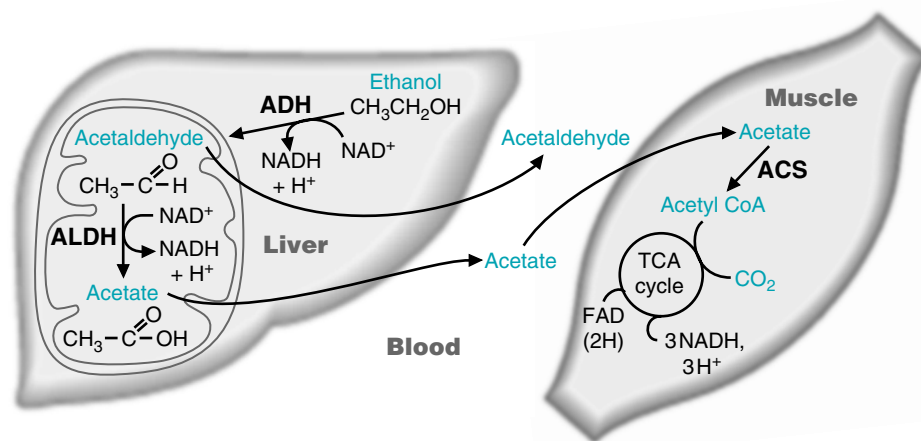# 25 Metabolism of Ethanol

*Ethanol is a **dietary fuel** that is metabolized to acetate principally in the liver, with the generation of NADH. The principal route for metabolism of ethanol is through hepatic **alcohol dehydrogenases**, which oxidize ethanol to **acetaldehyde** in the cytosol (Fig. 25.1). Acetaldehyde is further oxidized by **acetaldehyde dehydrogenases** to **acetate,** principally **in mitochondria**. Acetaldehyde, which is toxic, also may enter the blood. **NADH** produced by these reactions is used for adenosine triphosphate (ATP) generation through oxidative phosphorylation. Most of the acetate enters the blood and is taken up by skeletal muscles and other tissues, where it is activated to **acetyl CoA** and is oxidized in the TCA cycle.*

*Approximately 10 to 20% of ingested ethanol is oxidized through a microsomal oxidizing system **(MEOS)**, comprising **cytochrome P450** enzymes in the endoplasmic reticulum (especially **CYP2E1**). CYP2E1 has a high K_m for ethanol and is inducible by ethanol. Therefore, the proportion of ethanol metabolized through this route is greater at high ethanol concentrations, and greater after chronic consumption of ethanol.*

*__Acute effects__ of alcohol ingestion arise principally from the generation of NADH, which greatly increases the **NADH/NAD$^+$** ratio of the liver. As a consequence, **fatty acid oxidation is inhibited**, and **ketogenesis** may occur. The elevated **NADH/NAD$^+$** ratio may also cause **lactic acidosis** and inhibit **gluconeogenesis**.*

*Ethanol metabolism may result in **alchohol-induced liver disease**, including **hepatic steatosis** (fatty liver), **alcohol-induced hepatitis**, and **cirrhosis**. The principal toxic products of ethanol metabolism include **acetaldehyde** and **free radicals**. Acetaldehyde forms **adducts** with proteins and other compounds. The **hydroxyethyl radical** produced by MEOS and other radicals produced during*

Fig. 25.1. The major route for metabolism of ethanol and use of acetate by the muscle. (ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; ACS, acetyl-CoA synthetase).

*inflammation cause irreversible damage to the liver. Many other tissues are adversely affected by ethanol, acetaldehyde, or by the consequences of **hepatic dysmetabolism** and injury. **Genetic polymorphisms** in the enzymes of ethanol metabolism may be responsible for individual variations in the development of alcoholism or the development of liver cirrhosis.*

THE WAITING ROOM

A dietary history for **Ivan Applebod** showed that he had continued his habit of drinking scotch and soda each evening while watching TV, but he did not add the ethanol calories to his dietary intake. He justifies this calculation on the basis of a comment he heard on a radio program that calories from alcohol ingestion "don't count" because they are empty calories that do not cause weight gain.

Al Martini was found lying semiconscious at the bottom of the stairs by his landlady when she returned from an overnight visit with friends. His face had multiple bruises and his right forearm was grotesquely angulated. Nonbloody dried vomitus stained his clothing. Mr. Martini was rushed by ambulance to the emergency room at the nearest hospital. In addition to multiple bruises and the compound fracture of his right forearm, he had deep and rapid (Kussmaul) respirations and was moderately dehydrated.

Initial laboratory studies showed a relatively large anion gap of 34 mmol/L (reference range = 9–15 mmol/L). An arterial blood gas analysis confirmed the presence of a metabolic acidosis. Mr. Martini's blood alcohol level was only slightly elevated. His serum glucose was 68 mg/dL (low normal).

The anion gap is calculated by subtracting the sum of the value for serum chloride and for the serum HCO_3^- content from the serum sodium concentration. If the gap is greater than normal, it suggests that acids such as the ketone bodies acetoacetate and β-hydroxybutyrate are present in the blood in increased amounts.

Jean Ann Tonich, a 46-year-old commercial artist, recently lost her job because of absenteeism. Her husband of 24 years had left her 10 months earlier. She complains of loss of appetite, fatigue, muscle weakness, and emotional depression. She has had occasional pain in the area of her liver, at times accompanied by nausea and vomiting.

On physical examination she appears disheveled and pale. The physician notes tenderness to light percussion over her liver and detects a small amount of ascites (fluid within the peritoneal cavity around the abdominal organs). The lower edge of her liver is palpable about 2 inches below the lower margin of her right rib cage, suggesting liver enlargement, and feels somewhat more firm and nodular than normal. Jean Ann's spleen is not palpably enlarged. There is a suggestion of mild jaundice. No obvious neurologic or cognitive abnormalities are present.

After detecting a hint of alcohol on Jean Ann's breath, the physician questions her about possible alcohol abuse, which she denies. With more intensive questioning, however, Jean Ann admits that for the last 5 or 6 years she began drinking gin on a daily basis (approximately 4–5 drinks, or 68–85 g ethanol) and eating infrequently. Laboratory tests showed that her serum ethanol level on the initial office visit was 245 mg/dL (0.245%). A serum ethanol level above 150 mg/dL (0.15%) is considered indicative of inebriation.
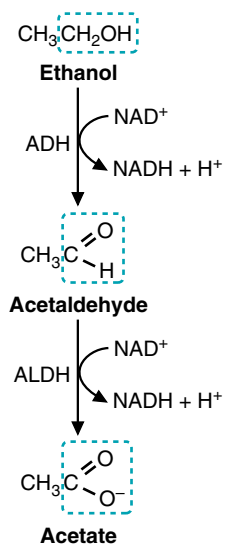
Jaundice is a yellow discoloration involving the sclerae (the "whites"' of the eyes) and skin. It is caused by the deposition of bilirubin, a yellow degradation product of heme. Bilirubin accumulates in the blood under conditions of liver injury, bile duct obstruction, and excessive degradation of heme.
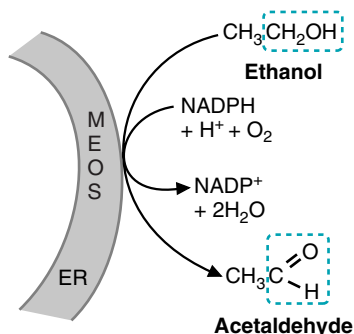
Jean Ann Tonich's admitted ethanol consumption exceeds the definition of moderate drinking. Moderate drinking is now defined as not more than two drinks per day for men, but only one drink per day for women. A drink is defined as 12 oz of regular beer, 5 oz of wine, or 1.5 oz distilled spirits (80 proof).

