CHEM 496 Topics in Chem & Biochem: Biochemistry, Physiology & Neurochemistry of Beer, Wine & Alcohol

Block III – EtOH metabolism, alcohol use disorder & pathology

1

Alcohol – who we metabolize EtOH, what are the effects (chronic and acute), and how does it impact our brain?

Alcohol as a Drug

- Alcohol is a psychoactive drug that is a **CNS depressant**.
- Some claim that alcohol is the most widely consumed drug in the world and for some is as much a part of daily life as eating.
- Alcohol is an addictive substance. Of the approximately 2 million receiving treatment for drug abuse, 64% are being treated for alcoholism.

2

Negative Impact of Alcohol

- 100,000 – 140,00 US deaths and 3 mil global deaths associated with alcohol each year (2.5 males for each female).
- Nearly 50% of all Americans will be involved in an alcohol-related traffic accident at some time during their lives.
- More than 2% of nighttime drivers have a blood-alcohol content that exceeds 0.08%, the legal amount in most states.

3

Negative Impact of Alcohol

- Shortened the lives of those lost by an average of 26 years for a total of nearly 3.6 million – most due to long term usage leading to cancer, liver disease, and heart disease
- Costs over 287 billion in the US each year
- Illness, accidents, violence, and crime related to alcohol use
- Consumption by college students causes approximately 2,000 deaths per year.
- Fetal alcohol syndrome
- Alcohol is the second leading cause of premature death in America
- 2 million people admitted for treatment of alcohol use disorder annually

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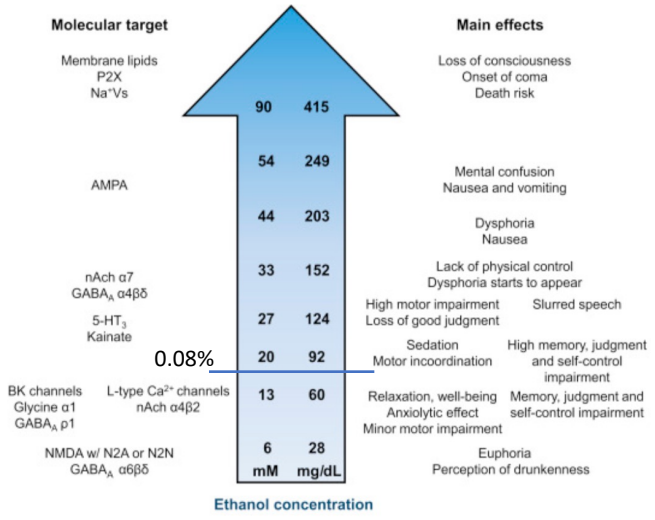
Main Ethanol Targets in the Brain



M.T. Marin, G. Morais-Silva, in Addictive Substances and Neurological Disease, 2017

0.08% is legal intoxication levels in most states = 17 mM (mmol/L)

- **Concentration dependent effects**
- Interaction with receptors
  - Receptors
    - GABAA-Rs,
    - NMDA-Rs
    - GlyRs
    - 5-HT_3Rs
  - Voltage gated channels
    - L-type Ca^{2+}Vs
    - BK (big potassium)

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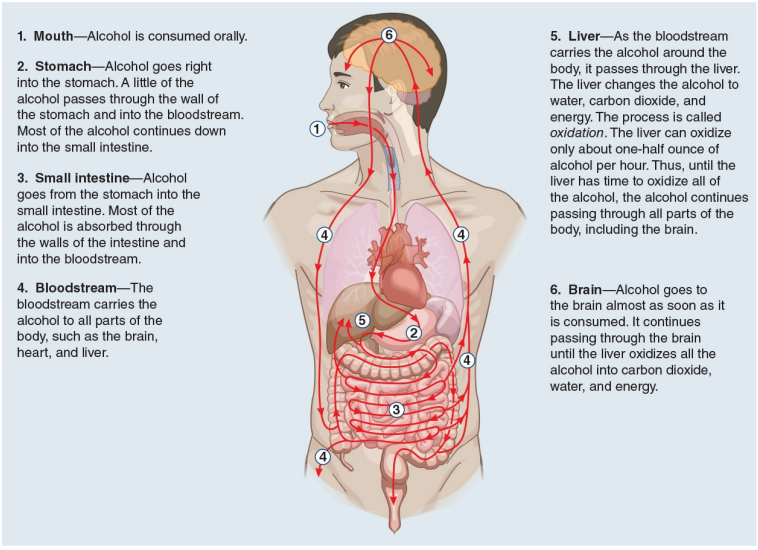
Blood Alcohol Concentration (BAC)

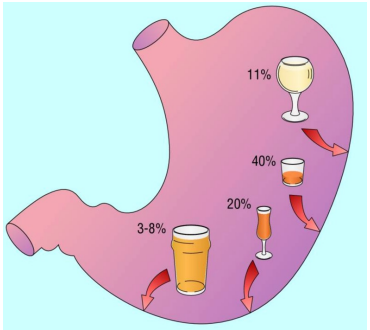
BAC depends on rate of EtOH 4 factors:

- Absorption, distribution, metabolism and excretion



Absorption: Rate of absorption

EtOH is crosses the gut into the blood stream by diffusion via concentration gradient – thus shots vs 3.2% beer effect



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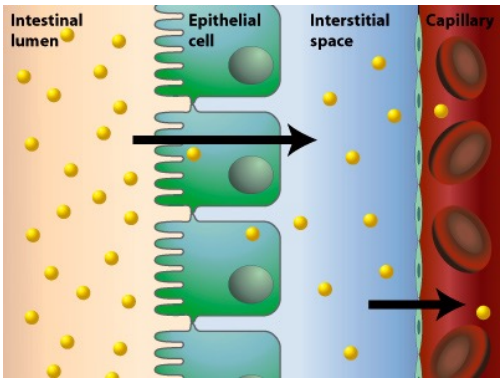
Blood Alcohol Concentration (BAC)

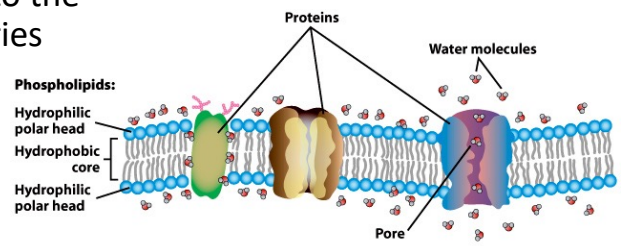
BAC depends on rate of EtOH 4 factors:

- Absorption, distribution, metabolism and excretion

Absorption: Crossing the membrane from the gut through the epithelial cells into the interstitial space and then the capillaries



Driven by concentration gradient



- EtOH's H-bonding OH group somewhat limits the free diffusion through polar membrane.
- EtOH can move through water channels and pores created by transmembrane proteins. A filtration process

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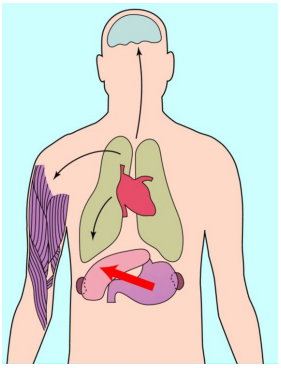
Blood Alcohol Concentration (BAC)

BAC depends on rate of EtOH 4 factors:

- Absorption, distribution, metabolism and excretion

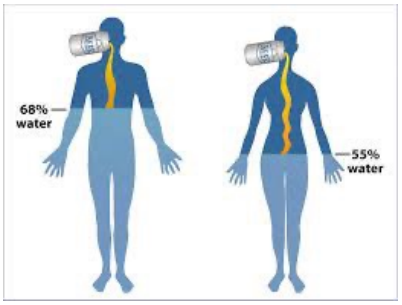
~20-30% of EtOH is absorbed in the Stomach and the rest in the small intestine



EtOH is very water soluble.

– all of the tissues are exposed EtOH in proportion to their relative con tent of water.

- Equilibrium in tissue is reached quickly with the concentration of EtOH in blood



- EtOH's is nearly insoluble in fat.
- Women generally have higher % body fat and less volume of body water – lower volume of distribution (CV=CV)
- Women will have higher peak BAC given the same dose as men.
- Men metabolize EtOH in stomach at a greater rate than females contributing to dynamics of BAC between genders

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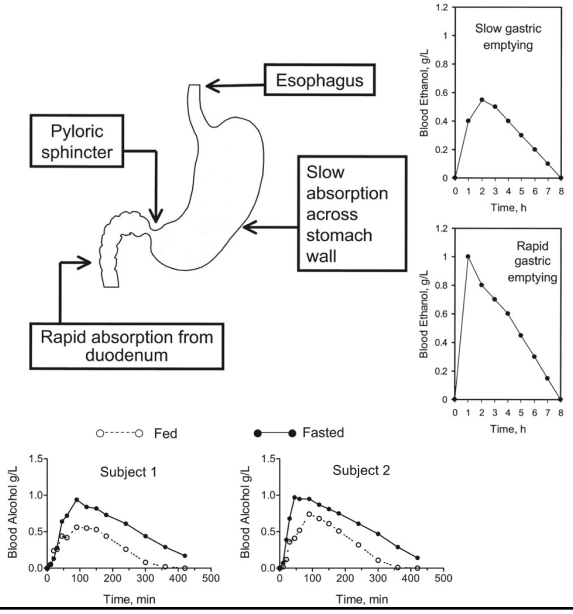
Blood Alcohol Concentration (BAC)

Factors Affecting Alcohol Absorption and distribution



- The more EtOH (% and volume) the greater the concentration gradient -> higher absorption
- Absorption from the duodenum and jejunum is more rapid than the stomach. The longer the EtOH is in the stomach the slower the uptake.
- Food slows gastric emptying and will reduce absorption (don't drink on an empty stomach).
  - Type of food (fatty vs carbo vs protein) has a lesser impact on absorption rate: fat doesn't "absorb" the EtOH
  - Cigarette smoking and high carbohydrate beer slows absorption
  - Carbonated beverages and drinking after fast increases absorption

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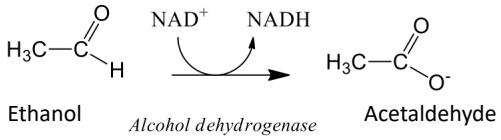
First Pass Metabolism

Some (minor fraction ~10-30%) of the EtOH is metabolized in the stomach (first pass metabolism) rest is metabolized in liver or a small amount is lost as liquid.

