

Slide 1

Ethanol Pharmacokinetics, Metabolism and Forensic Aspects

- The goal of this lecture is to describe the biochemical pathways which play a role in the metabolism of ethanol.
- The pathways will be described with respect to the enzymes involved, the factors which regulate the overall flux through the pathway, and how these pathways impact on normal physiological pathways involved in metabolism of nutrients and drugs.
- The effects of chronic ethanol consumption on ethanol and acetaldehyde metabolism will be discussed, including factors which may be responsible for metabolic tolerance.
- Factors which influence the absorption and the elimination of ethanol will be discussed.

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Slide 2

Why is Understanding Pathways of Ethanol Metabolism Important?

- Learn how the body disposes of ethanol and its metabolites.
- Discern some of the factors which influence this process.
- Learn how ethanol influences the metabolism of nutrients and drugs, and modulates the therapeutic effectiveness of drugs.
- May learn how ethanol damages various organs.
- May help to identify individuals who are at increased or decreased risk for alcohol toxicity.

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Slide 3

Distribution of Ethanol in the Body

- The equilibrium concentration of ethanol in a tissue depends on the relative water content of that tissue. The rate of equilibration of ethanol with a tissue depends on
 - Permeability (water content)
 - Rate of blood flow
 - Mass of the tissue
- Ethanol is practically insoluble in fats and oils, although like water, it can readily pass through biological membranes.
- Ethanol distributes from the blood into all tissues and fluids in proportion to their relative content of water. The concentration of ethanol in a tissue is dependent on the relative water content of the tissue, and reaches equilibrium quickly with the concentration of ethanol in the plasma. There is no plasma protein binding for ethanol.

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The same dose of ethanol per unit of body weight can produce very different blood ethanol concentrations in different individuals because of the large variations in proportions of fat and water in their bodies, and the low lipid: water partition coefficient of ethanol. Women generally have a smaller volume of distribution for ethanol than men because of their higher percentage of body fat. Women will have higher peak blood ethanol levels than men when given the same dose of ethanol as g per kg body weight but

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no differences occur when given the same dose per liter of body water. First pass metabolism of ethanol by the stomach, which may be greater in males, may also contribute to the higher blood ethanol levels found in women.

The breath analyzer test for estimating blood ethanol concentrations is dependent on the diffusion of ethanol from pulmonary arterial blood into the alveolar air. The ethanol vapor in breath is in equilibrium with the ethanol dissolved in the water of the blood at a blood : breath partition coefficient of about 2100:1.

The interplay between kinetics of absorption, distribution and elimination determines the extent of systemic exposure to ethanol.

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Factors Affecting Ethanol Absorption

1. Concentration of ethanol
2. Blood flow at site of absorption
3. Irritant properties of ethanol
4. Rate of ingestion
5. Type of beverage
6. Food

Absorption of ethanol from the duodenum and jejunum is much more rapid than from the stomach, hence the rate of gastric emptying is an important determinant of the rate of absorption of orally administered ethanol.

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.Ethanol crosses biological membranes by passive diffusion, down its concentration gradient. Therefore, the higher the concentration of ethanol, the greater is the resulting concentration gradient, and the more rapid is the absorption.

.Rapid removal of ethanol from the site of absorption by an efficient blood flow will help to maintain the concentration gradient and thereby promote absorption.

.Ethanol has irritant properties and high concentrations can cause superficial erosions, hemorrhages and paralysis of the stomach smooth muscle. This will decrease ethanol absorption.

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Peak blood ethanol concentrations develop more slowly if the alcohol containing beverage is ingested rapidly perhaps a reflection of the irritant properties of ethanol. However, peak blood ethanol levels are higher if a given dose of ethanol is ingested as a single dose rather than several smaller doses, probably because ethanol concentration gradient will be higher in the former case.

Certain congeners present in alcoholic beverages may decrease the absorption of ethanol; however, this is not a significant effect. In general, there is little difference in the rate of absorption of the same dose of ethanol administered in the form of different alcoholic beverages. This is important for forensic considerations, i.e., blood ethanol concentration is not significantly influenced by the type of alcoholic beverage consumed.

The presence of food in the stomach retards gastric emptying and thus will reduce the absorption of ethanol, the "don't drink on an empty stomach" concept. Recent studies indicate that meals high in either fat, or carbohydrate or protein are equally effective in retarding gastric emptying.

Several other factors influence ethanol absorption, including tobacco, certain drugs and physical exercise, which decrease gastric motility, temperature, perhaps variations in hormonal status.

The blood ethanol concentration is determined by the amount of ethanol consumed, by the presence or absence of food in the stomach, factors which affect gastric emptying and the rate of ethanol oxidation

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First Pass Metabolism of Ethanol in the Stomach

Some of the ethanol which is ingested orally does not enter the systemic circulation but may be oxidized in the stomach by ADH isoforms such as α - (or μ)-ADH and class I and class III ADH. This first pass metabolism could modulate ethanol toxicity since its efficiency determines the bioavailability of ethanol.

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Ethanol is rapidly passed into the duodenum from the stomach in the fasted state. This will minimize first pass metabolism and thereby play a role in the higher blood alcohol concentrations observed in the fasted versus the fed state.

First pass metabolism has been reported to be low in alcoholics, especially in alcoholic women because of decreased ADH activity. This may be important in the increased sensitivity to ethanol and the higher blood ethanol concentrations in women than in men after an equivalent oral dose of ethanol. Several drugs, including H₂ receptor blockers such as cimetidine or ranitidine, or aspirin inhibit stomach ADH activity. This will decrease first pass metabolism by the stomach, and hence, increase blood ethanol concentrations.

The overall significance of first pass metabolism by the stomach is controversial. Some first pass metabolism may also occur in the liver, particularly when the delivery of alcohol into the portal vein is slow, as occurs in the fed state. The relative contribution of

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stomach and liver first pass metabolism has not yet been defined. The speed of gastric emptying modulates gastric and hepatic first pass metabolism of ethanol.

References

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Ammon et al. Clin. Pharmacol. Ther. **59**, 503-13, 1996.
Levitt et al. Alcohol Clin. Exp. Res. **21**, 293-7, 1997.

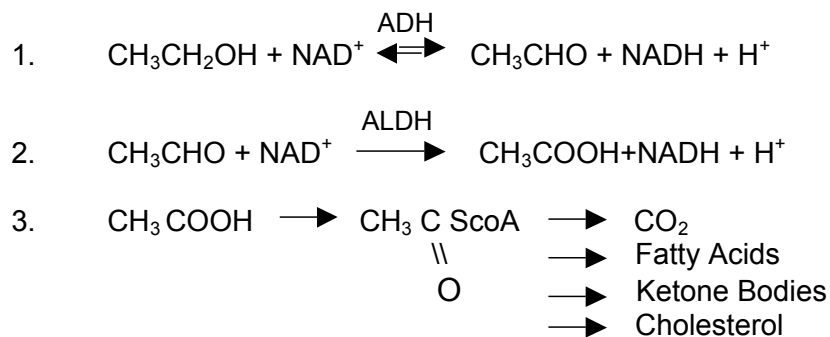
Slide 6

General Scheme for Ethanol Oxidation

1. < 10 % ethanol excreted in breath, sweat and urine.
2. ~ 90 % ethanol removed by oxidation.
3. Most of this ethanol oxidation occurs in the liver.
4. Ethanol cannot be stored in the liver.
5. No major feedback mechanisms to pace the rate of ethanol metabolism to the physiological conditions of the liver cell.