I. ETHANOL METABOLISM

Ethanol is a small molecule that is both lipid and water soluble. It is, therefore, readily absorbed from the intestine by passive diffusion. A small percentage of ingested ethanol (0-5%) enters the gastric mucosal cells of the upper GI tract (tongue, mouth,

CH_3CH_2OH
Ethanol

ADH, NAD$^+$ → NADH + H$^+$

$CH_3C \begin{smallmatrix} =O \\ -H \end{smallmatrix}$
Acetaldehyde

ALDH, NAD$^+$ → NADH + H$^+$

$CH_3C \begin{smallmatrix} =O \\ -O^- \end{smallmatrix}$
Acetate

Fig. 25.2. The pathway of ethanol metabolism (ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase).

CH_3CH_2OH
Ethanol

MEOS / ER

NADPH + H$^+$ + O$_2$ → NADP$^+$ + 2H$_2$O

$CH_3C \begin{smallmatrix} =O \\ -H \end{smallmatrix}$
Acetaldehyde

Fig. 25.3. The reaction catalyzed by MEOS (which includes CYP2E1) in the endoplasmic reticulum.

esophagus, and stomach), where it is metabolized. The remainder enters the blood. Of this, 85 to 98% is metabolized in the liver, and only 2 to 10% is excreted through the lungs or kidneys.

The major route of ethanol metabolism in the liver is through liver alcohol dehydrogenase, a cytosolic enzyme that oxidizes ethanol to acetaldehyde with reduction of NAD^+ to NADH (Fig.25.2). If it is not removed by metabolism, acetaldehyde exerts toxic actions in the liver and can enter the blood and exert toxic effects in other tissues.

Approximately 90% of the acetaldehyde that is generated is further metabolized to acetate in the liver. The major enzyme involved is a low K_m mitochondrial acetaldehyde dehydrogenase (ALDH), which oxidizes acetaldehyde to acetate with generation of NADH (see Fig. 25.2). Acetate, which has no toxic effects, may be activated to acetyl CoA in the liver (where it can enter either the TCA cycle or the pathway for fatty acid synthesis). However, most of the acetate that is generated enters the blood and is activated to acetyl CoA in skeletal muscles and other tissues (see Fig. 25.1). Acetate is generally considered nontoxic and is a normal constituent of the diet.

The other principal route of ethanol oxidation in the liver is the microsomal ethanol oxidizing system (MEOS), which also oxidizes ethanol to acetaldehyde (Fig. 25.3). The principal microsomal enzyme involved is a cytochrome P450 mixed-function oxidase isozyme (CYP2E1), which uses NADPH as an additional electron donor and O_2 as an electron acceptor. This route accounts for only 10 to 20% of ethanol oxidation in a moderate drinker.

Each of the enzyme activities involved in ethanol metabolism (alcohol dehydrogenase, acetaldehyde dehydrogenase, and CYP2E1) exist as a family of isoenzymes. Individual variations in the quantity of these isoenzymes influence a number of factors, such as the rate of ethanol clearance from the blood, the degree of inebriation exhibited by an individual, and differences in individual susceptibility to the development of alcohol-induced liver disease.

A. Alcohol Dehydrogenase

Alcohol dehydrogenase (ADH) exists as a family of isoenzymes with varying specificity for chain length of the alcohol substrate (Table 25.1). Ethanol is a small molecule that does not exhibit much in the way of unique structural characteristics and, at high concentrations, is nonspecifically metabolized by many members of the ADH family. The alcohol dehydrogenases that exhibit the highest specificity for ethanol are the class I alcohol dehydrogenases. We have three genes for class I alcohol dehydrogenases, each of which exists as allelic variants (polymorphisms).

Table 25.1. Isozymes of Medium-Chain-Length Alcohol Dehydrogenases

Class	Gene	Sub-Unit	Tissue Distribution	Properties
I	ADH 1 ADH 2 ADH 3	α β γ	Most abundant in liver and adrenal glands. Much lower levels in kidney, lung, colon, small intestine, eye, ovary, blood vessels. None in brain or heart	K_m of 0.05–4 mM for ethanol. Active only with ethanol. High tissue capacity.
II	ADH 4	π	Primarily liver, lower levels in GI tract	K_m of 34 mM for ethanol.
III	ADH 5	χ	Ubiquitously expressed, but at higher levels in liver. The only isozyme present in germinal cells.	Relatively inactive toward ethanol. Active mainly toward long-chain alcohols, and ω-OH fatty acids.
IV	ADH 7	σ	Present in highest levels in upper GI tract, gingiva and mouth, esophagus, down to the stomach. Not present in liver.	K_m of 28 mM. It is the most active of medium-chain alcohol DH toward retinal.
V	ADH 6	-	May be highest in fetal liver.	Some activity toward ethanol

The class I alcohol dehydrogenases are present in high quantities in the liver, representing approximately 3% of all soluble protein. These alcohol dehydrogenases, commonly referred to collectively as liver alcohol dehydrogenase, have low K_ms for ethanol between 0.05 and 4 mM (high affinities). Thus, the liver is the major site of ethanol metabolism and the major site at which the toxic metabolite acetaldehyde is generated.

Although the class IV and class II enzymes make minor contributions to ethanol metabolism, they may contribute to its toxic effects. Ethanol concentrations can be quite high in the upper GI tract (e.g., beer is approximately 0.8 M ethanol), and acetaldehyde generated here by class IV enzymes (gastric ADH) might contribute to the risk for cancer associated with heavy drinking. Class II ADH genes are expressed primarily in the liver and at lower levels in the lower gastrointestinal tract.

B. Acetaldehyde Dehydrogenases

Acetaldehyde is oxidized to acetate, with the generation of NADH, by acetaldehyde dehydrogenases (see Fig. 25.2). More than 80% of acetaldehyde oxidation in the human liver is normally catalyzed by mitochondrial acetaldehyde dehydrogenase (ALDH2), which has a high affinity for acetaldehyde and is highly specific. However, individuals with a common allelic variant of ALDH2 have a greatly decreased capacity for acetaldehyde metabolism

Most of the remainder of acetaldehyde oxidation occurs through a cytosolic acetaldehyde dehydrogenase (ALDH1). Additional aldehyde dehydrogenases act on a variety of organic alcohols, toxins, and pollutants.

C. Fate of Acetate

Metabolism of acetate requires activation to acetyl CoA by acetyl CoA synthetase in a reaction similar to that catalyzed by fatty acyl CoA synthetases (Fig. 25.4). In liver, the principle isoform of acetyl CoA synthetase (ACS I) is a cytosolic enzyme that generates acetyl CoA for the cytosolic pathways of cholesterol and fatty acid synthesis. Acetate entry into these pathways is under regulatory control by mechanisms involving cholesterol or insulin. Thus, most of the acetate generated enters the blood.

Acetate is taken up and oxidized by other tissues, notably heart and skeletal muscle, which have a high concentration of the mitochondrial acetyl CoA synthetase isoform (ACSII). This enzyme is present in the mitochondrial matrix. It therefore generates acetyl CoA that can directly enter the TCA cycle and be oxidized to CO_2.