First-pass metabolism is a pharmacokinetic concept where some of a drug is metabolized prior to entering the blood



Alcohol Dehydrogenase (ADH class I and II) expressed in stomach.

- Impact of ADH is diminished in rapid stomach emptying
- Alcoholics (especially women) have decreased ADH activity and may contribute to increased sensitivity in women
- Aspirin and anti-ulcer / acid pump blockers inhibit stomach ADH

2-5% of Ethanol is lost unchanged in urine, sweat or breath

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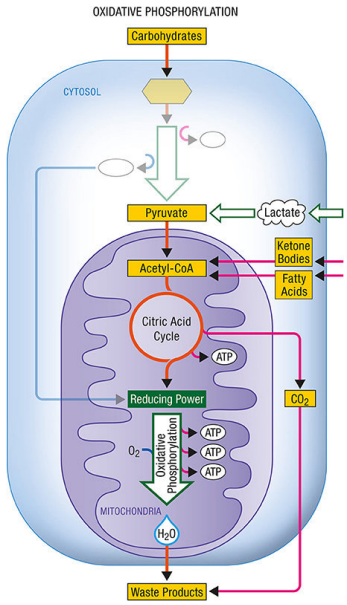
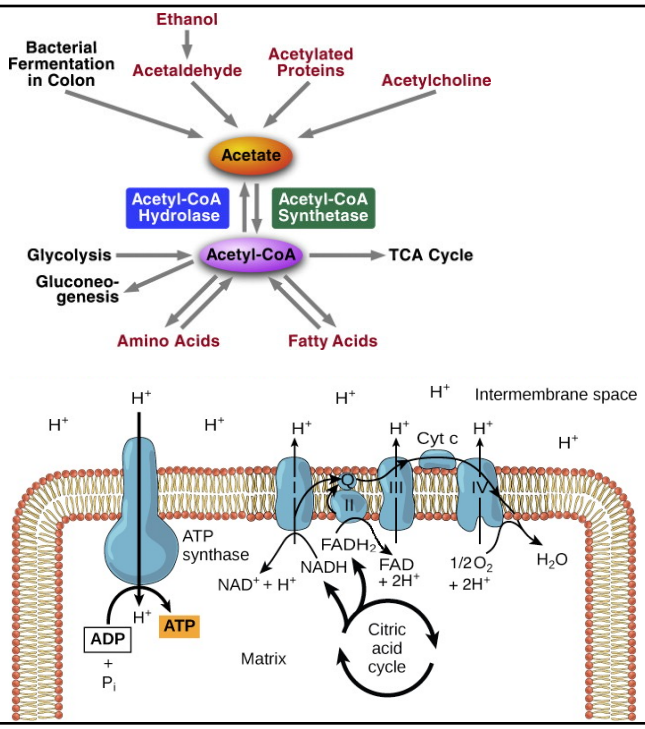
Simplified Metabolism





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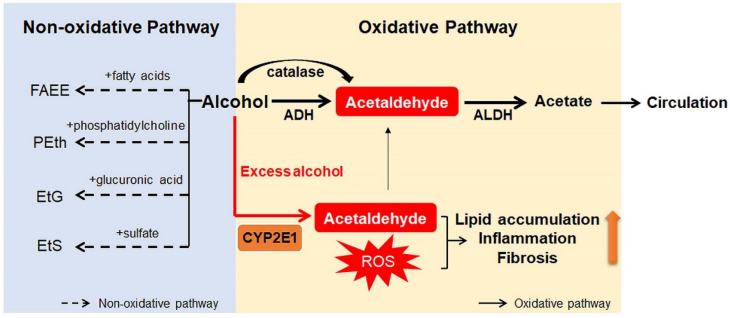
Alcohol Metabolism

Oxidative -Three systems

1) Alcohol dehydrogenase – steady rate based on amount of ADH present
2) Cytochrome P450 system (CYP2E1) - induced by heavy use
3) Catalase

- Occurs primarily in liver but brain can participate
- **Alcohol dehydrogenase is the major component**

Acetate used to make carbohydrate, fat, amino acids, ketone bodies and cholesterol



Non-Oxidative -minor pathway

1) Ethanol esters - conjugation to fatty acids (FAEE)

- PLD generation of PEth
- Conjugation with sugars and sulfates

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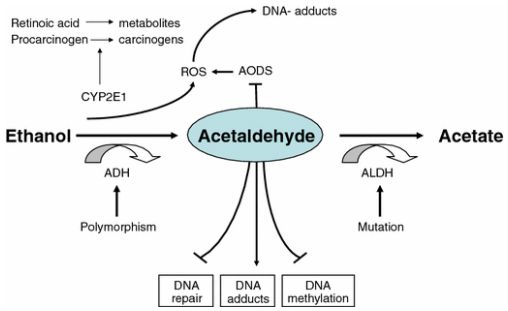
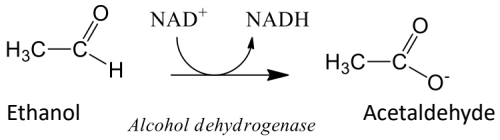
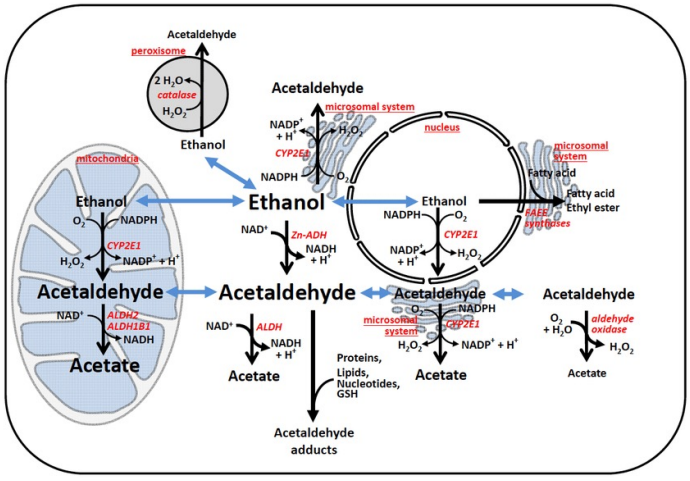
Acetaldehyde – one bad actor

The danger is due to the production of Acetaldehyde and ROS







Circulating acetaldehyde w/o EtOH barely detectable <3µM
In blood with acute dose up to 20 mM!

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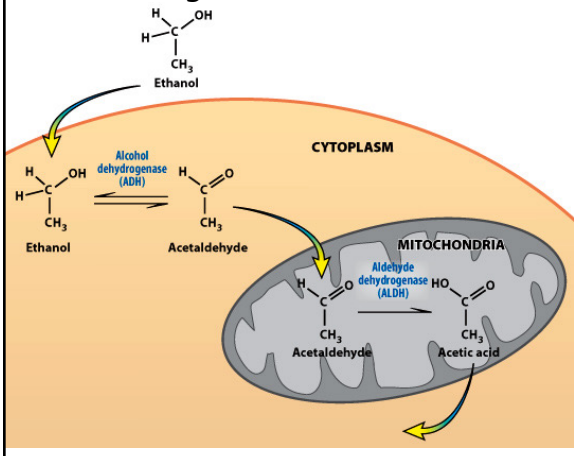
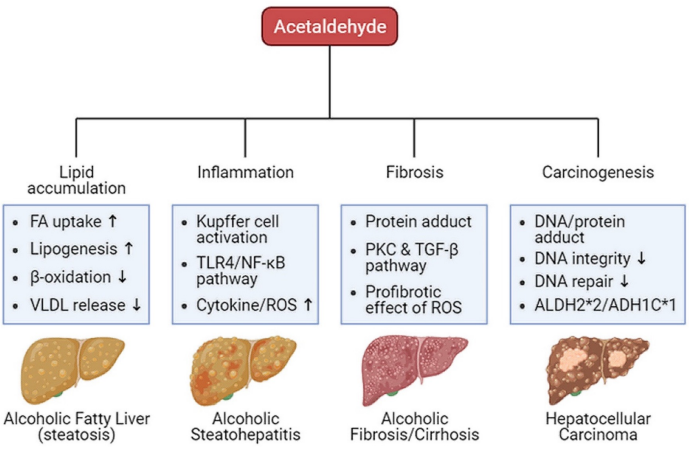
Acetaldehyde Metabolism

Alcohol liver metabolisms produces products that damage the liver resulting in alcoholic liver disease (ALD)