$\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \xrightleftharpoons{\text{ADH}} \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+$
 $\text{CH}_3\text{CHO} + \text{NAD}^+ \xrightarrow{\text{ALDH}} \text{CH}_3\text{COOH} + \text{NADH} + \text{H}^+$
 $\text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{C}(=\text{O})\text{SCoA} \rightarrow \begin{cases} \text{CO}_2 \\ \text{Fatty acids} \\ \text{Ketone bodies} \\ \text{Cholesterol} \end{cases}$

General Scheme for Ethanol Oxidation



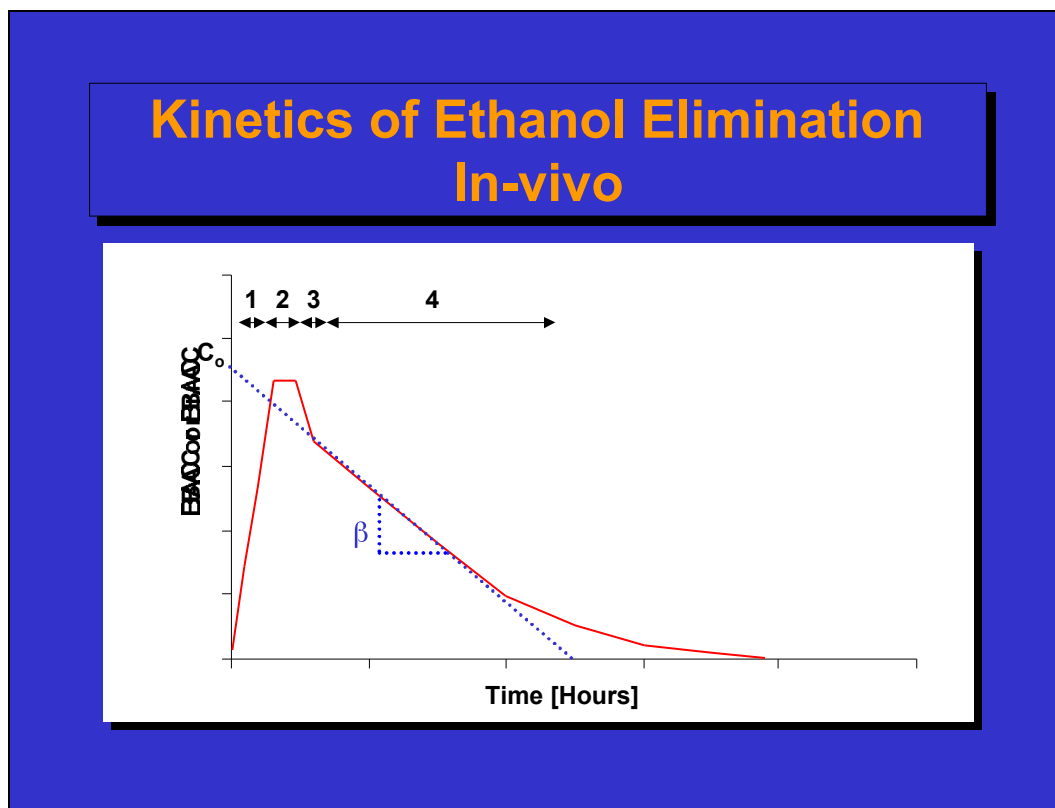
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Ethanol Metabolism – General Principles

The major enzyme system(s) responsible for the oxidation of ethanol, alcohol dehydrogenase, and to a lesser extent, the cytochrome P450-dependent ethanol-oxidizing system, are present to the largest extent in the liver. Liver damage lowers the rate of ethanol oxidation and hence, elimination from the body. Ethanol is a nutrient and has caloric value (about 7 kcalories per gram; carbohydrates and protein produce 4 kcal per gram, while fat produces 9 kcal). However, unlike carbohydrates, (glycogen in liver and muscle) and fat (triglycerides in adipose tissues and liver) which can be stored and utilized in time of need e.g. fasting, ethanol is not stored and remains in body water until eliminated. Whereas metabolism of the major nutrients is under hormonal control, e.g. Insulin/glucagon, leptin, catecholamine, thyroid hormones, generally, there is little hormonal regulation to pace the rate of ethanol elimination. In view of these considerations, there is a major burden on the liver to oxidize ethanol in order to remove this agent from the body.

In general, animals with small body weight metabolize ethanol at faster rates than larger animals e.g. the rate of ethanol elimination in mice is 5x greater than the rate in humans. These rates of ethanol metabolism correlate with the basal metabolic rate for that species, indicating that the capacity to oxidize ethanol parallels the capacity to oxidize the typical nutrients. However, it is important to note that alcohol-derived calories are produced at the expense of the metabolism of normal nutrients since ethanol will be oxidized preferentially over other nutrients.

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Kinetics of Ethanol Elimination In-vivo

Ethanol elimination was originally believed to be a zero-order process, meaning that ethanol was removed from the body at a constant rate, which was independent of the concentration of ethanol. Many studies have shown an essentially linear descent of the blood ethanol concentration with time. Since the K_m of ADH for ethanol is low (about 1 mM), ADH is saturated at relatively low concentrations of ethanol, hence, the overall elimination process is proceeding at maximal velocity and is independent of the ethanol concentration.

However, linearity is not observed at low ethanol concentration since ADH is no longer saturated with ethanol. Ethanol elimination now follows Michaelis-Menten kinetics; the rate of change in the concentration of ethanol is dependent upon the concentration of ethanol and the kinetic constants K_m and V_{max} .

In addition, because the metabolism of ethanol by CYP2E1 reflects a high K_m for ethanol system, a concentration-dependent rate of ethanol elimination can be observed, with higher rates of ethanol elimination at higher blood ethanol concentrations. The latter is exaggerated after chronic ethanol consumption since the high K_m CYP2E1 system is induced. Because of this concentration dependence, it is not possible to estimate one single rate of ethanol metabolism.

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Concentration-dependent metabolism of ethanol has been observed in some, but not all studies on ethanol elimination. Some studies have reported a more rapid decline in blood alcohol concentration at earlier times, right after peak blood alcohol concentrations have been reached, which may reflect concentration-dependent ethanol metabolism, or more rapid reoxidation of NADH.