D. Microsomal Ethanol Oxidizing System

Ethanol is also oxidized to acetaldehyde in the liver by the microsomal ethanol oxidizing system, which comprises members of the cytochrome P450 superfamily of enzymes. Ethanol and NADPH both donate electrons in the reaction, which reduces O_2 to $2H_2O$ (Fig. 25.5). The cytochrome P450 enzymes all have two

The accumulation of acetaldehyde causes nausea and vomiting, and, therefore, inactive acetaldehyde dehydrogenases are associated with a distaste for alcoholic beverages and protection against alcoholism. In one of the common allelic variants of ALDH2 (ALDH2*2), a single substitution increases the K_m for acetaldehyde 260-fold (lowers the affinity) and decreases the V_{max} 10-fold, resulting in a very inactive enzyme. Homozygosity for the ALDH2*2 allele affords absolute protection against alcoholism; no individual with this genotype has been found among alcoholics. Alcoholics are frequently treated with acetaldehyde dehydrogenase inhibitors (e.g., disulfiram) to help them abstain from alcohol intake. Unfortunately, alcoholics who continue to drink while taking this drug are exposed to the toxic effects of elevated acetaldehyde levels.

The human has at least seven, and possibly more, genes that code for specific isoenzymes of medium-chain-length alcohol dehydrogenases, the major enzyme responsible for the oxidation of ethanol to acetaldehyde in the human. These different alcohol dehydrogenases have an approximately 60 to 70% identity and are assumed to have arisen from a common ancestral gene similar to the class III isoenzyme many millions of years ago. The class I alcohol dehydrogenases (ADH 1, ADH 2, and ADH 3) are all present in high concentration in the liver, and have a relatively high affinity and capacity for ethanol at low concentrations. (These properties are quantitatively reflected by their low K_m, a parameter discussed in Chapter 9). They have a 90 to 94% sequence identity and are able to form both homo- and hetero-dimers, among themselves (e.g., ββ or βγ). However, none of the ADHs can form dimers with an ADH from another class. The three genes for class I alcohol dehydrogenases are arranged in tandem, head to tail, on chromosome 4. The genes for the other classes of alcohol dehydrogenase are also on chromosome 4 in nearby locations.

ADH 2 and ADH 3 are present as functional polymorphisms that differ in their properties. Genetic polymorphisms for ADH partially account for the observed differences in ethanol elimination rates among various individuals or populations. Although susceptibility to alcoholism is a complex function of genetics and socioeconomic factors, possession of the ADH 2*2 allele, which encodes a relatively fast ADH (high V_{max}), is associated with a decreased susceptibility to alcoholism—presumably because of nausea and flushing caused by acetaldehyde accumulation (because the aldehyde dehydrogenase gene cannot keep up with the amount of acetaldehyde produced). This particular allele has a relatively high frequency in the East Asian population and a low frequency among white Europeans. In contrast, the ADH 2*1/2*1 genotype (homozygous for allele 1 of the ADH 2 gene) is a risk factor for the development of Wernicke-Korsakoff syndrome, a neuropsychiatric syndrome commonly associated with alcoholism.
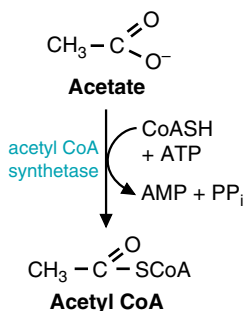
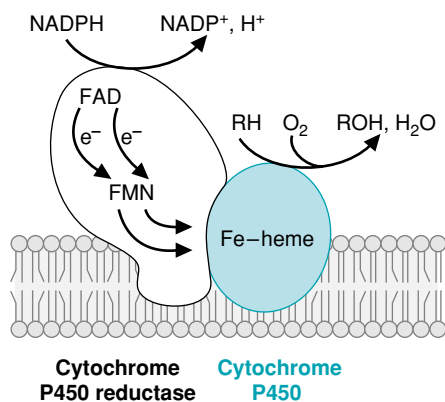**Fig. 25.4.** The activation of acetate to acetyl CoA

Fig. 25.5. General structure of cytochrome P450 enzymes. O_2 binds to the P450 Fe-heme in the active site and is activated to a reactive form by accepting electrons. The electrons are donated by the cytochrome P450 reductase, which contains an FAD plus an FMN or Fe-S center to facilitate the transfer of single electrons from NADPH to O_2. The P450 enzymes involved in steroidogenesis have a somewhat different structure. For CYP2E1, RH is ethanol (CH_3CH_2OH) and ROH is acetaldehyde (CH_3COH).

CYP represents **cy**tochrome **P**450. P450 is an Fe-heme similar to that found in the cytochromes of the electron transport chain ("P" denotes the heme **p**igment, and 450 is the wavelength of visible light absorbed by the pigment). In CYP2E1, the "2" refers to the gene family, which comprises isoenzymes with greater than 40% amino acid sequence identity. The "E" refers to the subfamily, a grouping of isoenzymes with greater than 55 to 60% sequence identity, and the "1" refers to the individual enzymes within this subfamily.

major catalytic protein components: an electron-donating reductase system that transfers electrons from NADPH (cytochrome P450 reductase) and a cytochrome P450. The cytochrome P450 protein contains the binding sites for O_2 and the substrate (e.g., ethanol) and carries out the reaction. The enzymes are present in the endoplasmic reticulum, which on isolation from disrupted cells forms a membrane fraction after centrifugation that was formerly called "microsomes" by biochemists.

1. CYP2E1

MEOS is part of the superfamily of cytochrome P450 enzymes, all of which catalyze similar oxidative reactions. Within the superfamily, at least 10 distinct gene families are found in mammals. More than 100 different cytochrome P450 isozymes exist within these 10 gene families. Each isoenzyme has a distinct classification according to its structural relationship with other isoenzymes. The isoenzyme that has the highest activity toward ethanol is called CYP2E1. A great deal of overlapping specificity exists among the various P450 isoenzymes, and ethanol is also oxidized by several other P450 isoenzymes. "MEOS" refers to the combined ethanol oxidizing activity of all the P450 enzymes.

CYP2E1 has a much higher K_m for ethanol than the class I alcohol dehydrogenases (11 mM [51 mg/dL] compared with 0.05–4 mM [0.23 to 18.4 mg/dL]). Thus, a greater proportion of ingested ethanol is metabolized through CYP2E1 at high levels of ethanol consumption than at low levels.

2. INDUCTION OF P450 ENZYMES

The P450 enzymes are inducible both by their most specific substrate and by substrates for some of the other cytochrome P450 enzymes. Chronic consumption of ethanol increases hepatic CYP2E1 levels approximately 5- to 10-fold. However, it also causes a twofold to fourfold increase in some of the other P450s from the same subfamily, from different subfamilies, and even from different gene families. The endoplasmic reticulum undergoes proliferation, with a general increase in the content of microsomal enzymes, including those that are not directly involved in ethanol metabolism.

The increase in CYP2E1 with ethanol consumption occurs through transcriptional, post-transcriptional, and post-translational regulation. Increased levels of mRNA, resulting from induction of gene transcription or stabilization of message, are found in actively drinking patients. The protein is also stabilized against degradation. In general, the mechanism for induction of P450 enzymes by their substrates occurs through the binding of the substrate (or related compound) to an intracellular receptor protein, followed by binding of the activated receptor to a response element in the target gene. Whether ethanol induction of CYP2E1 follows this general pattern has not yet been shown.