There must be a balance between ADH and ALDH to minimize acetaldehyde buildup

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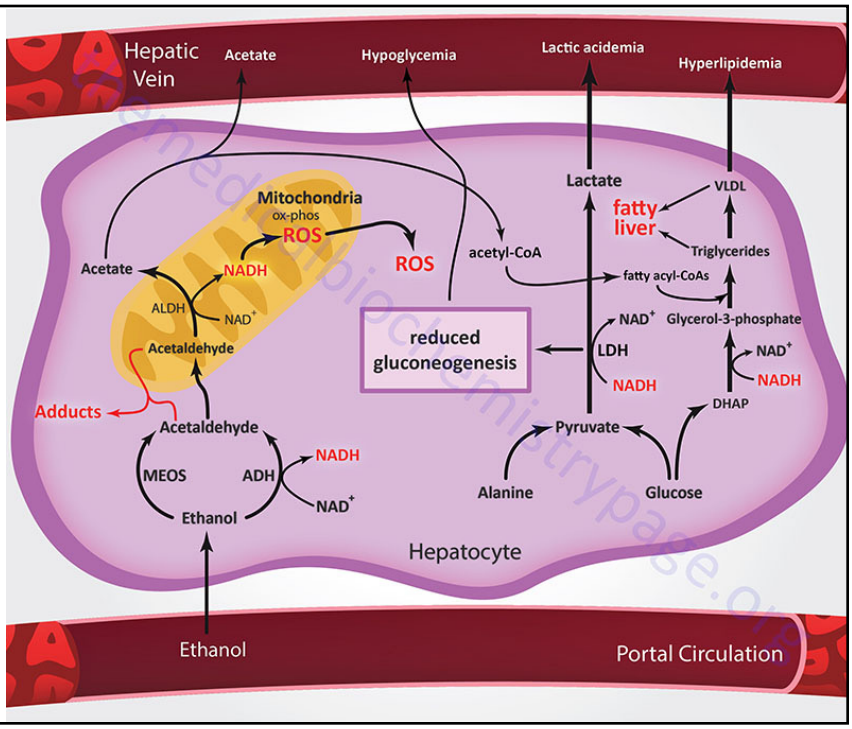
Chronic alcohol ingestion shifts pathways leading to a number of problematic issues for liver.

- Reduced glucose production and hypoglycemia
- Fatty liver via fatty acid synthesis from acetate and the NAD+/NADH ratio
- High VLDL and later LDL and TAG – leading to heart disease
- Metabolic acidosis with lactate production



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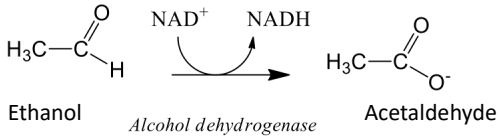
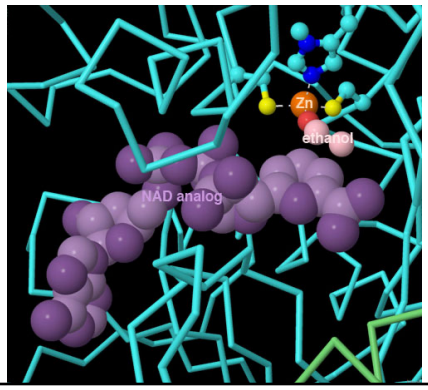
Alcohol Dehydrogenase (ADH)



The bulk of EtOH is metabolized by ADH in either stomach or liver.

- The redox reaction requires Zn^{2+} and NAD^+ cofactors to produce acetaldehyde (a more toxic compound than ethanol).



- *The zinc atom is cradled by three amino acids from the protein: cysteine 46 to the left, cysteine 174 to the right, and histidine 67 above. The ethanol binds to the zinc and is positioned next to the NAD cofactor, which extends below the ethanol molecule.*

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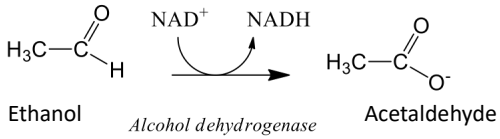
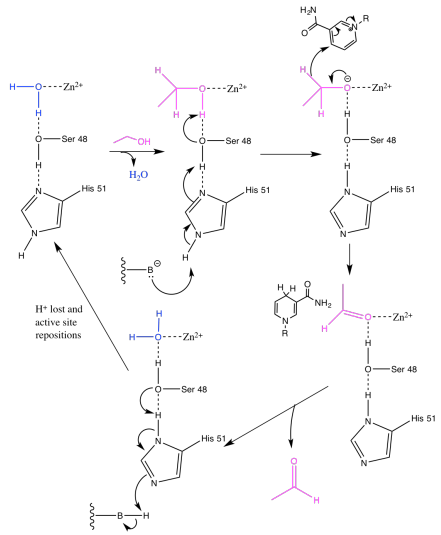
Alcohol Dehydrogenase (ADH)





The essential features of the ADH catalytic cycle

- Water stably is bound to Zn until displaced by Ethanol. Zn is held by two Cys residues.
- His and Ser coordinate to pull the proton from the –OH of Ethanol. This is supported by the hydrogen extraction from His by a deprotonated acidic functional group
- After binding of NAD^+, the water molecule is displaced from the Zn atom by the incoming alcohol substrate.
- Deprotonation of the coordinated alcohol yields a zinc alkoxide intermediate, which then undergoes hydride transfer to NAD^+ to give the zinc bound aldehyde and NADH.
- A water molecule then displaces the aldehyde to regenerate the original catalytic zinc center, and finally NADH is released to complete the catalytic cycle.

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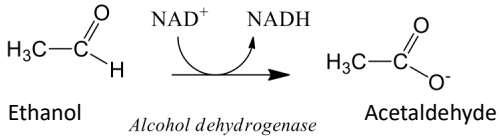
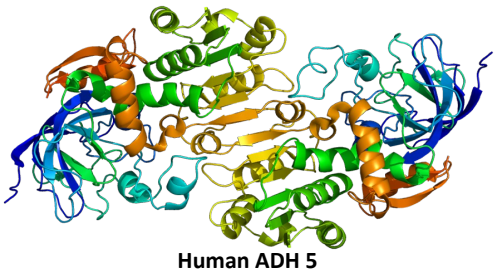
Alcohol Dehydrogenase (ADH)



Structure, kinetics and substrate specificity

ADH is a family of 5 isozymes (ADH1 [A,B&C], ADH4-7) each a dimer expressed in different tissues and with unique kinetic properties.

- Each dimer can be a homodimer or a mixed heterodimer with another isoform if expressed in the same tissue.
- Each of the five genes of ADH are found in close proximity to the others on chromosome 4



Human ADH 5

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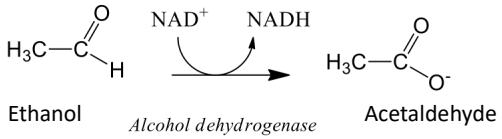
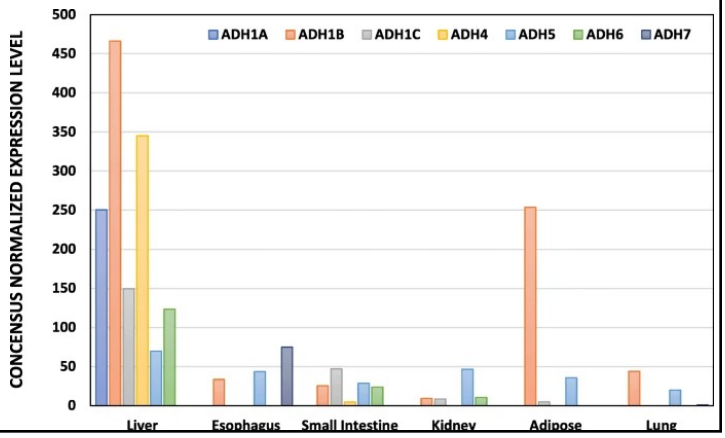
Alcohol Dehydrogenase (ADH)



Structure, kinetics and substrate specificity

- Several ADH isoforms (except ADH7) are expressed in high levels in Liver.
- ADH5 is found in several tissues at high levels
- ADH1B is expressed in adipose which is consistent with ADH role in obesity and insulin resistance.
- ADH5 and ADH7 are the prime enzymes found in stomach.

- There is little evidence of sex or age (after 1 yr) differences in ADH expression
- There is a significant co-occurrence of AUD and depression but while many look for an ADH polymorphism, none has yet been identified.



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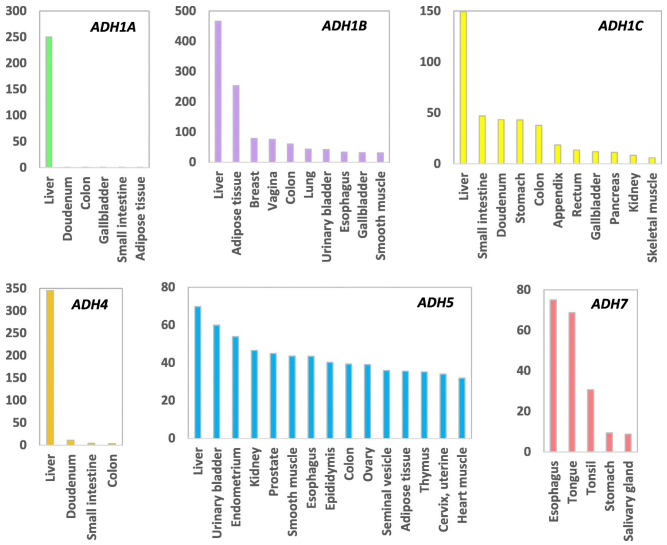
Alcohol Dehydrogenase (ADH)

Structure, kinetics and substrate specificity

In general at modest to high levels of EtOH most enzymes are saturated and metabolism is zero order
- At low concentration elimination slows as the EtOH falls near Km and maximum velocity is no longer taking place