Although rates vary widely, the "average" metabolic capacity to remove ethanol is about 170 to 240 g per day for a person with a body weight of 70 kg. This would be equivalent to an average metabolic rate of about 7 g/hr which translates to about one drink per hr. Since alcoholics may consume 200 to 300 g of ethanol per day, equivalent to 1400 to 2100 kcal, consumption of normal nutrients is usually significantly decreased (typically, 2000-3000 kcal consumed per day).

Reference

Salaspuro and Lieber, Ann Clin Res. 10: 294-297, 1978.

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Alcohol Dehydrogenase

Constant		1 1	2 2	3 3	1 1	2 2	
K_m NAD ⁺ , μ M	13	7.4	180	530	7.9	8.7	14
K_m ethanol, mM	4.2	0.049	0.94	24	1	0.63	34
K_i 4-methylpyrazole, μ M	1.1	0.13	-	2.1	0.1	-	2000
V_{max} min ⁻¹	27	9.2	400	3 00	87	35	20
pH-optimum	10.5	10.5	8.5	7.0	10.5	10.5	10.5

Crabb et. al 1987 & Bosron et. al 1993

- Physiological Function?
- Isoforms-Why so many?
- Localization-consequence on liver function.
- Development.

Alcohol Dehydrogenase

Some kinetic constants for human liver ADH are shown above.

Reference

Crabb et. al 1987 & Bosron et. al 1993

Physiological Function?

Isoforms-Why so many?

Localization-consequence on liver function

Development

ADH is a zinc-containing enzyme, consisting of two subunits of 40 kDa each. Functions of ADH may be to oxidize the small amount of endogenous ethanol produced by microorganisms in the body, to oxidize exogenous ethanol and other alcohols consumed in the diet, and perhaps to oxidize substrates involved in steroid and bile acid metabolism. The enzyme has broad substrate specificity, oxidizing many primary or secondary alcohols. ADH is localized in the cytosolic fraction of the cell. ADH is found in highest amount in the liver, followed by GI tract, kidneys, nasal mucosa, testes and uterus.

Multiple forms of ADH exist in human liver. There are seven known human ADH genes, two which show polymorphisms. Class I ADH contains three genes, *ADH1*, *ADH2* and *ADH3* which code for the following subunits α_1 , α_2 and α_3 and β_1 and β_2 . These

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different subunits and polymorphic forms can combine to produce a variety of homo- or hetero-dimers e.g., $\alpha_1\alpha_1$, $\alpha_2\alpha_2$ the forms are found primarily in the liver.

Class II ADH- The *ADH4* gene codes for the α_2 subunit, which produces $\alpha_2\alpha_2$ homodimers in the liver and to a lesser extent in kidney and lung. The high K_m for ethanol may make this enzyme more important in metabolism of high concentrations of ethanol.

Class III ADH- The *ADH5* gene codes for the α_X subunit which produces $\alpha_X\alpha_X$ homodimers. This enzyme was formerly known as the glutathione-dependent formaldehyde dehydrogenase, and is found in most tissues. This isoform has a very high K_m for ethanol ($>2M$).

Class V-ADH- The mRNA product produced by the *ADH6* gene is present in liver and stomach, but the protein has not been characterized.

Class IV ADH- The *ADH7* gene encodes the σ subunit which is very efficient in oxidizing retinol to retinal. This form is present in the stomach. A class VI ADH has recently been found but is not yet characterized.

The class I ADH isoforms play the most important role in ethanol oxidation. ADH is present in low levels in fetal liver, the α_a homodimer can be observed in early gestation, the α_b subunits start to be expressed in late gestation, and the α_g subunit is expressed after birth. The fetus eliminates ethanol very slowly because of this late maturation of ADH genes. The ability to form many isoforms, with varying kinetic properties, probably contributes to the large variability in the capacity for metabolizing ethanol that human populations exhibit. The strong sensitivity of the Class I ADH to pyrazole inhibition explains the powerful inhibition of ethanol metabolism by these agents.

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Factors Modifying the Ethanol Elimination Rate

There is a 3-4 fold variability in the rate of ethanol elimination by humans because of genetic and environmental factors, including sex, age, race, food, biological rhythms, exercise, alcoholism, and drugs.

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There is a 3-4 fold variability in the rate of ethanol elimination by humans because of genetic and environmental factors, including sex, age, race, food, biological rhythms, exercise, alcoholism, and drugs.

Sex- Generally, the earlier literature reported no effect of gender on ethanol elimination rates. More recent studies generally indicate a faster rate of ethanol elimination by women when rates are corrected for lean body mass. Since women have smaller body size and therefore smaller lean body mass, ethanol elimination per unit lean body mass is higher in women. Men and women generally have similar ethanol elimination rates when results are expressed as g per hr or g per liter liver volume. Because of first pass metabolism by the stomach, it is possible that a given oral dose of ethanol may produce a higher blood ethanol concentration in females than males- see slide on First Pass Metabolism.

Age- Very young animals have low ethanol elimination rates because ADH (and CYP2E1) are not fully developed. Fetal liver eliminates ethanol very poorly which may have consequences for fetal alcohol syndrome. There may be a small decline in ethanol elimination with aging, perhaps due to decreased liver mass, or body water content.

Race- Unclear literature. Ethanol elimination may be higher in subjects containing the b₃ class I ADH isoform compared to the b₁ isoform (see ADH allele slide). Some studies, but not all, suggest an increased rate of ethanol elimination by native Americans

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compared to Caucasians. Rate of ethanol elimination by Chinese is similar to that of Caucasians. One study reported that African Americans had a lower alcohol elimination rate than Caucasians, which reflected a lower liver weight per unit body weight. Liver mass may explain ethnic and gender differences in alcohol elimination rates. See slide on Acetaldehyde Metabolism concerning active and inactive variants of ALDH.

More research on possible population differences in ethanol elimination is required.

Food- Ethanol metabolism is higher in the fed nutritional state as compared to the fasted state because ADH levels are higher, and the ability of substrate shuttle mechanisms to transport reducing equivalents into the mitochondria is elevated. Food may also increase liver blood flow. Meals high in carbohydrates are more effective than high fat or high protein in increasing ethanol elimination. One explanation for this is the "fructose effect". The sugar fructose increases ethanol metabolism by providing substrates which help to convert NADH to NAD⁺, and by producing ADP which enhances mitochondrial oxygen uptake. Food also affects absorption of ethanol (slide).

Biological Rhythms- in rodents, the rate of ethanol elimination varies with the time of day, being maximal at the end of the daily dark period. This may be related to a temperature cycle. Few studies have been carried out in humans.

Exercise- unclear literature, most studies report a small increase in ethanol elimination rate, perhaps due to increased temperature or catecholamine release.

Alcoholism- See Metabolic Tolerance Slide. Damaged liver will decrease the rate of ethanol metabolism.

Drugs- Agents which inhibit ADH (pyrazoles, isobutyramide) or compete with ethanol for ADH (methanol, ethylene glycol) or which inhibit the mitochondrial respiratory chain will decrease the ethanol elimination rate. Most hormones do not significantly affect ethanol metabolism; there may be a small increase produced by thyroid hormones.