Overlapping specificity in the catalytic activity of P450 enzymes and in their inducers is responsible for several types of drug interactions. For example, phenobarbital, a barbiturate long used as a sleeping pill or for treatment of epilepsy, is converted to an inactive metabolite by cytochrome P450 monooxygenases CYP2B1 and CYP2B2. After treatment with phenobarbital, CYP2B2 is increased 50- to 100-fold. Individuals who take phenobarbital for prolonged periods develop a drug tolerance as CYP2B2 is induced, and the drug is metabolized to an inactive metabolite more rapidly. Consequently, these individuals use progressively higher doses of phenobarbital.

Ethanol is an inhibitor of the phenobarbital-oxidizing P450 system. When large amounts of ethanol are consumed, the inactivation of phenobarbital is directly or indirectly inhibited. Therefore, when high doses of phenobarbital and ethanol are consumed at the same time, toxic levels of the barbiturate can accumulate in the blood.

Although induction of CYP2E1 increases ethanol clearance from the blood, it has negative consequences. Acetaldehyde may be produced faster than it can be metabolized by acetaldehyde dehydrogenases, thereby increasing the risk of hepatic injury. An increased amount of acetaldehyde can enter the blood and can damage other tissues. In addition, cytochrome P450 enzymes are capable of generating free radicals, which also may lead to increased hepatic injury and cirrhosis (see Chapter 24).

E. Variations in the Pattern of Ethanol Metabolism

The routes and rates of ethanol oxidation vary from individual to individual. Differences in ethanol metabolism may influence whether an individual becomes a chronic alcoholic, develops alcohol-induced liver disease, or develops other diseases associated with increased alcohol consumption (such as hepatocarcinogenesis, lung cancer, or breast cancer). Factors that determine the rate and route of ethanol oxidation in individuals include:

- Genotype—Polymorphic forms of alcohol dehydrogenases and acetaldehyde dehydrogenases can greatly affect the rate of ethanol oxidation and the accumulation of acetaldehyde. CYP2E1 activity may vary as much as 20-fold between individuals, partly because of differences in the inducibility of different allelic variants.
- Drinking history—The level of gastric alcohol dehydrogenase (ADH) decreases and CYP2E1 increases with the progression from a naïve, to a moderate, and to a heavy and chronic consumer of alcohol.
- Gender—Blood levels of ethanol after consuming a drink are normally higher for women than for men, partly because of lower levels of gastric ADH activity in women. After chronic consumption of ethanol, gastric ADH decreases in both men and women, but the gender differences become even greater. Gender differences in blood alcohol levels also occur because women are normally smaller. Furthermore, in females, alcohol is distributed in a 12% smaller water space because a woman's body composition consists of more fat and less water than that of a man.
- Quantity—The amount of ethanol an individual consumes over a small amount of time determines its metabolic route. Small amounts of ethanol are metabolized most efficiently through the low K_m pathway of class I ADH and class II ALDH. Little accumulation of NADH occurs to inhibit ethanol metabolism via these dehydrogenases. However, when higher amounts of ethanol are consumed in a short period, a disproportionately greater amount is metabolized through MEOS. MEOS, which has a much higher K_m for ethanol, functions principally at high concentrations of ethanol. A higher activity of MEOS would be expected to correlate with tendency to develop alcohol-induced liver disease, because both acetaldehyde and free radical levels would be increased.

F. The Energy Yield of Ethanol Oxidation

The ATP yield from ethanol oxidation to acetate varies with the route of ethanol metabolism. If ethanol is oxidized by the major route of cytosolic ADH and mitochondrial ALDH, one cytosolic and one mitochondrial NADH are generated with a maximum yield of 5 ATP. Oxidation of acetyl CoA in the TCA cycle and electron transport chain leads to the generation of 10 high-energy phosphate bonds. However, activation of acetate to acetyl CoA requires two high-energy phosphate bonds (one in the cleavage of ATP to AMP+ pyrophosphate and one in the cleavage of pyrophosphate to phosphate), which must be subtracted. Thus the maximum total energy yield is 13 moles of ATP per mole of ethanol.

In contrast, oxidation of ethanol to acetaldehyde by CYP2E1 consumes energy in the form of NADPH, which is equivalent to 2.5 ATP. Thus, for every mole of

As blood ethanol concentration rises above 18 mM (the legal intoxication limit is now defined as 0.08% in most states of the United States, which is approximately 18 mM), the brain and central nervous system are affected. Induction of CYP2E1 increases the rate of ethanol clearance from the blood, thereby contributing to increased alcohol tolerance. However, the apparent ability of a chronic alcoholic to drink without appearing inebriated is partly a learned behavior.

At **Ivan Applebod's** low level of ethanol consumption, ethanol is oxidized to acetate via ADH and ALDH in the liver and the acetate is activated to acetyl CoA and oxidized to CO_2 in skeletal muscle and other tissues. The overall energy yield of 13 ATP per ethanol molecule accounts for the caloric value of ethanol, approximately 7 Cal/g. However, chronic consumption of substantial amounts of alcohol does not have the effect on body weight expected from the caloric intake. This is partly attributable to induction of MEOS, resulting in a proportionately greater metabolism of ethanol through MEOS with its lower energy yield (only approximately 8 ATP). In general, weight loss diets recommend no, or low, alcohol consumption because ethanol calories are "empty" in the sense that alcoholic beverages are generally low in vitamins, essential amino acids, and other required nutrients, but not empty of calories.

ethanol metabolized by this route, only a maximum of 8.0 moles of ATP can be generated (10 ATP from acetylCoA oxidation through the TCA cycle, minus 2 for acetate activation; the NADH generated by aldehyde dehydrogenase is balanced by the loss of NADPH in the MEOS step).

II. TOXIC EFFECTS OF ETHANOL METABOLISM

Alcohol-induced liver disease, a common and sometimes fatal consequence of chronic ethanol abuse, may manifest itself in three forms: fatty liver, alcohol-induced hepatitis, and cirrhosis. Each may occur alone, or they may be present in any combination in a given patient. Alcohol-induced cirrhosis is discovered in up to 9% of all autopsies performed in the United States, with a peak incidence in patients 40 to 55 years of age.

However, ethanol ingestion also has acute effects on liver metabolism, including inhibition of fatty acid oxidation and stimulation of triacylglycerol synthesis, leading to a fatty liver. It also can result in ketoacidosis or lactic acidosis and cause hypoglycemia or hyperglycemia, depending on the dietary state. These effects are considered reversible.

In contrast, acetaldehyde and free radicals generated from ethanol metabolism can result in alcohol-induced hepatitis, a condition in which the liver is inflamed and cells become necrotic and die. Diffuse damage to hepatocytes results in cirrhosis, characterized by fibrosis (scarring), disturbance of the normal architecture and blood flow, loss of liver function and, ultimately, hepatic failure.