Name/Variant	Km (mM)	Vmax	Tissue
ADH1A	4.0	30	Liver
ADH1B*1 (Arg/Arg) ADH1B*2 (His/Arg) ADH1B*3 (Arg/Cys)	0.05 0.9 40.0	4 350 300	Liver, adipose,
ADH1C*1 (Arg/Ile) ADH1C*2 (Gln/Val)	1.0 0.6	90 40	Liver, gut
ADH4	30.0	20	Liver
ADH5	>1,000	100	Ubiquitous
ADH6	--	--	mRNA no protein
ADH7	10.0	1,800	Throat tongue

mmol/L*	Effect†	BAC (mg/dL)
4–11	Decreased fine motor control	20–50
11–22	Decreased coordination	50–100
22–33	Difficulty standing	100–150
33–55	Difficulty sitting	150–250
66	Unresponsive to voice and/or pain	300
88	Respiratory depression	400



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Alcohol Dehydrogenase (ADH)

Structure, kinetics and substrate specificity

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ADH1A	4.0	30	Liver
ADH1B*1 (Arg/Arg) ADH1B*2 (His/Arg) ADH1B*3 (Arg/Cys)	0.05 0.9 40.0	4 350 300	Liver, adipose,
ADH1C*1 (Arg/Ile) ADH1C*2 (Gln/Val)	1.0 0.6	90 40	Liver, gut
ADH4	30.0	20	Liver
ADH5	>1,000	100	Ubiquitous
ADH6	--	--	mRNA no protein
ADH7	10.0	1,800	Throat tongue

After 4 cans of beer or 6 shots BAC will reach ~110 mg/dl (23 mM) in about an hour after consumption

Legal intoxication in the US (0.08%) corresponds to 17 mM

Levels at 43.2 mM may lose consciousness and above 86 mM is fatal

First Pass Metabolism

- ADH1C: Low Km and moderate Vmax
  - Slow and steady EtOH metabolism
- ADH5: High Km (low affinity) med Vmax
  - This enzyme is important right after drinking when EtOH conc can reach molar levels
  - Helps prevent production of acetaldehyde until EtOH is present
- Those with ADH1B*2 mutations with high Vmax and low Km results in high levels of acetaldehyde at even moderate level of drinking
- Those with ADH1B*3 will have higher EtOH clearance w/ potentially high levels of acetaldehyde but will not metabolize as much EtOH until high levels of BAC

Liver Metabolism

- Major forms ADH1A, ADH1B, ADH4
- Covers a range of Km and moderate clearance rates
- Allows quick and steady metabolism at all levels

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Special Cases

Liver ADH is very low in fetus and elimination of EtOH in fetal tissues is slow (**FAS**).

- There is some evidence that mothers homozygous with **ADH1B*1** (low km low Vmax) mutations had a higher risk of children with FAS.
  - These mothers report higher drinking at and after conception (2.45 vs 1.82 drinking days/week)
  - Slow metabolism in mother leads to sustained EtOH throughout body including fetus
- Mothers and infants who lack **ADH1B*3** (high Km high Vmax) and drink are at greater risk of FAS syndromes (compared to ADH1B*3 who didn't drink or ADH1B who did drink)

EtOH and FAS:

- Alcohol consumption decreases folate (Vit B9 and B12) limiting DNA methylation and production of important cofactors.
- Increased acetate from EtOH metabolism increased histone acetylation in areas of brain that may lead to memory, learning and addiction

ADH1B*2 (low Km high Vmax) has a high frequency in China and Japan (~50%).

- Heterozygous mutations reduced the risk of alcoholism
- Homozygous was even more protective from alcohol abuse
- Presumably because of high levels of acetaldehyde which causes several negative outcomes including – hangovers!

ADH1B*3 (high Km high Vmax) is found mostly in African origin people. They metabolize EtOH faster than other but only when high levels of EtOH. This is associated with reduced risk of alcohol dependence

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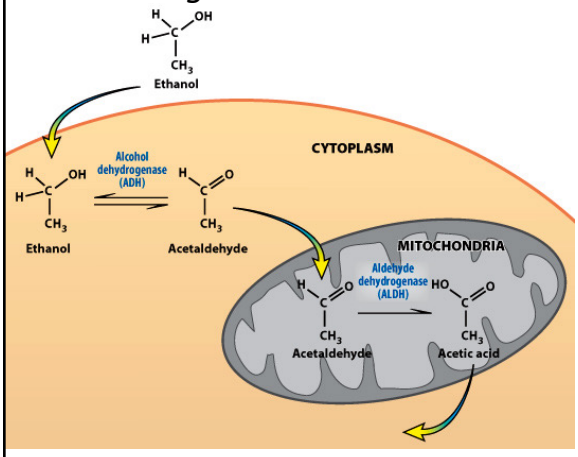
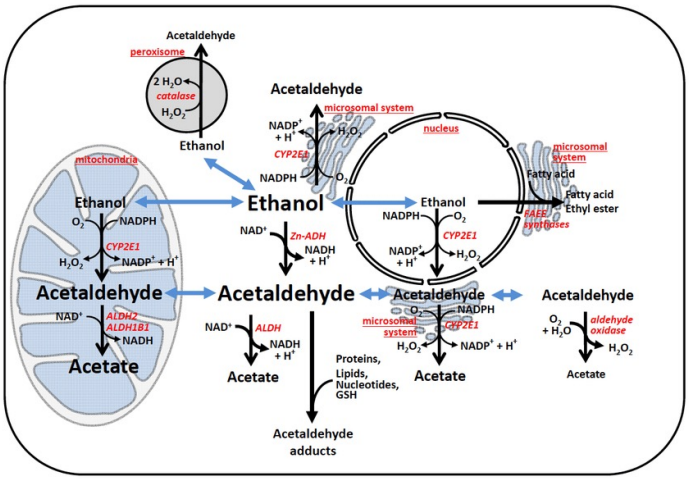
Acetaldehyde Metabolism: Part 2 - ALDH

Alcohol liver metabolisms produces products that damage the liver resulting in alcoholic liver disease (ALD)





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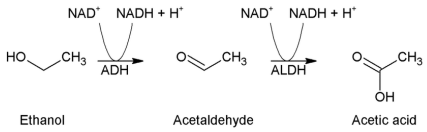
Acetaldehyde Metabolism: Part 2 - ALDH



ALDH catalyzes the NAD(P)+ dependent oxidation of an aldehyde to a carboxylic acid.

There are 19 ALDH genes grouped into 9 families, each coding for ALDH with a broad specificity of substrate and expression level / tissue.

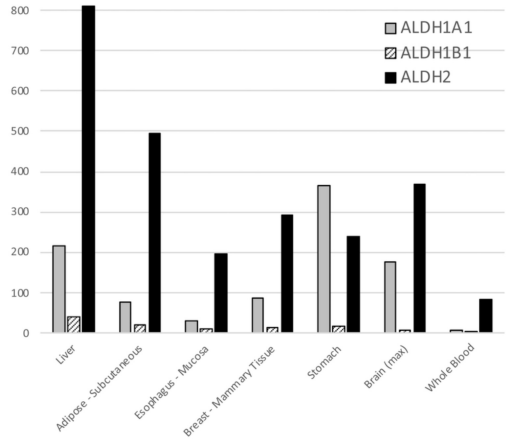
- ALDH1A1, ALDH1B1 and ALDH2 are most relevant to metabolism of acetaldehyde.

ALDH highest expression in liver with significant amounts found in adipose, stomach and brain.

ALDH1A1 is cytosolic. ALDH1B1 & ALDH2 are mitochondrial

Gene Name	Km (mM)	Max (min^{-1})
ALDH1B1	55.0	655
ALDH1A1	180.0	380
ALDH2	0.2	280
ALDH2*1. (Glu504)	X	X
ALDH2*2. (Lys 504)	X	X

Acetaldehyde Dehydrogenase – (ALDH)



The inhibition of ALDH in the brain may have implications in the pathogenesis of Parkinson's disease. ALDH is responsible for the detoxification of acetaldehyde containing metabolites of dopamine and build up of these metabolites can cause neurotoxicity.

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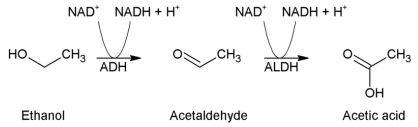
Acetaldehyde Metabolism: Part 2 - ALDH

Acetaldehyde Dehydrogenase – (ALDH)

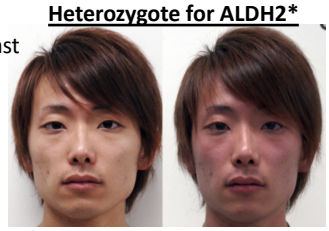


Acute (short term) effect of Acetaldehyde:
Elevated blood acetaldehyde causes facial flushing, severe headache, palpitations, tachycardia, hypertension, respiratory distress, nausea and vomiting. These symptoms commence within 15–30 min of ingesting alcohol and persist for several hrs.

The main isozyme responsible for detoxification is ALDH2

- Low Km (very high affinity) and high reaction velocity (Vmax) rapidly eliminates most of the acetaldehyde as fast as ADH can produce it.
- Any mutation to E504 (results in a large increase in the K_m for NAD^+, which almost inactivates the enzyme.
- About half of Taiwanese, Han Chinese and Japanese populations have high levels of acetaldehyde after drinking.
- People with both heterozygous and homozygous mutated ALDH2 show virtually no acetaldehyde metabolism and experience negative physiological responses to alcohol
- Alcoholic cirrhosis is reduced by 70% in this population as well as low esophageal and had and neck cancers.