References

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Alcohol Dehydrogenase

- Control of ADH activity is complex
 - dissociation of the product NADH is rate limiting step.
 - Subject to product inhibition by NADH and acetaldehyde.
 - Subject to substrate inhibition by high concentrations of ethanol.

Alcohol Dehydrogenase

Control of ADH activity is complex:

- a) dissociation of the product NADH is rate limiting step
- b) subject to product inhibition by NADH and acetaldehyde
- c) subject to substrate inhibition by high concentrations of ethanol

Ethanol oxidation is generally limited by the maximum capacity of ADH. Amount of ADH in the liver is greater in the fed than the fasted state which plays a major role in the increased rate of ethanol oxidation in the fed state. Inhibitors of ADH such as 4-methylpyrazole or isobutyramide inhibit ethanol oxidation in direct proportion to their potency as inhibitors of ADH. Hormonal effects on ADH are complex; Some stimulation is found after treatment with growth hormone, epinephrine or estrogens. Thyroid hormones and androgens inhibit ADH activity.

References

- Edenberg et al. Pharmacogenetics **9**: 25-30, 1999.
Crabb et. al. Arch. Biochem. Biophys. **224**: 299-399, 1983.
Dawson, A. G. Trends Biochem. Sci. **8**: 195-197, 1983.

Slide 11

Frequency of ADH Alleles in Racial Populations

Frequency of ADH Alleles in Racial Population				ADH 3*1	ADH 3*2
	ADH ¹ ₂	ADH ² ₂	ADH ³ ₂	γ_1	γ_2
White-American	>95%	< 5%	< 5%	50%	50%
White-European	85%	15%	< 5%	60%	40%
Japanese	15%	85%	< 5%	95%	5%
Black-American	85%	< 5%	15%	85%	15%

Bosron et. al. 1993 & Crabb 1995

Frequency of ADH Alleles in Racial Populations

The polymorphic forms of ADH (Class I ADH₁, ADH₂ and ADH₃ genes) vary to some extent in different racial groups as shown in the above Table. To date, there are no clear associations between the various ADH isozymes and the development of alcoholic liver disease, or the susceptibility to alcohol actions, or the propensity to consume ethanol. Further research in this area is required, as is research on what other substrates the various ADH isoforms oxidize and the influence of nutrition and hormones on content and activity of these ADH isoforms. In view of the high V_{max} for the ₂ and ₃ isoforms compared to the ₁ isoforms, rates of ethanol oxidation should theoretically be higher in individuals with the ₂ and/or ₃ alleles. Some studies have supported this but more research is required.

An interesting recent study suggested that individuals carrying 1 or 2 copies of the ₂ allele and 1 copy of the inactive ALDH 2 allele (see acetaldehyde slide) had the lowest risk for alcoholism, followed by individuals with the ₂ allele and the normally active ALDH, with the most sensitive being individuals with the ₁ allele plus the normally active ALDH. This “protection” against alcoholism afforded by the ₂ plus inactive ALDH alleles was suggested to reflect high acetaldehyde levels, which would accumulate and cause aversive reactions if ethanol was consumed.

Reference

Bosron et. al. 1993 & Crabb 1995.

Crabb et. al. J. Lab-Clin. Med **122**: 234-240, 1993.

McCarver et. al. J. Pharmacol Exp. Ther. **283**: 1095-1101, 1997.

Chen et. al. Amer J. Human Genet. **65**: 795-807, 1999.

Hepatic Redox State

- ADH and ALDH reactions use NAD^+ and produce NADH.
- Cytosolic Redox State.
- Mitochondrial Redox State.
- Effects on Liver Metabolism.

Hepatic Redox State

ADH and ALDH reactions use NAD^+ and produce NADH

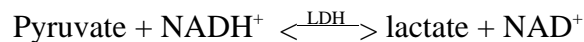
Cytosolic Redox State

Mitochondrial Redox State

Effects on Liver Metabolism

Because the ADH and ALDH reactions reduce NAD^+ to NADH, the cellular NAD^+/NADH redox ratio is lowered as a consequence of ethanol metabolism. This has profound effects on other liver metabolic pathways which require NAD^+ .

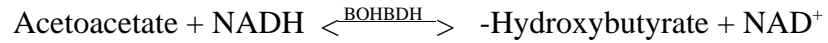
Since the ADH reactions occur in the cytosol, the cytosolic NAD^+/NADH redox ratio will be lowered. This ratio is reflected by the pyruvate/lactate ratio because of the reaction.



The high activity of lactate dehydrogenase (LDH) keeps the pyruvate/lactate ratio equilibrated with the NAD^+/NADH ratio.

Since the ALDH reaction occurs largely in the mitochondria, the mitochondrial NAD^+/NADH redox ratio will be lowered. This reaction is reflected by the b-hydroxybutyrate/Acetoacetate ratio because of the reaction.

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LDH is a cytosolic enzyme, whereas -hydroxybutyrate dehydrogenase is mitochondrial.

Some important reactions which are inhibited because of this decreased NAD^+/NADH redox ratio are:

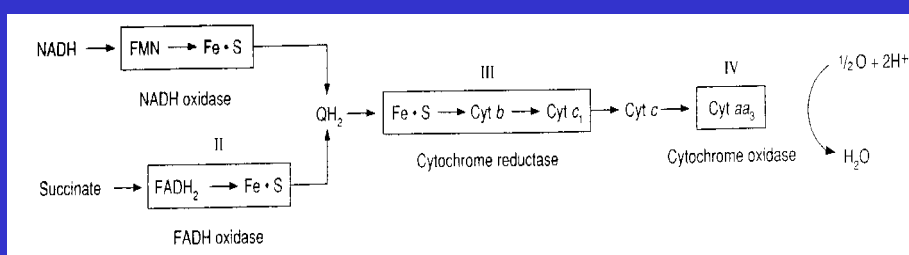
- .Glycolysis
- .Citric Acid Cycle (Ketogenesis favored)
- .Pyruvate Dehydrogenase
- .Fatty Acid Oxidation
- .Gluconeogenesis

References

- Williamson et al. J. Biol. Chem. 246: 7632-7641, 1971.
Veech et al. Biochem. J. 127: 387-397, 1972.

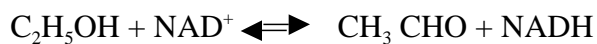
Reoxidation of NADH Generated by the ADH Reaction

- There is a need to reoxidize NADH back to NAD⁺.
- Cytosolic pathways are not sufficient.
- NADH must be reoxidized by the mitochondrial electron transfer pathway shown below.