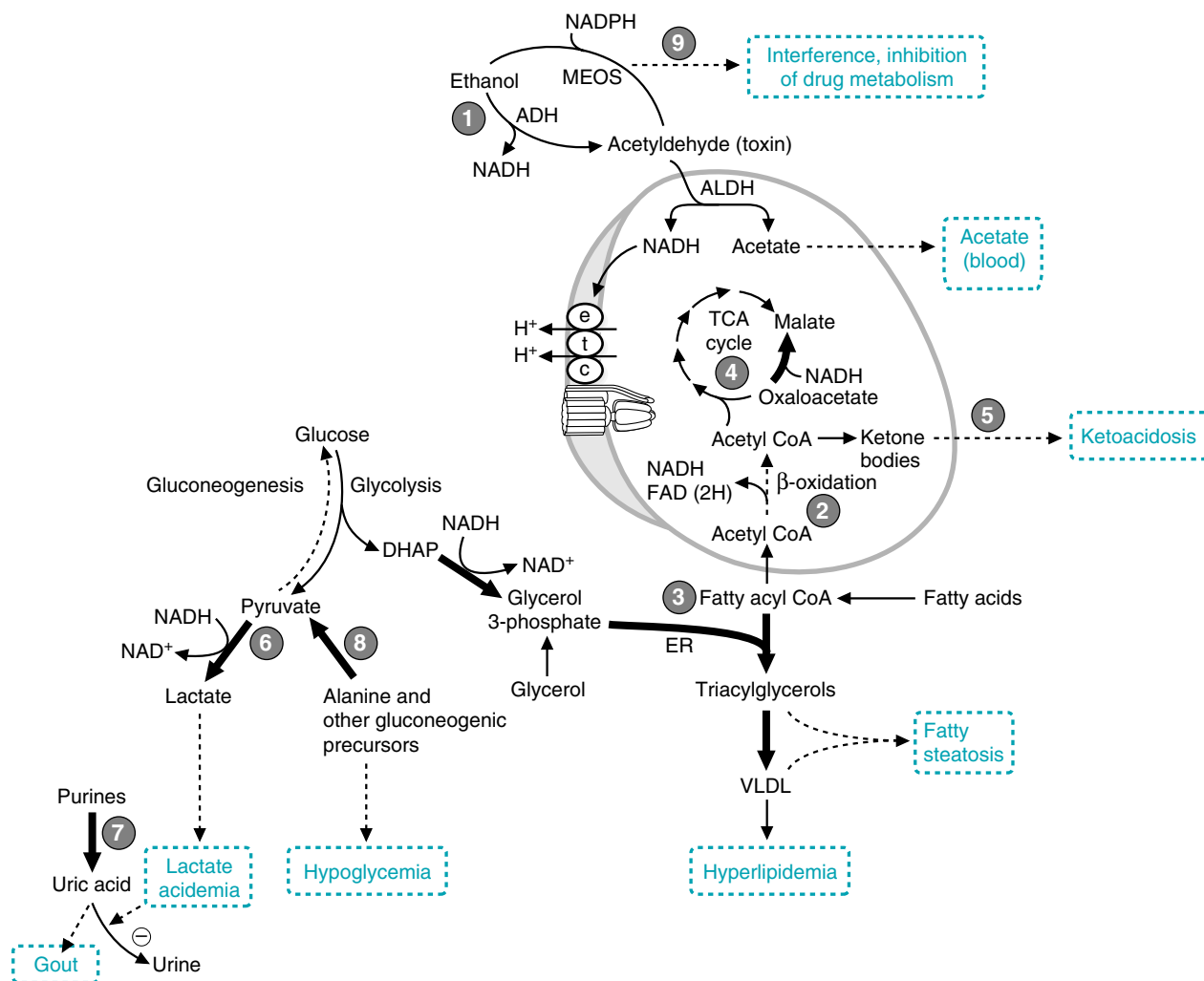### A. Acute Effects of Ethanol Arising from the Increased NADH /NAD$^+$ Ratio

Many of the acute effects of ethanol ingestion arise from the increased NADH/NAD$^+$ ratio in the liver (Fig. 25.6). At lower levels of ethanol intake, the rate of ethanol oxidation is regulated by the supply of ethanol (usually determined by how much ethanol we consume) and the rate at which NADH is reoxidized in the electron transport chain. NADH is not a very effective product inhibitor of ADH or ALDH, and there is no other feedback regulation by ATP, ADP, or AMP. As a consequence, NADH generated in the cytosol and mitochondria tends to accumulate, increasing the NADH/NAD$^+$ ratio to high levels (see Fig. 25.6, circle 1). The increase is even greater as the mitochondria become damaged from acetaldehyde or free radical injury.

1. CHANGES IN FATTY ACID METABOLISM

The high NADH/NAD$^+$ ratio generated from ethanol oxidation inhibits the oxidation of fatty acids, which accumulate in the liver (see Fig. 25.6, circles 2 and 3) These fatty acids are re-esterified into triacylglycerols by combining with glycerol 3-P. The increased NADH/NAD$^+$ ratio increases the availability of glycerol 3-P by promoting its synthesis from intermediates of glycolysis. The triacylglycerols are incorporated into VLDL (very-low-density lipoproteins), which accumulate in the liver and enter the blood, resulting in an ethanol-induced hyperlipidemia.

Although just a few drinks may result in hepatic fat accumulation, chronic consumption of alcohol greatly enhances the development of a fatty liver. Re-esterification of fatty acids into triacylglycerols by fatty acyl CoA transferases in the ER is enhanced (see Fig. 25.6). Because the transferases are microsomal enzymes, they are induced by ethanol consumption just as MEOS is induced. The result is a fatty liver (hepatic steatosis).

The source of the fatty acids can be dietary fat, fatty acids synthesized in the liver, or fatty acids released from adipose tissue stores. Adipose tissue lipolysis increases after ethanol consumption, possibly because of a release of epinephrine.

The hyperlipidemia is greatly enhanced if fat is ingested with ethanol. Thus, "happy hour" foods (e.g., pizza, fried potato skins with sour cream, nachos, and deep-fried peppers stuffed with cream cheese and wrapped in bacon) are exactly the wrong things to eat while drinking. Steamed vegetables or salads with your beer would be much better for your liver.

Fig. 25.6. Acute effects of ethanol metabolism on lipid metabolism in the liver. (1) Metabolism of ethanol generates a high $NADH/NAD^+$ ratio. (2) The high $NADH/NAD^+$ ratio inhibits fatty acid oxidation and the TCA cycle, resulting in accumulation of fatty acids. (3) Fatty acids are re-esterified to glycerol 3-P by acyltransferases in the endoplasmic reticulum. Glycerol 3-P levels are increased because a high $NADH/NAD^+$ ratio favors its formation from dihydroxyacetone phosphate (an intermediate of glycolysis). Ethanol-stimulated increases of endoplasmic reticulum enzymes also favors triacylglycerol formation. (4) NADH generated from ethanol oxidation can meet the requirements of the cell for ATP generation from oxidative phosphorylation. Thus, acetyl CoA oxidation in the TCA cycle is inhibited. (5) The high $NADH/NAD^+$ ratio shifts oxaloacetate (OAA) toward malate, and acetyl CoA is directed into ketone body synthesis. Options 6–8 are discussed in the text.

2. ALCOHOL-INDUCED KETOACIDOSIS.

Fatty acids that are oxidized are converted to acetyl CoA and subsequently to ketone bodies (acetoacetate and β-hydroxybutyrate). Enough NADH is generated from oxidation of ethanol and fatty acids that there is no need to oxidize acetyl CoA in the TCA cycle. The very high $NADH/NAD^+$ ratio shifts all of the oxaloacetate in the TCA cycle to malate, leaving the oxaloacetate levels too low for citrate synthase to synhesize citrate (see Fig. 25.6, circle 4). The acetyl CoA enters the pathway for ketone body synthesis instead of the TCA cycle.

Although ketone bodies are being produced at a high rate, their metabolism in other tissues is restricted by the supply of acetate, which is the preferred fuel. Thus, the blood concentration of ketone bodies may be much higher than found under normal fasting conditions.

Al Martini's admitting physician suspected an alcohol-induced ketoacidosis superimposed on a starvation ketoacidosis. Tests showed that his plasma free fatty acid level was elevated, and his plasma β-hydroxybutyrate level was 40 times the upper limit of normal. The increased $NADH/NAD^-$ ratio from ethanol consumption inhibited the TCA cycle and shifted acetyl CoA from fatty acid oxidation into the pathway of ketone body synthesis.