Heterozygote for ALDH2*



The alcohol flush reaction. The primary feature of the alcohol flush reaction is a red face—or flush—but it can also be accompanied by hives, nausea, low blood pressure, the worsening of asthma, or an episode of migraine.

Alcohol flush is due to high acetaldehyde in people with ALDH2 mutations

- Due to the unpleasant effects, these people are protected from drinking

25

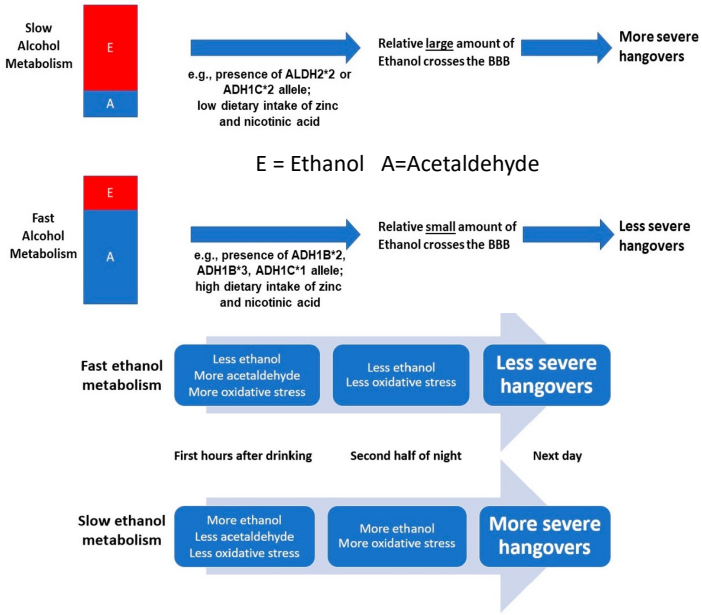
Acetaldehyde Metabolism

The Hangover – not the movie

- Methanol in some alcohols is slowly metabolized to formaldehyde by ADH.
- Presence of formaldehyde and acetaldehyde along with dehydration and hypoglycemia lead to hangovers

Data suggest that the ethanol elimination rate is a critical determinant of hangover severity

- Significant correlations have been found between ethanol concentration (but not acetaldehyde) and hangover severity.
- ROS metabolism of ethanol leads to oxidative stress and high inflammatory response to alcohol consumption.
- Nutrients, microbiota, and hangover treatments that speed up the conversion of ethanol into acetaldehyde are associated with less severe hangovers.



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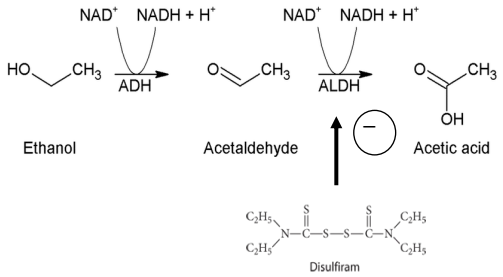
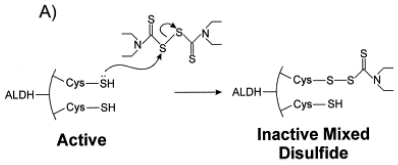
Acetaldehyde Metabolism

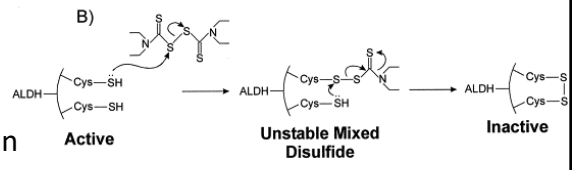


Disulfiram irreversibly inhibits aldehyde dehydrogenase (ALDH1A1) by competing with nicotinamide adenine dinucleotide (NAD) at the cysteine residue in the active site of the enzyme.

Disulfiram discourages drinking by inhibiting ALDH and inducing the immediate unpleasantness associated with acetaldehyde.

- Similar effect to ALDH mutations
- About 5 min after drinking: fall in blood pressure, sweating, difficulty in breathing, headache, nausea and vomiting
- Aversion therapy
- Second line therapy –first line drugs interact in brain receptors





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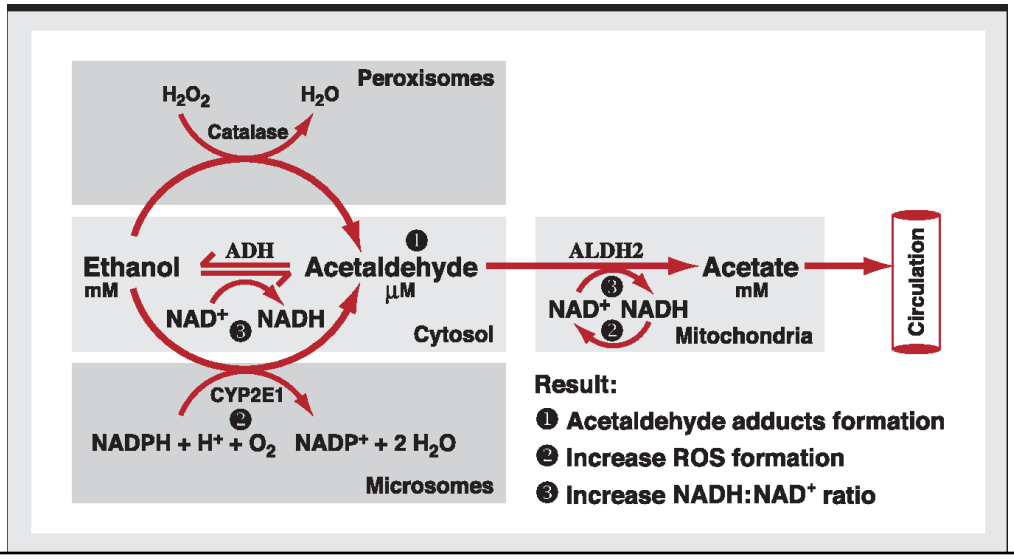
OK. What next (Alcohol Metabolism cont.)

How else is Ethanol oxidized to Acetaldehyde?



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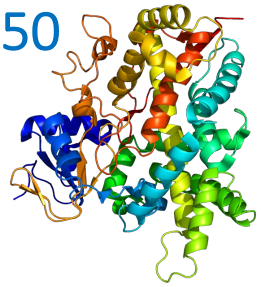
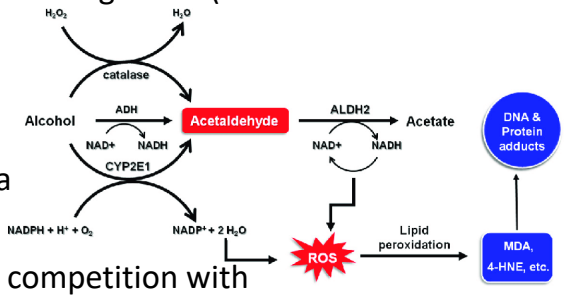
Acetaldehyde Metabolism: Cytochrome P450



CYP2E1, CYP21A2 and CYP23A4

Cytochrome P450 (CYP) is an iron-containing protein involved in metabolism of drugs and foreign compounds (xenobiotics).

- Found in microsomes of liver (endoplasmic reticulum fragments)
- Chronic alcohol intake leads to increase expression
- May play a larger role in non-liver tissue like brain w/o ADH.
- The Km is high and will be "active" during social drinking levels (Km 8-12 mM. (ADH 0.4- 2 mM))
- In people with Alcohol Use Disorder, there are as many as 13 mutations (polymorphisms) which, like ADH can alter protection.
- Reactive Oxygen Species (ROS) are produced as a side reaction: Superoxide anion ($O2^{\bullet -}$) H_2O_2, $^{\bullet}OH$
- CYP2E1 activates/processes many drugs and the competition with EtOH alters the half-life or the drug.



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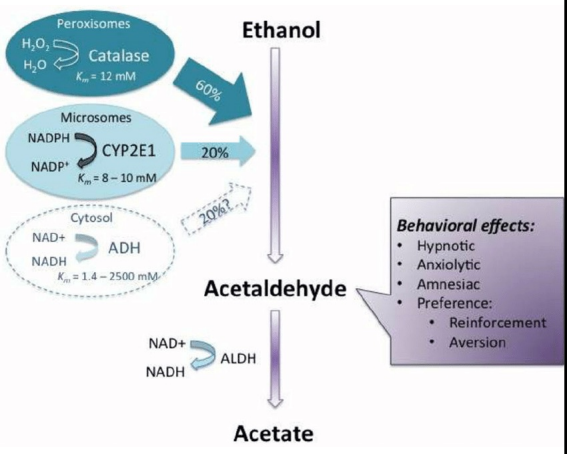
Acetaldehyde Metabolism: Catalase

Catalase expressed in peroxisomal fraction of the cell.

- Normal role is to reduce hydrogen peroxide using the oxidation/reduction potential of the iron-heme created during fatty acid synthesis
- Due to low rates of H_2O_2 production and the higher Km 12 mM (ADH 0.4- 2 mM), catalase plays a minor role in liver metabolism of alcohol.
- How ever, in neural tissue, due to lack of ADH,



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Metabolic Adaptation – Alcohol Tolerance

"Because I could handle my drinking – or so I thought – and could consume a lot of alcohol without becoming uncontrollably inebriated, I refused to see it as a problem." Buzz Aldrin. One of the first people to walk on the moon.

The simple definition of alcohol tolerance is when the amount of alcohol that is consumed does not change but results in less of an effect or when higher amounts of alcohol are needed to produce the same effect.