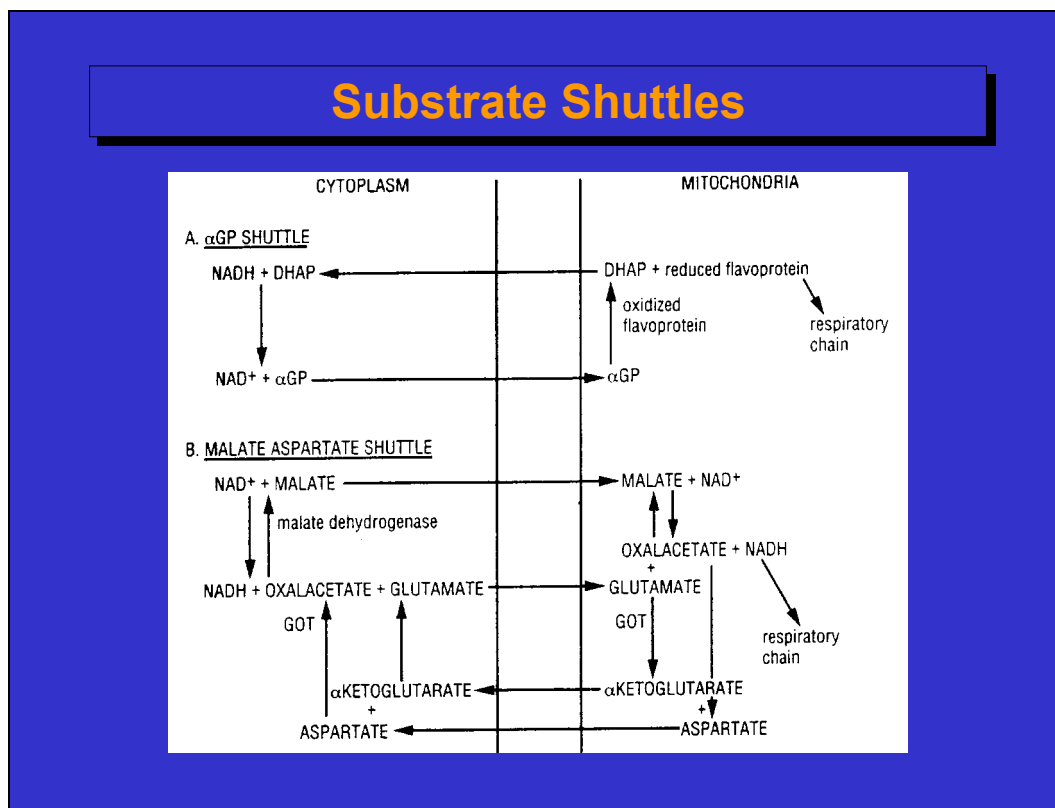
Reoxidation of NADH Generated by the ADH Reaction

- There is a need to reoxidize NADH back to NAD⁺
- Cytosolic pathways are not sufficient
- NADH must be reoxidized by the mitochondrial electron transfer pathway shown below. To maintain effective rates of ethanol oxidation by ADH, it is important to regenerate NAD⁺ from the NADH produced by the ADH reaction.



Under certain conditions, the rate of oxidation of ethanol can be limited by the reoxidation of NADH. The major system for reoxidizing NADH is the mitochondrial electron transfer system. By coupling NADH reoxidation to this system, energy will be produced from ethanol metabolism (7 kcal per g ethanol).

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**Substrate Shuttles**

Because intact mitochondria are not permeable to NADH, it is necessary to transfer the reducing equivalents of NADH present in the cytosol into the mitochondria by substrate shuttle mechanisms. The two major substrate shuttles are the α -glycerophosphate shuttle and the malate-aspartate shuttle, shown above. Based upon studies with enzyme inhibitors, transport carrier inhibition and calculations of flux, the malate-aspartate shuttle plays the major role in transferring reducing equivalents into the mitochondria.

The rate of ethanol oxidation can be limited by the transfer of reducing equivalents into mitochondria (shuttle capacity) or by the actual capacity of the mitochondrial respiratory chain to oxidize these reducing equivalents. Shuttle capacity may become limiting under fasting metabolic states as the levels of shuttle components decrease. This may contribute to the lower rates of ethanol oxidation (in addition to lower ADH content) in the fasting metabolic state. Agents or conditions which enhance reoxidation of NADH by the respiratory chain can increase the rate of ethanol metabolism e.g. uncoupling agents can accelerate ethanol oxidation in the fed metabolic state.

References

- Meijer et. al. Biochem. J. 150: 205-209, 1975.
Cederbaum et. al. Arch. Biochem. Biophys. 183: 638-646, 1977.

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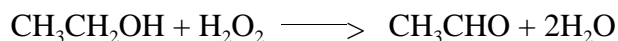
Catalase-Dependent Oxidation of Ethanol



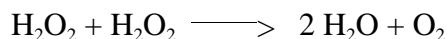
Catalase, a heme enzyme, is found in the peroxisomal fraction of the cell. This is an important antioxidant enzyme since it normally catalyzes the removal of H_2O_2 .



Catalase-Dependent Oxidation of Ethanol



Catalase, a heme enzyme, is found in the peroxisomal fraction of the cell. This is an important antioxidant enzyme since it normally catalyzes the removal of H_2O_2



This pathway is limited by the rather low rates of H_2O_2 generation produced under physiological cellular conditions (less than 4 $\mu\text{mol/g}$ liver/hr, only 2% that of ethanol oxidation) and appears to have an insignificant role in ethanol oxidation by the liver. However, some oxidation of ethanol by catalase may occur in tissues where ADH or CYP2E1 (discussed next) are absent or low in content, e.g. brain. The peroxisomal fatty acid oxidation system produces H_2O_2 , which may promote some oxidation of ethanol by catalase.

Reference

Thurman & Handler, Drug Metab. Rev. 20: 679-688, 1989.

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Microsomal (Cytochrome P450) Oxidation of Ethanol

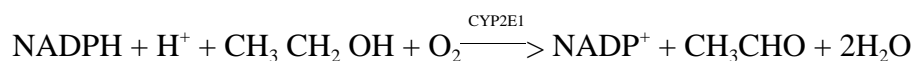
$$\text{NADPH} + \text{CH}_3\text{CH}_2\text{OH} \xrightarrow[\text{+ O}_2 + \text{H}^+]{\text{CYP2E1}} \text{NADP}^+ + \text{CH}_3\text{CHO} + 2\text{H}_2\text{O}$$

CYP2E1 - Function

Role in Ethanol Oxidation

Inducibility

Microsomal (Cytochrome P450) Oxidation of Ethanol



CYP2E1- Function
 Role in ethanol oxidation
 Inducibility

Cytochrome P450s are a family of heme enzymes which are involved in the oxidation of steroids, fatty acids and numerous xenobiotics ingested from the environment. Highest levels of cytochrome P450 are in the liver, where they are present mainly in the endoplasmic reticulum (microsomal fraction). Some P450 is also found in mitochondria. P450 functions in conjunction with other microsomal enzymes such as NADPH-cytochrome P450 reductase and cytochrome b₅.