3.   LACTIC ACIDOSIS, HYPERURICEMIA, AND HYPOGLYCEMIA

Another consequence of the very high NADH/NAD$^+$ ratio is that the balance in the lactate dehydrogenase reaction is shifted toward lactate, resulting in a lacticacidosis (see Fig. 25.6, circle 6). The elevation of blood lactate may decrease excretion of uric acid (see Fig. 25.6, circle 7) by the kidney. Consequently patients with gout (which results from precipitated uric acid crystals in the joints) are advised not to drink excessive amounts of ethanol. Increased degradation of purines also may contribute to hyperuricemia.

The increased NADH/NAD$^+$ ratio also can cause hypoglycemia in a fasting individual who has been drinking and is dependent on gluconeogenesis to maintain blood glucose levels (Fig. 25.6, circles 6 and 8). Alanine and lactate are major gluconeogenic precursors that enter gluconeogenesis as pyruvate. The high NADH/NAD$^+$ ratio shifts the lactate dehydrogenase equilibrium to lactate, so that pyruvate formed from alanine is converted to lactate and cannot enter gluconeogenesis. The high NADH/NAD$^+$ ratio also prevents other major gluconeogenic precursors, such as oxaloacetate and glycerol, from entering the gluconeogenic pathway.

In contrast, ethanol consumption with a meal may result in a transient hyperglycemia, possibly because the high NADH/NAD$^+$ ratio inhibits glycolysis at the glyceraldehyde-3-P dehydrogenase step.

B. Acetaldehyde Toxicity

Many of the toxic effects of chronic ethanol consumption result from accumulation of acetaldehyde, which is produced from ethanol both by alcohol dehydrogenases and MEOS. Acetaldehyde accumulates in the liver and is released into the blood after heavy doses of ethanol (Fig. 25.7). It is highly reactive and binds covalently to amino groups, sulfhydryl groups, nucleotides, and phospholipids to form "adducts."

The noncaloric effect of heavy and chronic ethanol ingestion that led **Ivan Applebod** to believe ethanol has no calories may be partly attributable to uncoupling of oxidative phosphorylation. The hepatic mitochondria from tissues of chronic alcoholics may be partially uncoupled and unable to maintain the transmembrane proton gradient necessary for normal rates of ATP synthesis. Consequently, a greater proportion of the energy in ethanol would be converted to heat. Metabolic disturbances such as the loss of ketone bodies in urine, or futile cycling of glucose, also might contribute to a dimished energy value for ethanol.

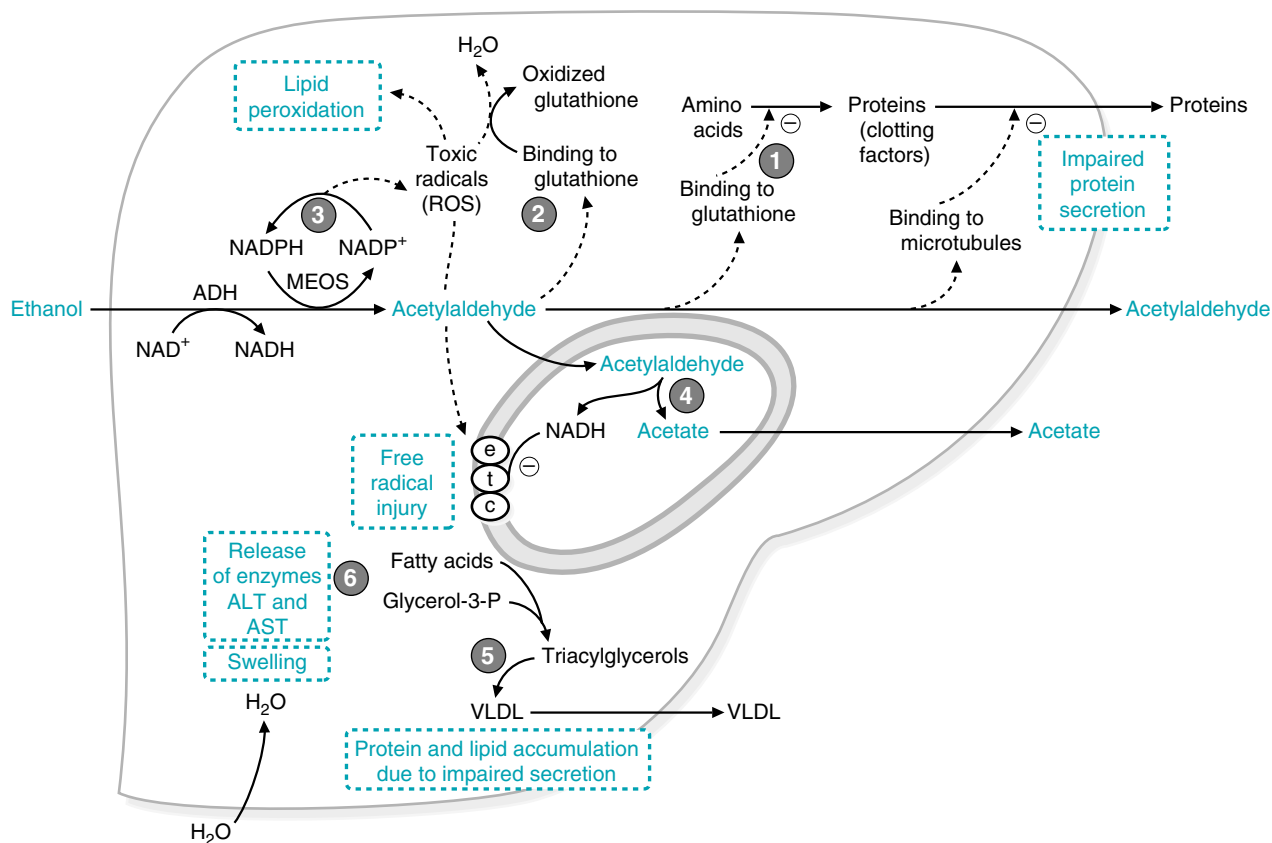### 1.   ACETALDEHYDE AND ALCOHOL-INDUCED HEPATITIS

One of the results of acetaldehyde-adduct formation with amino acids is a general decrease in hepatic protein synthesis (see Fig. 25.7, circle 1). Calmodulin, ribonuclease, and tubulin are some of the proteins affected. Proteins in the heart and other tissues also may be affected by acetaldehyde that appears in the blood.

As a consequence of forming acetaldehyde adducts of tubulin, there is a diminished secretion of serum proteins and VLDL lipoproteins from the liver. The liver synthesizes many blood proteins, including serum albumin, blood coagulation factors, and transport proteins for vitamins, steroids, and iron. These proteins accumulate in the liver, together with lipid. The accumulation of proteins results in an influx of water (see Fig. 25.7, circle 6) within the hepatocytes and a swelling of the liver that contributes to portal hypertension and a disruption of hepatic architecture.

2.   ACETALDEHYDE AND FREE RADICAL DAMAGE

Acetaldehyde adduct formation enhances free radical damage. Acetaldehyde binds directly to glutathione and diminishes its ability to protect against H_2O_2 and prevent lipid peroxidation (see Fig. 25.7, circle 2). It also binds to free radical defense enzymes.