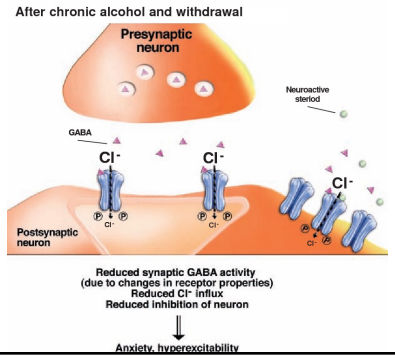
- This <u>used</u> to be part of the criterion for diagnosis of Alcoholic Use Disorder (AUD).

Instead consider both metabolic adaptation and brain/CNS adaptation.

The development of alcohol dependence is posited to involve numerous changes in brain chemistry (i.e., neurotransmission) that lead to physiological signs of withdrawal upon abstinence from alcohol as well as promote vulnerability to relapse in dependent people.

Blood Alcohol Level	Normal Brain	Addicted Brain
>.1*	Tipsy	Usually, no effect
>.2	Potentially on ventilator or in coma	Many times only minimal effect
>.3	Usually in coma or dead	Many times only moderate effect, but "functional"
.4-.5	Barely alive	At times can function to a certain degree

Alcoholic - significantly greater tolerance



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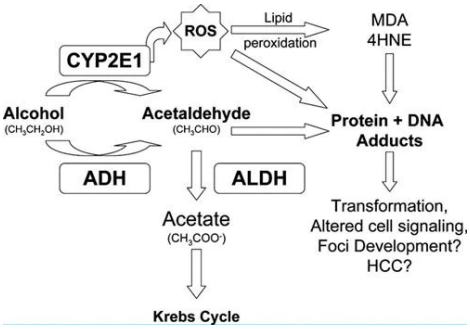
Metabolic Adaptation – Alcohol Tolerance



- < 10% of ethanol is eliminated via breath, sweat and urine
- ~90% is removed by oxidation
- Some removed in "first pass"
- Most of the oxidation occurs in liver
- Most immediate changes. Once higher levels of EtOH is ingested, the system is zero order in elimination/metabolizing the alcohol

Tolerance in metabolic adaptation terms means the rate of elimination of ethanol from the body

- **Induction of ADH**
- **Induction of CYP2E1**
- **Hyperbolic metabolic state**
- **Increased release of cytokines or prostaglandins which elevate oxygen consumption by liver cells.**

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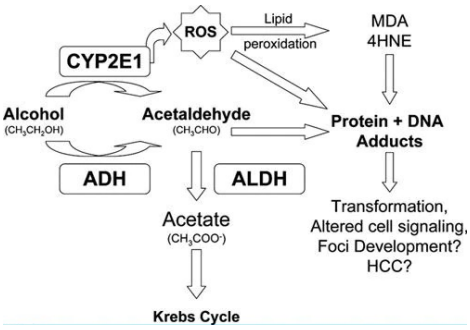
Metabolic Adaptation – Alcohol Tolerance



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Tolerance in metabolic adaptation terms means the rate of elimination of ethanol from the body.

Induction of ADH – chronic alcohol ingestion can decrease thyroid hormones, one of the regulators of ADH expression. Yet some studies show that ADH expression can increase depending on intake.

Induction of CYP2E1

Hyperbolic metabolic state

Increased release of cytokines or prostaglandins which elevate oxygen consumption by liver cells.

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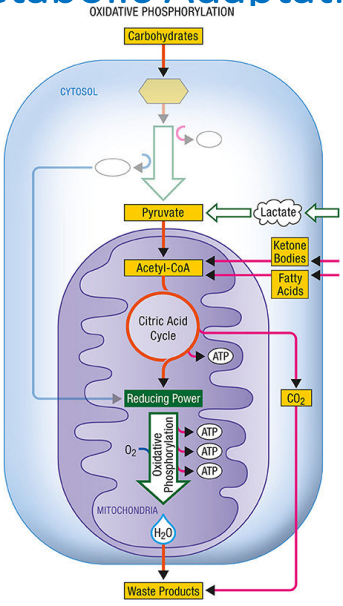
Metabolic Adaptation – Alcohol Tolerance



Tolerance in metabolic adaptation terms means the rate of elimination of ethanol from the body.

Induction of ADH

Induction of CYP2E1

Hyperbolic metabolic state – Increased activity of ATPase O_2 consumption and NADH oxidation to NAD^+ . This can also lead to hypoxia and liver damage.

Increased release of cytokines or prostaglandins which elevate oxygen consumption by liver cells.

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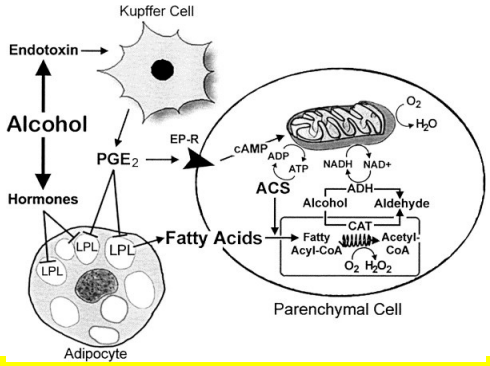
Metabolic Adaptation – Alcohol Tolerance



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Tolerance in metabolic adaptation terms means the rate of elimination of ethanol from the body.

Induction of ADH

Induction of CYP2E1

Hyperbolic metabolic

Increased release of cytokines or prostaglandins which elevate oxygen consumption by liver cells- Ethanol increases release of hormones, cytokines and prostaglandins to further increase metabolism and increase oxygen consumption

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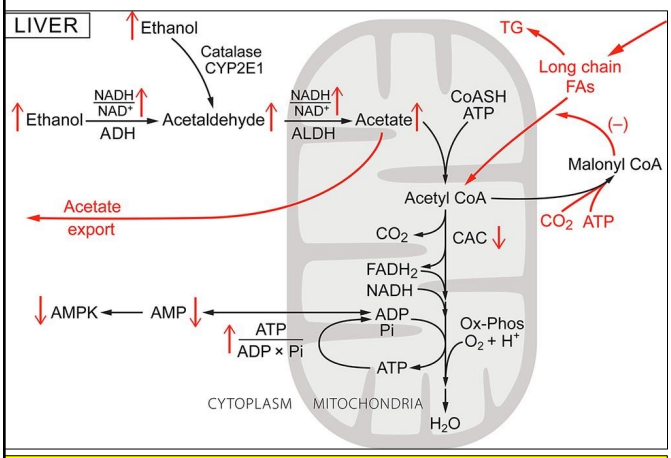
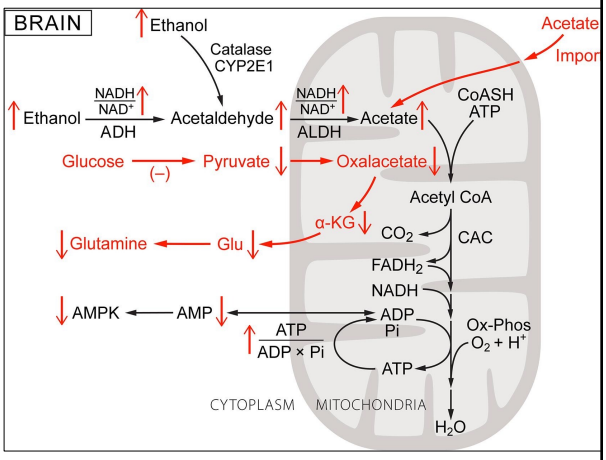
Pathophysiology and biochemistry of alcohol metabolism



Excessive ADH-ALDH use drives a high NADH:NAD+ ratio limiting glycolysis. High levels of acetate are released into the blood. Acetate takes place of fatty acid use and high fatty acid production. The TCA and gluconeogenesis pathways are decreased resulting in low blood sugar.



Acetate from blood (liver) and CYP/ADH – ALDH produce Acetyl-CoA for Krebs Cycle/OxPhos ATP production. This is similar to the metabolic changes seen during starvation. High levels of Acetate leads to acetylation of histone and epigenetic (gene) changes in brain.

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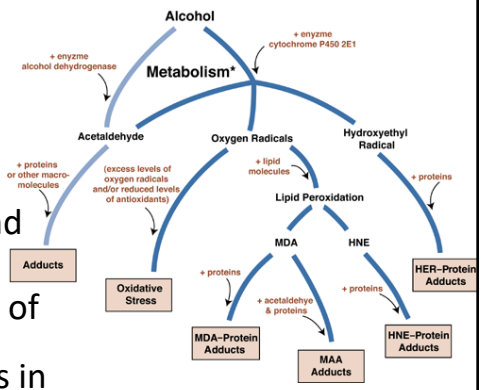
Pathophysiology and biochemistry of alcohol metabolism: Acetaldehyde Adducts

Combination of ROS and Acetaldehyde react with biological molecules to form "adducts"

- Is an "addition product" meaning some compound is covalently added to another compound. Protein, lipid, RNA or DNA.
- In cancer, an adduct is when a piece of DNA is modified by adding a part of another compound leads to cancer.
- Adducts lead to DNA strand breaks, miss-reads of DNA-RNA transcription, miss-reads in DNA replication, blocking DNA repair. If this happens in a gene that regulates cell growth- Cancer can result.