There are many isoforms of P450; over 100 gene families have been identified. The P450s are arranged in families based on sequence homologies. CYP2E1 is a P450 which has the highest activity for oxidizing ethanol to acetaldehyde. Besides ethanol, CYP2E1 can oxidize many other compounds including acetone, benzene, other alcohols. A clear

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physiological function for CYP2E1 has not been identified, although it may metabolize acetone eventually to glucose.

The K_m of CYP2E1 for ethanol is about 10 mM, which is about 10-fold higher than the K_m of ADH for ethanol. At low ethanol concentrations, CYP2E1 may account for about 10% of the total ethanol oxidizing capacity of the liver. However in view of its higher K_m , the relevance of CYP2E1 in ethanol oxidation increases as blood ethanol concentrations increase. Ethanol oxidation increases at higher ethanol concentrations, and much of this increase is probably due to CYP2E1 metabolism of ethanol.

Many P450s are induced by their substrates; this helps to remove the xenobiotic from the body. CYP2E1 levels are increased by chronic ethanol administration by a mechanism largely involving protection of the enzyme against proteolysis by the macromolecular proteasome complex. CYP2E1 is also induced in diabetics, in the fasted nutritional state and by certain drugs, including the ADH inhibitors pyrazole and 4-methylpyrazole. Because of its inducibility, CYP2E1 may play an important role in ethanol metabolism after chronic ethanol consumption, i.e. in alcoholics. As many as 13 different CYP2E1 polymorphisms have been identified. Some of these may be important as risk factors for carcinogenicity of tobacco or certain toxins, however, there is no evidence linking any of these polymorphisms to the frequency of alcohol liver damage.

References

- Lieber, C.S. Alcoholism: Clin. Exp Res. **23**: 991-1007, 2000.
Koop and Tierney Bio Essays **12**: 429-435, 1990.

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Alcohol-Drug Interactions: The CYP2E1 system can explain

- Increased sensitivity of active drinkers to certain drugs.
- Resistance of alcoholics, in the absence of ethanol, to certain drugs.
- Increased toxicity of certain chemicals in alcoholics.
- Ethanol-dependent oxidative stress.

Alcohol-Drug Interactions-The CYP2E1 system can explain

- a) .Increased sensitivity of active drinkers to certain drugs.
- b) Resistance of alcoholics, in the absence of ethanol, to certain drugs.
- c) .Increased toxicity of certain chemicals in alcoholics.
- d) .Ethanol-dependent oxidative stress.

Since ethanol and certain drugs compete for metabolism by CYP2E1, active drinkers will often display an enhanced sensitivity to certain drugs as ethanol will inhibit the metabolism of the drug and thereby prolong its half-life.

Conversely, since CYP2E1 is induced after chronic ethanol consumption, metabolism of drugs which are also substrates for CYP2E1 will be increased. This will decrease the half-life of the drug, thus, decrease the effectiveness of the drug when ethanol is not present.

CYP2E1 is very active in oxidizing many chemicals to reactive intermediates, e.g. carbon tetrachloride, benzene, nitrosamines, acetaminophen, halothane. Toxicity of these agents is enhanced in alcoholics.

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The CYP2E1 catalytic turnover cycle results in the production of relatively large amount of reactive oxygen intermediates such as the superoxide radical and hydrogen peroxide. This may be important in mechanisms of alcoholic liver injury involving oxidative stress.

Reference

- Lieber, C.S. *Physiol Rev* **77**: 517-544, 1994.
Koop, D.R. *Faseb J.* **6**: 724-730, 1992.
Dai. et. al. *Biochemistry* **32**: 6928-6937, 1993.

Metabolic Adaptation (Tolerance)

Besides CNS adaptation, alcoholics (in the absence of liver disease) often display an increased rate of blood alcohol clearance. This is called metabolic tolerance or adaptation. Suggested mechanisms include:

1. Induction of ADH.
2. Increased shuttle capacity.
3. Increased reoxidation of NADH.
4. Induction of CYP2E1.
5. Release of cytokines or prostaglandins which increase oxygen consumption by the hepatocytes.

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- e) Release of cytokines or prostaglandins which increase oxygen consumption by the hepatocytes.

Class I ADH is generally not inducible. Further work with the many human isoforms is necessary.

Substrate shuttle capacity and transport of reducing equivalents into the mitochondria is not altered by chronic ethanol consumption, nor is the normal impermeability of mitochondria to NADH.

A major theory to explain metabolic adaptation – the “Hypermetabolic state hypothesis “ – postulates that changes in thyroid hormone levels increases $(\text{Na}^+ + \text{K}^+)$ -activated ATPase, with the subsequent increase of ADP levels. This increases the state 3

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mitochondrial oxygen consumption, therefore, increasing NADH reoxidation. Increased oxygen consumption may cause hypoxia, especially to hepatocytes of zone 3 of the liver acinus, the region where ethanol toxicity originates (centrilobular hypoxia hypothesis). CYP2E1 levels are enhanced after ethanol treatment and since CYP2E1 is the most active cytochrome P450 for oxidizing ethanol, this may play an important role in metabolic tolerance.

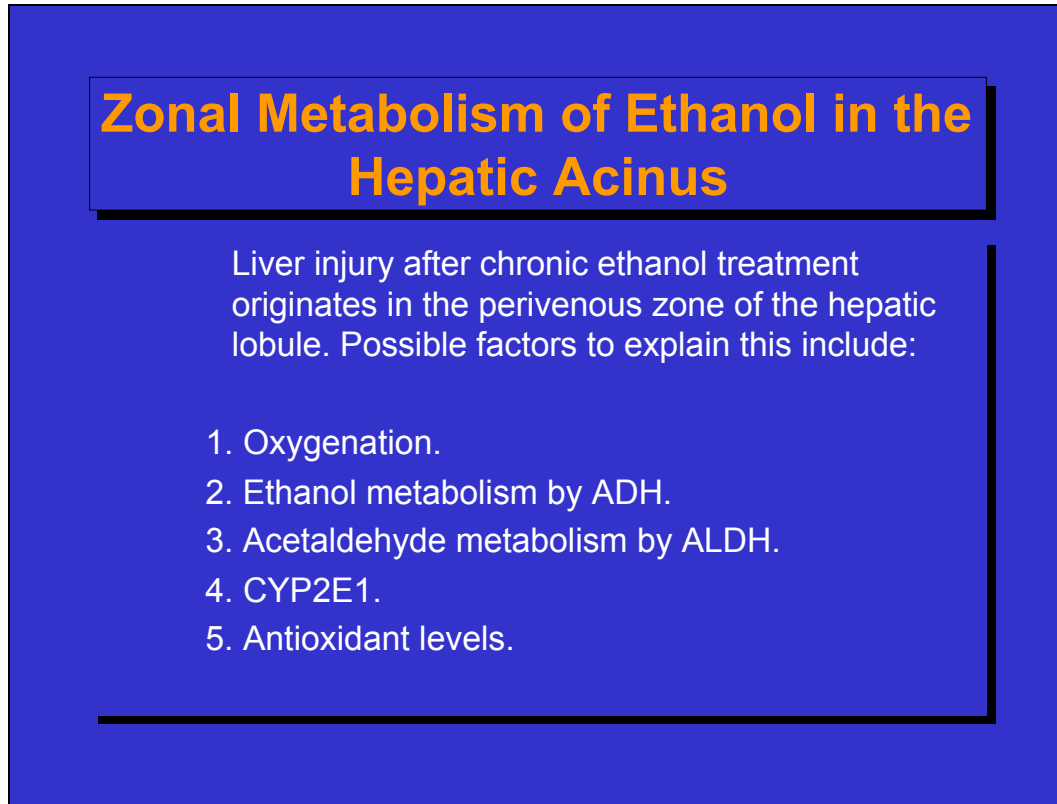
Ethanol, perhaps via increasing endotoxin levels, may activate non-parenchymal cells such as Kupffer cells to release mediators (cytokines and prostaglandins) which stimulate oxygen consumption, thereby NADH reoxidation, by parenchymal cells.