Damage to mitochondria from acetaldehyde and free radicals perpetuates a cycle of toxicity (see Fig. 25.7, circles 3 and 4). With chronic consumption of ethanol, mitochondria become damaged, the rate of electron transport is inhibited, and oxidative phosphorylation tends to become uncoupled. Fatty acid

Fig. 25.7. The development of alcohol-induced hepatitis. (1) Acetaldehyde adduct formation decreases protein synthesis and impairs protein secretion. (2) Free radical injury results partly from acetaldehyde adduct formation with glutathione. (3) Induction of MEOS increases formation of free radicals, which leads to lipid peroxidation and cell damage. (4) Mitochondrial damage inhibits the electron transport chain, which decreases acetaldehyde oxidation. (5) Microtubule damage increases VLDL and protein accumulation. (6) Cell damage leads to release of the hepatic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

oxidation is decreased even further, thereby enhancing lipid accumulation (see Fig. 25.7, circle 5). The mitochondrial changes further impair mitochondrial acetaldehyde oxidation, thereby initiating a cycle of progressively increasing acetaldehyde damage.

C. Ethanol and Free Radical Formation

Increased oxidative stress in the liver during chronic ethanol intoxication arises from increased production of free radicals, principally by CYP2E1. FAD and FMN in the reductase and heme in the cytochrome P450 system transfer single electrons, thus operating through a mechanism that can generate free radicals. The hydroxyethyl radical ($CH_3CH_2O\cdot$) is produced during ethanol metabolism and can be released as a free radical. Induction of CYP2E1, as well as other cytochrome P450 enzymes, can increase the generation of free radicals from drug metabolism and from the activation of toxins and carcinogens (see Fig. 25.7, circle 3). These effects are enhanced by acetaldehyde-adduct damage.

Phospholipids, the major lipid in cellular membranes, are a primary target of peroxidation caused by free radical release. Peroxidation of lipids in the inner mitochondrial membrane may contribute to the inhibition of electron transport and uncoupling of mitochondria, leading to inflammation and cellular necrosis. Induction of CYP2E1 and other P450 cytochromes also increases formation of other radicals and the activation of hepatocarcinogens.

Because of the possibility of mild alcoholic hepatitis and perhaps chronic alcohol-induced cirrhosis, the physician ordered liver function studies on **Jean Ann Tonich**. The tests indicated an alanine aminotransferase (ALT) level of 46 units/L (reference range = 5–30) and an aspartate aminotransferase (AST) level of 98 units/L (reference range = 10–30). The concentration of these enzymes is high in hepatocytes. When hepatocellular membranes are damaged in any way, these enzymes are released into the blood. Jean Ann Tonich's serum alkaline phosphatase level was 151 units/L (reference range = 56–155 for an adult female). The serum total bilirubin level was 2.4 mg/dL (reference range = 0.2–1.0). These tests show impaired capacity for normal liver function. Her blood hemoglobin and hematocrit levels were slightly below the normal range, consistent with a toxic effect of ethanol on red blood cell production by bone marrow. Serum folate, vitamin B12 and iron levels were also slightly suppressed. Folate is dependent on the liver for its activation and recovery from the enterohepatic circulation. Vitamin B12 and iron are dependent on the liver for synthesis of their blood carrier proteins. Thus, **Jean Ann Tonich** shows many of the consequences of hepatic damage.

In liver fibrosis, disruption of the normal liver architecture, including sinusoids, impairs blood from the portal vein. Increased portal vein pressure (portal hypertension) causes capillaries to anastomose (to meet and unite or run into each other) and form thin-walled dilated esophageal venous conduits known as esophageal varices. When these burst, there is hemorrhaging into the gastrointestinal tract. The bleeding can be very profuse because of the high venous pressure within these varices in addition to the adverse effect of impaired hepatic function on the production of blood clotting proteins.

D. Hepatic Cirrhosis and Loss of Liver Function

Liver injury is irreversible at the stage that hepatic cirrhosis develops. Initially the liver may be enlarged, full of fat, crossed with collagen fibers (fibrosis), and have nodules of regenerating hepatocytes ballooning between the fibers. As liver function is lost, the liver becomes shrunken (Laennec's cirrhosis). During the development of cirrhosis, many of the normal metabolic functions of the liver are lost, including biosynthetic and detoxification pathways. Synthesis of blood proteins, including blood coagulation factors and serum albumin, is decreased. The capacity to incorporate amino groups into urea is decreased, resulting in the accumulation of toxic levels of ammonia in the blood. Conjugation and excretion of the yellow pigment bilirubin (a product of heme degradation) is diminished, and bilirubin accumulates in the blood. It is deposited in many tissues, including the skin and sclerae of the eyes, causing the patient to become visibly yellow. Such a patient is said to be jaundiced.

CLINICAL COMMENTS

Ivan Applebod. When ethanol consumption is low (less than 15% of the calories in the diet), it is efficiently used to produce ATP, thereby contributing to **Ivan Applebod's** weight gain. However, in individuals with chronic consumption of large amounts of ethanol, the caloric content of ethanol is not converted to ATP as effectively. Some of the factors that may contribute to this decreased efficiency include mitochondrial damage (inhibition of oxidative phosphorylation and uncoupling) resulting in the loss of calories as heat, increased recycling of metabolites such as ketone bodies, and inhibition of the normal pathways of fatty acid and glucose oxidation. In addition, heavier drinkers metabolize an increased amount of alcohol through MEOS, which generates less ATP.

Al Martini. **Al Martini** was suffering from acute effects of high ethanol ingestion in the absence of food intake. Both heavy ethanol consumption and low caloric intake increase adipose tissue lipolysis and elevate blood fatty acids. As a consequence of his elevated hepatic NADH/NAD$^+$ ratio, acetyl CoA produced from fatty acid oxidation was diverted from the TCA cycle into the pathway of ketone body synthesis. Because his skeletal muscles were using acetate as a fuel, ketone body utilization was diminished, resulting in ketoacidosis. **Al Martini's** moderately low blood glucose level also suggests that his high hepatic NADH level prevented pyruvate and glycerol from entering the gluconeogenic pathway. Pyruvate is diverted to lactate, which may have contributed to his metabolic acidosis and anion gap.

Rehydration with intravenous fluids containing glucose and potassium was initiated. His initial potassium was low, possibly secondary to vomiting. An orthopedic surgeon was consulted regarding the compound fracture of his right forearm.

Jean Ann Tonich. Jean Ann Tonich's signs and symptoms, as well as her laboratory profile, were consistent with the presence of mild reversible alcohol-induced hepatocellular inflammation (alcohol-induced hepatitis) superimposed on a degree of irreversible scarring of liver tissues known as chronic alcoholic (Laennec's) cirrhosis of the liver. The chronic inflammatory process associated with long-term ethanol abuse in patients such as **Jean Ann Tonich** is accompanied by increases in the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Her elevated bilirubin and alkaline phosphatase were consistent with hepatic damage. Her values for ALT and

AST were significantly below those seen in acute viral hepatitis. In addition, the ratio of the absolute values for serum ALT and AST often differ in the two diseases, tending to be greater than 1 in acute viral hepatitis and less than 1 in chronic alcohol-induced cirrhosis. The reason for the difference in ratio of enzyme activities released is not understood, but a lower level of ALT in the serum may be attributable to an alcohol-induced deficiency of pyridoxal phosphate. In addition, serologic tests for viral hepatitis were nonreactive. Her serum folate, vitamin B12, and iron levels were also slightly suppressed, indicating impaired nutritional status.

