyde, in which a product or substrate of a reaction can affect the gene expression of an enzyme. Alcohol oxidase from the methylotrophic yeast *Pichia pastoris* was shown to undergo expression changes in the presence of methanol (482a). Further investigation revealed a formaldehyde-sensitive transcriptional factor that can alter the expression of the methanol-oxidizing enzymes in yeasts (65, 374). At the same time, increased methanol in the blood of mice led to overexpression of the brain genes involved in formaldehyde clearance (*Aldh2*, *Gsto1*, *Mgst3*, *Gstp1*) and downregulation of *Adh*1, which participates in formaldehyde formation (246, 413). These changes in gene expression of brain tissue in mice may control toxic formaldehyde concentrations. However, *Cyp2E1*, which oxidizes ethanol and methanol to aldehydes, was upregulated (246). Thus methanol or formaldehyde can alter the expression of the genes involved in methanol oxidation.

B. Alcoholism

Polymorphisms in genes that affect ethanol utilization are of particular interest in heavy drinkers. Mutations in *ADH* and *ALDH* genes that affect the enzymatic activity and

protein structure could be associated with the risk of alcoholism. Twin surveys revealed that the genetic influence on the development of alcoholism was as important as that of environmental factors (172, 173). Polymorphisms in enzymes such as ADH1b and ALDH2 that metabolize ethanol are mostly associated with alcoholism. Genotypes with *ADH1* and *ALDH2* alleles that lead to greater acetaldehyde accumulation are protective against excessive ethanol consumption. For example, the *ADH1B*2* (*Arg48His, rs1229984*) and *ADH1B*3* (*Arg370Cys, rs2066702*) variants are able to oxidize ethanol to acetaldehyde much faster (122) and are associated with a protective effect against alcoholism in East Asian (122), African American (121), and American Indian populations (372, 496).

Another way to increase acetaldehyde formation is to block the activity of ALDH2, which is the primary enzyme that oxidizes acetaldehyde to acetic acid. East Asian populations with inactive ALDH2 accumulate acetaldehyde to a greater extent while drinking, which confers a protective effect against ethanol dependence (182). Conversely, carriers of the SNPs in the *ADH4* promoter were found to have a higher risk of alcohol dependence (158, 294). Edenberg et al. (123) demonstrated that one of the SNPs (−75A) increased the expression of corresponding gene compared with the −75C variant (123). Individuals with cysteine at the −75 position are three times more likely to develop ethanol dependence (158).

C. Neurodegenerative Diseases

Thus far, the scheme of Alzheimer's disease pathogenesis is incomplete; however, there is strong evidence that oxidative stress triggers and participates in Alzheimer's disease development. Mitochondrial dysfunction is strongly associated with oxidative stress and neurodegenerative disorders, including Alzheimer's disease (279). Thus changes in mitochondrial proteins with antioxidative stress implications may be involved in neurodegenerative diseases. Examples include the cytochrome *c* oxidizing complex, manganese superoxide dismutase, and catalase and ALDH2, two enzymes that are important in alcohol metabolism. Whereas catalase mainly oxidizes ethanol and methanol at high concentrations, ALDH2 is a central protein in the oxidation of relational aldehydes. One of the major polymorphisms of *ALDH2* is the substitution of glutamate at the 487th position by lysine. This variant, *ALDH2*2*, is widespread among the Japanese population, in which up to 30% of people are heterozygous (447). In individuals who carry at least one *ALDH2*2* allele, a lower K_m results in higher acetaldehyde concentrations, whereas *ALDH2*2* homozygotes show no ALDH2 activity (447). Because ALDH2 localizes to the mitochondrial matrix and is implicated in the oxidation of aldehydes generated in oxidative stress, ALDH2 was proposed to play a role in Alzheimer's disease pathogenesis. The low activity of ALDH2*2 causes the accumulation of highly toxic 4-hydroxy-2-nonenal (4-HNE), which was shown to cause neuronal death (254) and was observed in Alzheimer's and Parkinson's disease patients (325, 404, 522). Analysis of the *ALDH2*2* polymorphism in the 472 Alzheimer's disease patients and 472 nondemented controls revealed a small but statistically significant difference in the frequency of the allele (48.1 and 37.4%, respectively) (221). Interesting, APOE-ε4 homozygotes carrying at least one ALDH2*2 allele have a 31-fold higher chance of developing Alzheimer's disease than those with neither allele (352). Because both proteins control the concentration of 4-HNE, the dysfunction of *ALDH2*2* dramatically increases the levels of 4-HNE. While the apolipoprotein E-ε4 (APOE-ε4) polymorphism is found in more Alzheimer's disease patients compared with healthy individuals than the *ALDH2*2* allele, the coexistence of both alleles dramatically increases the probability of developing Alzheimer's disease. Thus *ALDH2*2* itself is not a strong prognostic factor for Alzheimer's disease, but dramatically increases the risk of the Alzheimer's disease development in *APOE-ε4* homozygotes (352).