The so-called swift increase in alcohol metabolism (SIAM) refers to an increased rate of ethanol metabolism within a few hours after ethanol administration in vivo or in vitro. SIAM may be mediated by catecholamines, endotoxin or eicosanoids, each causing elevated oxygen uptake by the liver.

References

- Israel et al. (1975) *Fed. Proc.* **34**, 2052-9.
Cederbaum et al. (1978) *Biochem. Pharmacol.* **27**, 7-15.
Bradford et al. (1999) *J. Pharmacol. Exp. Ther.* **288**, 254-9.

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Zonal Metabolism of Ethanol in the Hepatic Acinus

Liver injury after chronic ethanol treatment originates in the perivenous zone of the hepatic lobule. Possible factors to explain this include:

1. Oxygenation.
2. Ethanol metabolism by ADH.
3. Acetaldehyde metabolism by ALDH.
4. CYP2E1.
5. Antioxidant levels.

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- c) Acetaldehyde metabolism by ALDH.
- d) CYP2E1.
- e) Antioxidant levels.

Oxygenation is low in this zone since there is an oxygen gradient across the liver lobule and less oxygen reaches these hepatocytes in the perivenous zone. This is exacerbated after chronic ethanol administration which increases hepatic oxygen uptake, so even less oxygen reaches the perivenous hepatocytes.

2 & 3- ADH and ALDH, and rates of ethanol and acetaldehyde metabolism appear to be evenly distributed across the liver lobule. However, because of the lower oxygen tension, there is a more pronounced reduction of the hepatic redox state produced by ethanol in the perivenous zone.

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CYP2E1 is largely in the perivenous zone. This may explain why toxicity of drugs which are metabolized by CYP2E1 to reactive metabolites, e.g. CCl₄, halothane or acetaminophen occurs in the perivenous zone.

.Level of antioxidants, such as glutathione and glutathione peroxidase, are lower in the perivenous zone.

References

- Lindros, K. O. (1997) *Gen. Pharmacol.* **28**, 191-6.
Vaananen et al. (1985) *Alcohol Clin. Exp. Res.* **9**, 315-21.
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Other Possible Pathways of Ethanol Metabolism

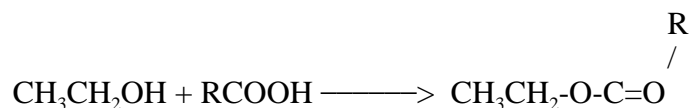
- Conjugation reactions.
- Fatty acid ethyl esters.
- Oxygen radical-dependent reactions.

Other Possible Pathways of Ethanol Metabolism

- a) Conjugation reactions.
- b) Fatty acid ethyl esters.
- c) Oxygen radical-dependent reactions.

Ethanol can react with glucuronic acid or with sulfate to form ethylglucuronide or ethylsulfate. Such soluble conjugates are readily excreted. Cofactor availability and the poor affinity for ethanol by most conjugation enzymes limit these pathways. Ethyl glucuronide has been suggested to be a marker for alcohol consumption because it can persist and be detected for an extended time period after alcohol has been completely eliminated from the body. Detection of this conjugated metabolite of ethanol may serve as a marker for relapse control.

Enzymes known as fatty acid ethyl ester synthases can catalyze the following reaction:

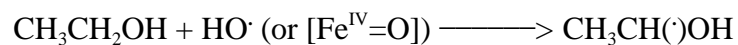


FAEES are present in brain, heart, pancreas, adipose tissue. The K_m for ethanol is usually very high. Some FAEES are isoforms of glutathione transferase. FAEE may disrupt

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biological membranes and could contribute to the toxicity of ethanol in tissues lacking ADH or CYP2E1.

Ethanol is an oxygen radical scavenging agent and can be oxidized to acetaldehyde upon reaction with hydroxyl radical or ferryl type species:



While not likely to be metabolically significant in view of the toxicity of such radicals, ethanol radicals such as the 1-hydroxyethyl radical can be generated from such reactions.

References

- Wurst et al. (2000) *Alcohol* **20**, 111-6.
Laposata, M. Prostaglandins, Leukotrienes and Essential Fatty Acids. **60**, 313-5, 1996.
Cederbaum, A. I. (1987) *Ann N. Y. Acad. Sci.* **492**, 35-49.

Acetaldehyde Metabolism

The balance between the various ADH and ALDH isoforms regulates the concentration of acetaldehyde, which is important as a key risk factor for the development of alcoholism.

1. Isoforms of ALDH.
2. Effects of Alcohol Consumption.
3. Alcohol-aversive drugs.
4. Significance of Acetaldehyde Removal.

Acetaldehyde Metabolism

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- a) Isoforms of ALDH
- b) Effects of Alcohol Consumption
- c) Alcohol-aversive drugs
- d) Significance of Acetaldehyde Removal

.Most of the acetaldehyde produced from the oxidation of ethanol is further oxidized in the liver by a family of ALDH isoforms. Major ALDH isoforms exist in the mitochondrial, microsomal, and cytosolic compartments. Mitochondria contain a low K_m ALDH in the matrix space (class II ALDH) and a high K_m ALDH in the outer membrane, microsomes contain a high K_m ALDH, while the cytosol contains an intermediate (class I ALDH) and a high K_m (class III ALDH) ALDH. The high K_m cytosolic ALDH can be induced by certain drugs such as phenobarbital and dioxins and is found in high amounts in tumor cells. Acetaldehyde can also be oxidized by aldehyde oxidase, xanthine oxidase, and by CYP2E1, but these are insignificant pathways. The low K_m mitochondrial ALDH is responsible for oxidizing the majority of acetaldehyde produced from the oxidation of ethanol, although in human liver, the class I cytosolic

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ALDH may also contribute. The class I and II ALDHs are tetrameric enzymes, with subunit molecular weights of 54 kDa.