Jean Ann Tonich was strongly cautioned to abstain from alcohol immediately and to improve her nutritional status. In addition, Jean Ann was referred to the hospital drug and alcohol rehabilitation unit for appropriate psychological therapy and supportive social counseling. The physician also arranged for a follow-up office visit in 2 weeks.

Although the full spectrum of alcohol-induced liver disease may be present in a well-nourished individual, the presence of nutritional deficiencies enhances the progression of the disease. Ethanol creates nutritional deficiencies in a number of different ways. The ingestion of ethanol reduces the gastrointestinal absorption of foods containing essential nutrients, including vitamins, essential fatty acids, and essential amino acids. For example, ethanol interferes with absorption of folate, thiamine, and other nutrients. Secondary malabsorption can occur through gastrointestinal complications, pancreatic insufficiency, and impaired hepatic metabolism or impaired hepatic storage of nutrients, such as vitamin A. Changes in the level of transport proteins produced by the liver also strongly affect nutrient status.

BIOCHEMICAL COMMENTS

Fibrosis in Chronic Alcohol-Induced Liver Disease Fibrosis is the excessive accumulation of connective tissue in parenchymal organs. In the liver, it is a frequent event following a repeated or chronic insult of sufficient intensity (such as chronic ethanol intoxication or infection by a hepatitis virus) to trigger a "wound healing–like" reaction. Regardless of the insult, the events are similar: an overproduction of extracellular matrix components occurs, with the tendency to progress into sclerosis, accompanied by a degenerative alteration in the composition of matrix components. (Table 25.2) Some individuals (fewer than 20% of those who chronically consume alcohol) go on to develop cirrhosis.

The development of hepatic fibrosis after ethanol consumption is related to stimulation of the mitogenic development of stellate (Ito) cells into myofibroblasts, and stimulation of the production of collagen type I and fibronectin by these cells. The stellate cells are perisinusoidal cells lodged in the space of Disse that produce extracellular matrix protein. Normally the space of Disse contains basement membrane–-like collagen (collagen type IV) and laminin. As the stellate cells are activated, they change from a resting cell filled with lipids and vitamin A to one that proliferates, loses its vitamin A content, and secretes large quantities of extracellular matrix components.

One of the initial events in the activation and proliferation of stellate cells is the activation of Kupffer cells, which are macrophages resident in the liver sinusoids

Table 25.2. Hepatic Injury

Stage of Injury	Main Features
Fibrosis: Increase of connective tissue	
	Accumulation of both fibrillar and basement membrane–like collagens
	Increase of laminen and fibronectin
	Thickening of connective tissue septae
	Capillarization of the sinusoids
Sclerosis: Aging of fibrotic tissue	
	Decrease of hyaluronic acid and heparan sulfate proteoglycans
	Increase of chondroitin sulfate proteoglycans
	Progressive fragmentation and disappearance of elastic fibers
	Distortion of sinusoidal architecture and parenchymal damage
Cirrhosis: End-stage process of liver fibrotic degeneration	
	Whole liver heavily distorted by thick bands of collagen surrounding nodules of hepatocytes with regenerative foci
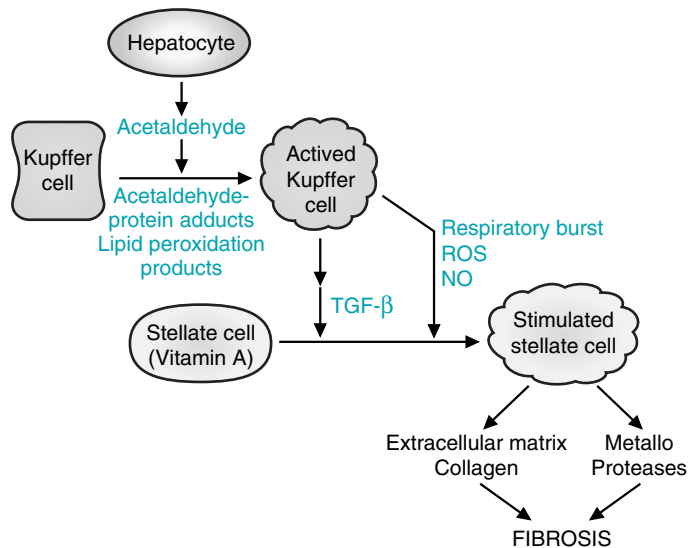
Fig. 25.8. Proposed model for the development of hepatic fibrosis involving hepatocytes, Kupffer cells, and stellate (Ito) cells. ROS, reactive oxygen species; NO, nitric oxide: TGFβ1, transforming growth factor β1.

Cytokines are proteins produced by inflammatory cells that serve as communicators with other cells. Chemokines are even smaller proteins produced by inflammatory cells that promote migration of other inflammatory cells (e.g., from the blood into the site of injury).

(Fig.25.8). The Kupffer cells are probably activated by a product of the damaged hepatocytes, such as necrotic debris, iron, ROS, acetaldehyde, or aldehyde products of lipid peroxidation. Kupffer cells also may produce acetaldehyde from ethanol internally through their own MEOS pathway.

Activated Kupffer cells produce a number of products that contribute to activation of stellate cells. They generate additional ROS through NADPH oxidase during the oxidative burst and NOS through inducible NO synthase (see Chapter 24). In addition, they secrete an impressive array of growth factors, such as cytokines, chemokines, prostaglandins, and other reactive molecules. The cytokine transforming growth factor β1 (TGFβ1), produced by both Kupffer cells and sinusoidal endothelial cells, is a major player in the activation of stellate cells. Once activated, the stellate cells produce collagen and proteases, leading to an enhanced fibrotic network within the liver.

Suggested References

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REVIEW QUESTIONS—CHAPTER 25

1. The fate of acetate, the product of ethanol metabolism, is which of the following?

   (A) It is taken up by other tissues and activated to acetyl CoA.
   (B) It is toxic to the tissues of the body and can lead to hepatic necrosis.
   (C) It is excreted in bile.
   (D) It enters the TCA cycle directly to be oxidized.
   (E) It is converted into NADH by alcohol dehydrogenase.

2. Which of the following would be expected to occur after acute alcohol ingestion?

   (A) The activation of fatty acid oxidation
   (B) Lactic acidosis
   (C) The inhibition of ketogenesis
   (D) An increase in the NAD^+/NADH ratio
   (E) An increase in gluconeogenesis

3. A chronic alcoholic is in treatment for alcohol abuse. The drug disulfiram is prescribed for the patient. This drug deters the consumption of alcohol by which of the following mechanisms?

   (A) Inhibiting the absorption of ethanol so that an individual cannot become intoxicated, regardless of how much he drinks
   (B) Inhibiting the conversion of ethanol to acetaldehyde, which would cause the excretion of unmetabolized ethanol
   (C) Blocking the conversion of acetaldehyde to acetate, which causes the accumulation of acetaldehyde
   (D) Activating the excessive metabolism of ethanol to acetate, which causes inebriation with consumption of a small amount of alcohol
   (E) Preventing the excretion of acetate, which causes nausea and vomiting

4. Induction of CYP2E1 would result in which of the following?

   (A) A decreased clearance of ethanol from the blood
   (B) A decrease in the rate of acetaldehyde production
   (C) A low possibility of the generation of free radicals
   (D) Protection from hepatic damage
   (E) An increase of one's alcohol tolerance level

5. Which one of the following consequences of chronic alcohol consumption is irreversible?

   (A) Inhibition of fatty acid oxidation
   (B) Activation of triacylglycerol synthesis
   (C) Ketoacidosis
   (D) Lactic acidosis
   (E) Liver cirrhosis