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Compound	Injury Effect/Disease	Mechanism
Ethanol	Steatosis	Triglyceride accumulation
	Oxidative stress[29,30]	ER Stress, Insulin resistance, Mitochondrial dysfunction, CYP2E1 induction
	Cirrhosis	Acetaldehyde generation
	Carcinogenesis[10,11,25,26]	DNA damage
Acetaldehyde	Protein/Enzyme dysfunction	Protein,[11,12,16,22,38,39] DNA[16,22,37] and hybrid adducts[18,43,58]
	Oxidative stress	Lipid peroxidation adducts,[18,22,56] Pro-inflammatory cytokine activation, Glutathione scavenger function inhibited, ROS production increased[37,52]
	Fibrogenesis/Cirrhosis	Adducts in HSCs and myofibroblasts[11,64,65]
	Carcinogenesis	Mutagenesis by binding with DNA[37]
	Atherosclerosis	Oxidation of LDL[79,80,81,82,83]
	Cardiomyopathy	Impairs contractile function of cardiomyocytes[86,88,90]
	Erythrocyte macrocytosis	Acetaldehyde-modified erythrocyte membrane protein[107]
	Anemia and iron overload in liver	Immune mediated attack on erythrocytes[107]
	Impairs coagulation function	Inactivates clotting factors, e.g thrombin, fibrinogen, Factors II, VII, X, Xa, XIIIa[110,111,112]
Lipid Adducts (MDA; 4-HNE)	Cell death	Oxidative/ER Stress[11]
	Hepatic fibrosis/cirrhosis	Hepatic stellate cell activation with induction of collagen 1 synthesis and inhibition of pro-collagen negative feedback loop[77, 78]
	Carcinogenesis	Mutagenesis, including inhibition of oncosuppressor genes, e.g. p53[22]
	Atherosclerosis	Auto-immune response to modified proteins[82,83] Oxidation of lipoproteins forming plaques[80,81]

Abbreviations: ER stress, endoplasmic reticulum stress; CYP2E1, cytochrome P450 2E1; ROS, reactive oxygen species; HSCs, hepatic stellate cells; LDL, low-density lipoproteins; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal.

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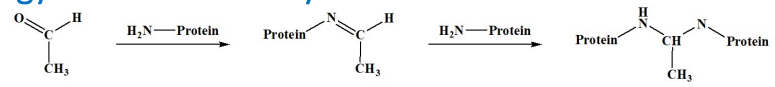
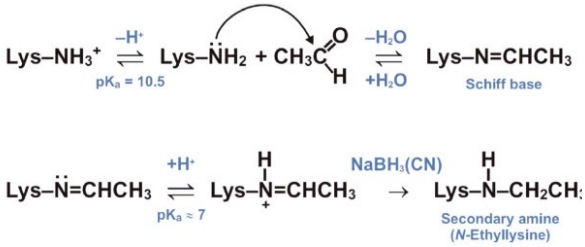
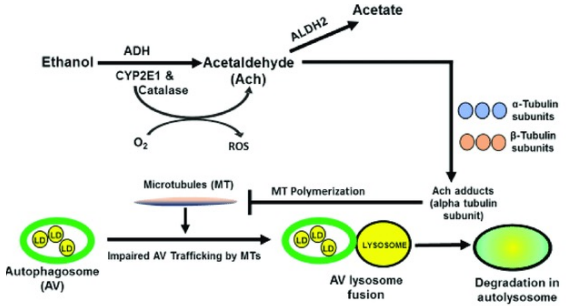
Pathophysiology and biochemistry of alcohol metabolism: Acetaldehyde Adducts



Protein Adduct: Schiff base formed between the reactive aldehyde carbon and the N of a lysine side chain or the N terminus of a protein. Some aromatic amino acids will also react and form a bond to acetaldehyde.

- Proteins once modified will not function properly. Some adducts increase the oxidative stress by removing radical scavengers (GSH).
- Acetaldehyde-Tubulin blocks polymerization leading to decrease protein trafficking and blocking proper lysosomal and phagosomal regulation and liver cell death





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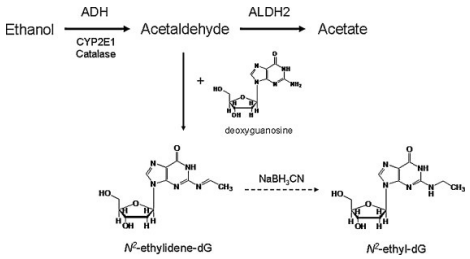
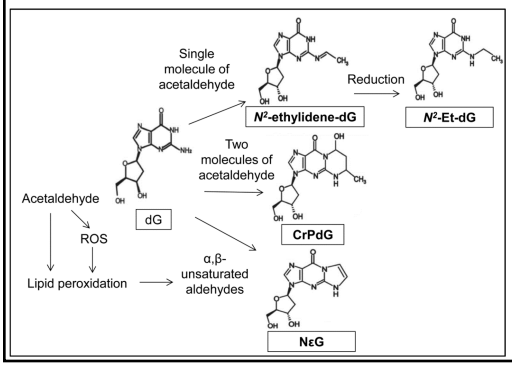
Pathophysiology and biochemistry of alcohol metabolism: Acetaldehyde Adducts

DNA Adduct: The formation of acetaldehyde-derived DNA adducts plays an important role in carcinogenesis. The major DNA adduct in the human body is a Schiff base N^2-ethylidene-2′-deoxyguanosine (N^2-ethylidene-dG) adduct. In reactions of acetaldehyde with deoxyribonucleosides, deoxyguanosine (dG) was most reactive, though products are also observed in reactions with deoxyadenosine (dA) and deoxycytidine (dC).





- N^2-Et-dG blocks DNA synthesis and induces frameshift deletions and G:C > T:A transversions.
- N^2-Et-dG can rotate around the exocyclic nitrogen and the alpha carbon of acetaldehyde because it has a single bond, whereas N^2-ethylidene-dG has a double bond, which makes it more hydrophobic than N^2-Et-dG. These differences may result in significantly different mutagenic potential between N^2-Et-dG and N^2-ethylidene-dG.
- CrPdG induces DNA interstrand and intrastrand cross-links. The ring-opened form of CrPdG can react with dG on the opposite strand of the DNA to form DNA interstrand cross-links.
- CrPdG-mediated disruption of the DNA replication process is thought to cause DNA damage

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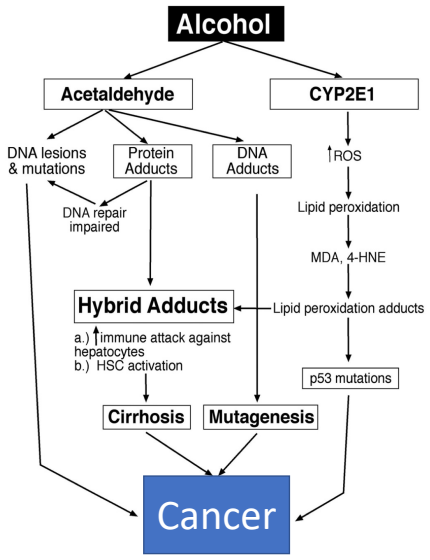
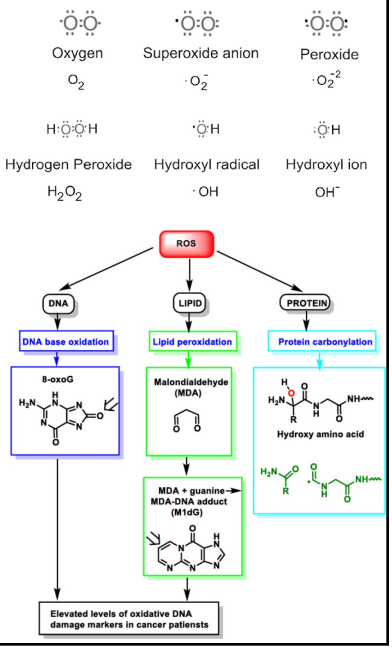
Pathophysiology and biochemistry of alcohol metabolism



Reactive Oxygen Species are generated as part of the P450 (CYP2E1) reaction and in very active mitochondria. The radicals have a very short half-life as they will oxidize proteins and other biomolecules.

Oxidative stress is the result of ROS and the damage to cell function as a result of unchecked/repaired free radical damage.

- The immune system is challenged and activated immune system supporting cancer and other diseases with increases in cytokines.
- Peroxidation of mitochondrial membranes alters the integrity of the membrane allowing cytochrome C to be released and starting cell death



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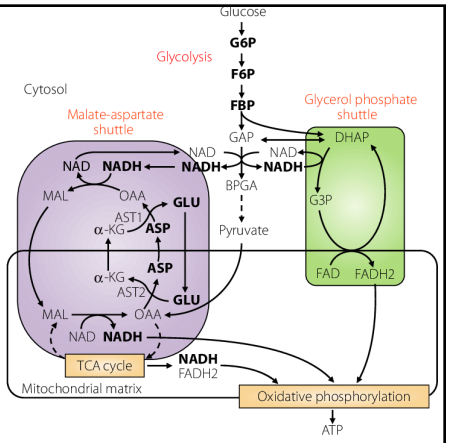
Hypoxia and Liver Damage

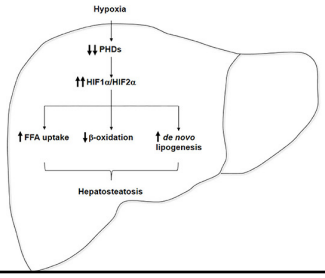
Excessive ADH and ALDH reduce the cell's cytosolic and mitochondrial NAD^+ stores to NADH

- NADH is oxidized in mitochondria as part of the electron transport system / ATPase (O_2 utilizing)
- The results are high oxygen consumption in liver. Liver cells close to the arterial supply will strip the blood of much of the oxygen leaving areas low of oxygen (hypoxia)
- Hypoxic tissue show damage in chronic alcohol consumption.

Non-alcoholic fatty liver disease is the most common chronic liver disease along with alcohol liver disease (ALD). In both cases, HIF1α, a transcriptional protein, activates a series of genes leading to cell death in hypoxia.