.In general, the capacity of ALDH to remove acetaldehyde exceeds the capacity of acetaldehyde generation by the various pathways of ethanol oxidation. Therefore, circulating levels of acetaldehyde are usually very low. Chronic ethanol consumption decreases acetaldehyde oxidation, either due to decreased ALDH activity or to impaired mitochondrial function. Acetaldehyde generation is increased by chronic ethanol consumption because of metabolic adaptation. As a result, circulating levels of acetaldehyde are usually elevated in alcoholics because of increased production, decreased removal or both.

The basis of action for certain alcohol-aversive drugs such as disulfiram (Antabuse) or cyanamide is to inhibit ALDH, and therefore ethanol oxidation. The resulting accumulation of acetaldehyde causes a variety of unpleasant effects such as nausea, sweating, vomiting, and increased heart rate, if ethanol is consumed with these drugs. Certain individuals, usually of Asian extraction, have an inactive mitochondrial ALDH because of a single amino acid substitution; glutamate 487 is converted to a lysine residue; this causes a large decrease in affinity for the NAD⁺ cofactor. Acetaldehyde is poorly eliminated by these individuals and as a consequence, little ethanol is consumed. ALDH2 deficient individuals are at lower risk for alcoholism. They may have possible increased risk for liver damage if alcohol continues to be consumed.

Acetaldehyde is a reactive compound and can interact with thiol and amino groups of amino acids in proteins. Formation of acetaldehyde adducts with proteins may cause inhibition of that protein's function and/or cause an immune response. ALDH is important not only for removing acetaldehyde, but also for the removal of other aldehydes, including biogenic aldehydes and lipid peroxidation-derived aldehydes. Effective removal of acetaldehyde is important not only to prevent cellular toxicity, but also to maintain efficient removal of ethanol, e.g., acetaldehyde is a product inhibitor of ADH. The class I ALDH can oxidize retinal to retinoic acid; the possibility that high levels of acetaldehyde compete with retinal for oxidation by class I ALDH may be of developmental significance.

Forensic Considerations

- Time lag.
- Ethanol elimination rates.
- Partition ratio.
- Fluctuations and anomalies.
- Back extrapolation procedures.
- Other factors.

Forensic Considerations

- a) Time lag.
- b) Ethanol elimination rates.
- c) Partition ratio.
- d) Fluctuations and anomalies.
- e) Back extrapolation procedures.
- f) Other factors.

The concentration of alcohol in blood (or breath) may be important for evaluating the degree of acute alcohol-induced impairment of driving ability or other actions. It may also be necessary to calculate the blood alcohol concentration at the relevant time e.g. an accident occurring 2 hours previously, not the present time.

There is a time lag from the period of intake of alcohol until the peak blood alcohol concentration is reached. This is regulated by the rate of alcohol absorption after oral intake. Alcohol absorption is regulated by numerous factors (absorption slide). Because of these factors, the time to reach peak blood alcohol concentrations varies greatly e.g. 14 min to 130 min found in a controlled study (mean time = 57 min for men, 42 min for women). Hence, if alcohol absorption is not complete, peak alcohol levels will not yet be reached.

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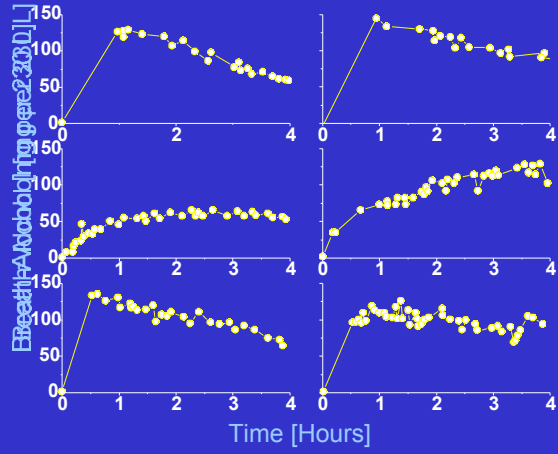
There is a 3-4 fold variation in ethanol elimination rates because of genetic and environment influences. In one controlled study assaying the rate of decrease of breath alcohol concentration in 134 men, rates varied from 5.9 to 27.9 mg per 230 liters air per hour. The absence or presence of food is very important as a regulatory factor as is sex, age, body weight, and even the time of day can influence the rate of ethanol metabolism.

Breath alcohol is usually determined to reflect blood alcohol. The typical value assumed to reflect the mean partition factor between blood alcohol and breath alcohol in the post absorptive state is 2300 : 1. In a controlled study of 393 men, this value ranged from 1706 : 1 to 3063 : 1. Blood : urine partition ratio shows an even greater variability (0.21 : 1 to 2.66 : 1).

Not all blood or breath alcohol curves follow the idealized Widmark pattern shown in panel A. Alcohol absorption is not always complete at 60 to 90 min (panel D), peak alcohol concentrations cannot always be predicted (panel C), ethanol elimination is not always linear as there are fluctuations in the decay curves (panel E, F).

To back extrapolate from a blood alcohol concentration taken at one time to a value at an earlier time, one needs to know an accurate value for the ethanol elimination rate, which as discussed in B, is not likely in view of the large inter-individual variability in ethanol elimination kinetics. Also, to back extrapolate, one uses linear kinetics, which may not be correct, especially for the concentration range under consideration. The variability in individual rates of ethanol elimination, the difficulty in knowing exactly when absorption of ethanol was completed, and the newer model showing that rates of ethanol elimination can change at different blood ethanol concentrations casts doubt on the validity of such retrograde calculations to an earlier blood ethanol concentration.

Breath Alcohol Levels



Questions for Consideration

- What limits and regulates ethanol metabolism in vivo?
- What is the mechanism(s) responsible for metabolic tolerance?
- Is it ethanol *per se*, or ethanol-derived metabolites which play a key role in organ damage? What might be the consequences of attempting to accelerate ethanol metabolism?
- What is the significance of first pass metabolism by the stomach?
- What is the role, if any, of the various ADH in oxidation of endogenous substrates, ethanol metabolism and ethanol toxicity? The hypothesis that ethanol or acetaldehyde inhibit the oxidation of physiologically important endogenous substrates of ADH and ALDH (e.g. retinol → retinal → retinoic acid) and that this may contribute to the adverse actions of ethanol requires further studies.

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Questions for Consideration

- Can the various ADH and ALDH or polymorphic forms of CYP2E1 be of predictive value or serve as markers to identify individuals who are susceptible to developing alcoholism? Can non-invasive probes be developed to measure the various isoforms present?
- Are there population and gender differences in rates of ethanol elimination, and if so, are such differences explained by the varying ADH and ALDH isoforms present in that population?
- What controls the expression of the various isoforms at transcriptional level, and are there posttranscriptional modifications? What dictates the turnover of these enzymes which may be important in regulating the amount of active enzyme present in the cells e.g. CYP2E1.

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Questions for Consideration

- Why are calories from ethanol less efficient in providing energy than calories from other nutrients? What is the mechanism by which food increases ethanol metabolism?
- What role, if any, does acetate play in the metabolic actions of ethanol.
- Can we build appropriate models and rate equations to kinetically describe the process of ethanol elimination under various conditions?

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