D. Cardiovascular Disease

Recent data revealed a crucial protective role of *ALDH2* in ischemic injury. ALDH2 is activated by protein kinase C-ε via phosphorylation (71) and then detoxifies poisonous aldehydes such as 4-HNE and acetaldehyde (36, 88). Because 4-HNE can trigger necrotic cell death in the reperfused myocardium (338) and blocks important metabolic proteins such as glyceraldehyde 3-phosphate dehydrogenase (475), Na^+-K^+-ATPase (417), and the 20S proteasome (134), its deactivation by ALDH2 has a cytoprotective effect in ischemic patients. Additionally, ALDH2 participates in the bioactivation of nitroglycerin, leading to increased blood flow (321). Based on these data, ALDH2 is a promising target for the treatment of ischemic injury by activators such as ALDH2 activator 1 (Alda-1) and ALA (71, 171a). Alda-1 not only increases ALDH2 levels and restores ALDH2*2 activities (53), but also prevents 4-HNE-mediated blocking the function of ALDH2 in ischemic conditions (71). Undoubtedly, the mechanisms regulating the transcription, translation, and enzymatic activity of ALDH2 require further investigation.

E. Tumoral Diseases

ADH1 and ALDH1 can oxidize a variety of the alcohol metabolites, including aliphatic alcohols and retinol (vitamin A). ADHs and ALDHs are implicated in developmental, apoptotic, metabolic, and signaling processes. The dysfunction of ADHs and ALDHs on different levels can lead to or be associated with different disorders. Due to the crucial role of ADH1 and ALDH1 in the retinoic acid pathway, this pathway is of particular interest. The retinoic acid

pathway controls the processes of differentiation, proliferation, and apoptosis and was found to participate in the development of various tumors (132, 280, 339).

1. Uterine fibroid

Among women, uterine fibroid (UF) is a widespread benign tumor of the smooth muscle cells that rarely transforms into malignant leiomyosarcoma. Due to uncontrolled fibroid growth, UF can cause various pains, frequent urination, and infertility in 1.0–2.4% of patients (109). Molecular factors involved in UF development include retinoic acid, insulin growth factor 2, transforming growth factor β pathways, and extracellular matrix formation. In UF samples, *ADH*, *ALDH1, CRBP1, RXR*α, and *RXR*γ mRNA and protein levels are significantly lower than in healthy controls (93, 529). ADH1 and ALDH1 oxidize retinol to retinal and retinoic acid; thus decreased RA levels are observed in UF samples. The reduced concentration of retinoic acid could lead to typical tumor characteristics: decreased differentiation and apoptosis together with increased proliferation (529). Thus ADH and ALDH may play an important role in UF development and could be targets in UF treatment.

2. Head and neck cancer

Acetaldehyde is a carcinogenic metabolite, and its concentration is controlled by the enzymatic activities of ADHs, ALDHs, catalase, and CYP2E1 (49). Because the main source of acetaldehyde is ethanol consumption, this carcinogen mostly affects the upper aerodigestive tract (408). Variants of the *ADH* and *ALDH* genes that lead to changes in the activities of their protein products are associated with the development of head and neck, esophageal, and liver cancers. Although variants that produce acetaldehyde in greater concentration would be expected to increase the risk of cancer, this is not always the case. Homozygotes for the *ADH1B*2* (Arg48His; *rs1229984*) and *ADH1C*1* (Arg272Gln; *rs1693482*) allele variants were shown to produce 40- and 2.5-fold more acetaldehyde from ethanol, respectively (39). However, meta-analyses revealed an inverse correlation between the head and neck cancer risk and carrying the variants of ADH1C and ADH1B that metabolize acetaldehyde faster (68). The authors provide three possible explanations for this unexpected finding: *1*) a decreased opportunity for the oral microflora to produce acetaldehyde locally by the prolonged systemic circulation of ethanol, *2*) the prevention of ethanol acting as a solvent for other carcinogens, and *3*) decreased amounts of ethanol consumption due to the discomfort caused by the consequent peak in systemic acetaldehyde. We propose that formaldehyde might also affect the results: large concentrations of formaldehyde can be found in human body after alcoholic beverage consumption, and formaldehyde is known to have a carcinogenic effect. Although little evidence is available regarding formaldehyde causing NPC (445), studies have demonstrated that chronic exposure to formaldehyde causes squamous cell carcinoma in rats (323, 324, 444).

3. Colorectal cancer

ADH1 and *ALDH* may contribute to colorectal cancer (CC). Biochemical tests reveal increased ADH1 activity in CC samples compared with healthy controls (205). Moreover, ALDH activity in cancer cells is slightly lower than in healthy tissues; the ratio between ADH1 and ALDH activities is 20.5:1.0 in cancer cells and 10:1 in healthy cells. Acetaldehyde seems to affect the relationship between the ethanol-oxidizing system and CC. In CC cells, carcinogenic acetaldehyde accumulates roughly twofold faster than in healthy cells, indicating that acetaldehyde may participate in the progression of CC. Additionally, ADH1 activity in the sera of CC patients is increased and rises with the stage of cancer (206).

VIII. CONCLUDING COMMENTS

Over the past decade, significant progress has been made in elucidating the functions and physiological roles of human metabolic methanol. Moreover, recent advances in the field of metabolic methanol research have provided insights into the mechanisms by which low levels of formaldehyde are maintained in human plasma. However, numerous aspects of metabolic methanol synthesis and the regulation of methanol-metabolizing genes are not understood.

Methanol is an integral and inevitable component of human life. In the morning woods (193) or on a mowed meadow (95a, 230, 505), inhaled air features a noticeable content of plant methanol vapors. The blood of fasting individuals contains small amounts of methanol and its oxidative product, formaldehyde; the levels of these molecules increase sharply even after the ingestion of vegetable pectin (112, 413). There are several metabolic sources of methanol **(FIGURE 1)**. In addition to fruit and vegetables, methanol can be consumed in soft drinks containing aspartame and alcoholic beverages, which contain methanol as a product of the fermentation of vegetative raw materials with PME. In addition to the processes of methylation-demethylation, an important source of metabolic methanol is the intestinal microflora. The detection of methanol even after inhibition of liver ADH1b by 4-methylpyrazole (246, 403) or administration of ethanol-free methanol (413) clearly indicates the existence of internal sources of methanol.

The mechanisms of phase I of the catabolism of methanol share several features of ethanol catabolism. In humans, the oxidation of methanol and ethanol requires several stages of conversion. In the first stage, methanol is oxidized to formaldehyde in three ways. The first is via oxidation by CYP450s (57, 87, 495). The contribution of CYP450s to

the metabolism of methanol and ethanol in the liver is low (<9%) and increases at higher doses of methanol and ethanol. The second form of methanol oxidation occurs via the catalase-H_2O_2 complex (64, 97). Its contribution to the metabolism of methanol and ethanol in the liver is small (<1–2%) but increases at higher doses of methanol and ethanol. The third means of methanol oxidation involves ADH1b, which catalyzes up to 90% of methanol and ethanol oxidation in the liver (63, 168, 297). ADH1b activity has not been detected in brain extracts (143). The enzymatic reaction occurs in two stages. The first is the formation of the methanol/ADH1b/NAD^+ ternary complex with the participation of Zn. At this stage, methanol and ethanol enter the complex equally effectively; thus ethanol functions as an antidote for methanol poisoning (382). In the second stage, hydride transfer occurs with the formation of CHO + NADH + H^+. Because ADH1b is involved in both methanol and ethanol catabolism, ethanol functions as a powerful competitive inhibitor at low concentrations, and the enzyme has a strong preference for converting ethanol to acetaldehyde over converting methanol to formaldehyde.

Methanol itself is not toxic to human cells; however, its oxidative product, formaldehyde, is a toxin that is believed to play a role in carcinogenesis and age-related neuronal damage in the brain (472). Although formaldehyde is primarily produced by the oxidation of methanol, there are several sources of metabolic formaldehyde, including SSAO-mediated oxidative deamination and the removal of methyl groups from lysine residues in histones catalyzed by histone demethylases.

The second phase of methanol catabolism, i.e., the conversion of formaldehyde to formic acid, occurs via three mechanisms, including the participation of CYP2E1 (27, 183, 255, 289, 452), cytosolic AlDH1 and mitochondrial AlDH2 (142, 297, 452, 453, 473, 485), and FDH (432).

There are two mechanisms by which metabolic formaldehyde can be maintained at low levels. The first is to decrease ADH activity to prevent the oxidation of methanol to formaldehyde. The second method is the rapid and effective oxidation of formaldehyde to the end products carbon dioxide and water. These two methods are implemented in the brain and embryo, both of which are highly sensitive to formaldehyde. ADH activity is decreased or absent in the brain and the embryo (143), and increased FDH (432) and ALDH2 (472) activity oxidize formaldehyde introduced via the bloodstream or formed in situ.

In conclusion, human methanol and formaldehyde catabolism is complex, multilayered, and highly effective. Metabolic control can be achieved at the gene or protein level, i.e., at the level of enzyme activity. Methanol is not poisonous itself but rather acts as a signaling molecule that regulates the activity of a gene cluster involved in the maintenance of a low level of formaldehyde (112, 246, 413). The disruption of formaldehyde metabolism control may be a causative factor in neurodegenerative diseases. Low levels of formaldehyde have been associated with some human pathologies. Increased formaldehyde content in the blood and brain has been detected in neurological patients as well as in the blood of the elderly, suggesting a disruption of the genetic and biochemical mechanisms responsible for maintaining low formaldehyde levels (463, 466). To determine the beneficial or detrimental effects of methanol, we must also consider the favorable role of fruits and raw vegetables in human health. A vegetarian diet is the main source of exogenous methanol in a healthy individual (112). The role of methanol-generated pectin in atherosclerosis and cancer prophylaxis is well-known (389). It has been suggested that pectin can modulate detoxifying enzymes, stimulate the immune system, modulate cholesterol synthesis, and act as an antibacterial, antioxidant, or neuroprotective agent. There is also increasing evidence to suggest that regular fruit and vegetable consumption may play an important role in preventing or delaying the onset of dementia, age-associated cognitive decline, and Alzheimer's disease (127).

Thus advances in modeling and an analysis of human methanol metabolism will extend our knowledge of the role of methanol in health and disease, permitting the customization of existing and future therapeutic and prophylactic modalities.

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