- ROS activates lipopolysaccharides which stabilize HIF1α, leading to greater damage.





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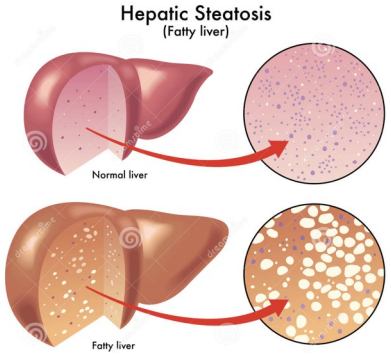
Fatty Liver (Steatosis), Fibrosis and Cirrhosis

The first stage of liver damage following chronic alcohol consumption is the appearance of fatty liver, followed by inflammation, apoptosis, fibrosis and finally cirrhosis.

- Lipid oversupply leads to liver increasing uptake of fatty acids and the production of new fatty acids from acetate.
- Acetaldehyde induced increases in the NADH/NAD+ ratio and increases in NADPH (from P450) and other changes (AMP) promotes further fatty acid synthesis.
- As a part of the process VLDL (lipid vesicles) are produced but not released from liver cells, leaving an enlarged and fatty liver.



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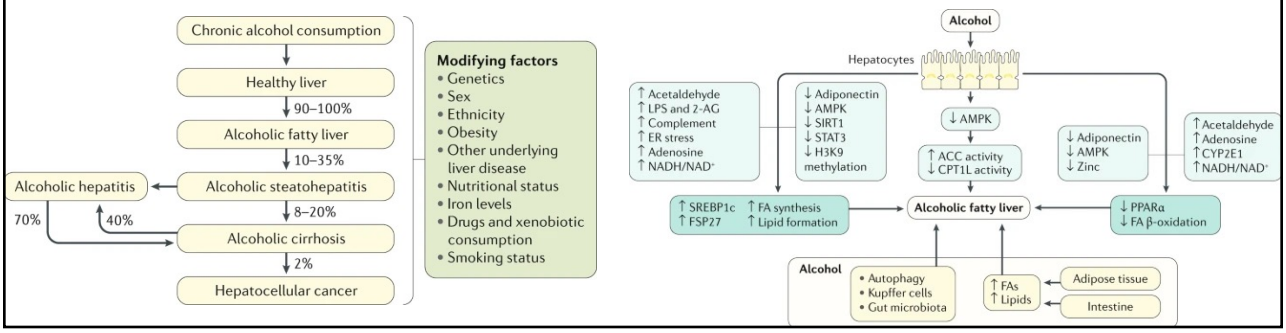
Fatty Liver (Steatosis), Fibrosis and Cirrhosis

Chronic alcohol exposure also activates hepatic macrophages, which then produce tumor necrosis factor-alpha (TNF-alpha).

- TNF-alpha induces mitochondria to increase the production of reactive oxygen species. This oxidative stress promotes hepatocyte necrosis and apoptosis, which is exaggerated when poor nutrition does not supply antioxidant vitamins and nutrients.
- Free radicals initiate lipid peroxidation, which causes inflammation and fibrosis.
- Inflammation is also incited by acetaldehyde that, when bound covalently to cellular proteins, forms adducts that are antigenic. Leaving the liver to be attacked by its own immune system.



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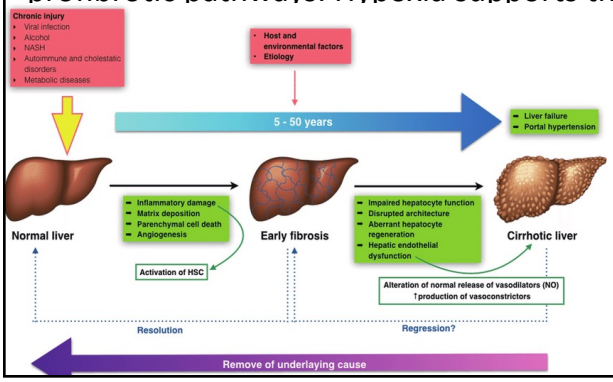
Fatty Liver (Steatosis), Fibrosis and Cirrhosis

Continued chronic alcohol exposure will cause a subset of steatosis livers to develop fibrosis and then cirrhosis.

- Fibrosis is a result of hepatocyte death. This causes the release of tumor necrosis factor and other hormones that cause damaged and normal cells express and secrete extracellular matrix proteins such as collagen and fibronectin. Essentially making the liver a scar tissue.

- Many of the protein adducts generated with acetaldehyde and ROS turn on these profibrotic pathways. Hypoxia supports the further stimulation of fibrosis.



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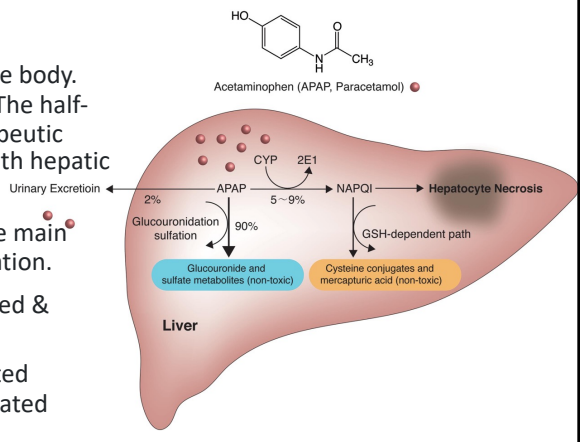
Alcohol and Acetaminophen (Tylenol) Toxicity

Acetaminophen toxicity is one of the most common causes of both intentional and unintentional poisoning in the United States. It has become the most common cause of acute liver failure and the second most prevalent cause of liver failure requiring transplantation.

Upon ingestion, acetaminophen is rapidly absorbed from the gastrointestinal (GI) tract and quickly distributed throughout the body. Peak plasma concentrations are achieved within 30 to 60 min. The half-life of acetaminophen is approximately 2 to 3 hours after therapeutic doses, yet can be increased to more than 4 hours in patients with hepatic injury.

Acetaminophen is extensively metabolized by the liver via three main hepatic pathways: glucuronidation, sulfation, and **CYP2E1** oxidation.

- Approximately 90% of acetaminophen is conjugated to sulfated & glucuronidated metabolites that are eliminated.
- Of the remaining acetaminophen, approximately 2% is excreted unchanged in the urine and the rest undergoes CYP2E1-mediated oxidation to form a reactive imine (NAPQI).



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Alcohol and Acetaminophen (Tylenol) Toxicity

With large acute doses or with chronic use, the major metabolic pathways—the glucuronide and sulfate conjugation systems—become saturated, and more acetaminophen is metabolized by the CYP2E1 system. When glutathione is approximately 70% depleted, NAPQI begins to accumulate in the hepatocytes, resulting in hepatic damage.

Acetaminophen overdose here is defined as intake of more than 5gm of APAP in one sitting APAP when ingested, is rapidly absorbed in the body leading to the synthesis of toxic intermediate NAPQI within hepatocytes by several P450

Under normal circumstances, this toxic metabolite reacts with sulfhydryl groups in glutathione, converting it to harmless metabolites before being excreted in the urine.

NAPQI then binds with glutathione (GSH), a well regulated antioxidant found within hepatocytes and is easily eliminated. However when a high dose of acetaminophen is ingested, NAPQI becomes the key pathway and exhausts the GSH(G) reserve and unconjugated NAPQI binds to hepatocellular proteins and other cellular components, damaging hepatocytes.

Patients with AUD or excess alcohol intake induce PY2E1 speeding up the production of NAPQI leading

In 1986, six alcoholics who developed toxicity after taking APAP and reviewed nineteen similar case reports. In 1995, an additional 67 alcoholic patients who developed liver toxicity after taking moderate doses of APAP.

However, the possibility that APAP may be toxic in alcoholics at moderate doses has been disputed The problems in obtaining proper controls makes it difficult to know if alcoholics are more susceptible to APAP hepatotoxicity.

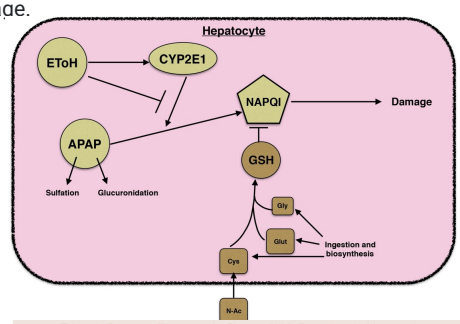

Table 1. Common Therapeutic Classes With Drug-Alcohol Interactions

Drug Class	Specific Medications	Interaction Mechanism	Clinical Significance
Antibiotics	Second-generation cephalosporins,[a] metronidazole, trimethoprim/sulfamethoxazole, isoniazid	Disulfiram-like reaction	Buildup of acetaldehyde can cause facial flushing, tachycardia, diaphoresis, and pounding headache
Cardiovascular	Vasodilators, nitroglycerin	Exacerbation of adverse effects	Increased risk of hypotension and falls
	Sulfonylureas, insulin	Exacerbation of adverse effects	Increased risk of hypoglycemia
	Warfarin	Increased metabolism with chronic heavy alcohol use; decreased metabolism with acute alcohol use	Decreased anticoagulant effect and increased risk of clots; increased anticoagulant effect and increased risk of hemorrhage
Analgesics	Opioids	Exacerbation of adverse effects	Increased sedation and CNS depression
	Nonnarcotic: NSAIDs/aspirin	Exacerbation of adverse effects	Increased risk of developing an ulcer or GI bleed
	Nonnarcotic: APAP	Increased production of APAP's toxic metabolites with chronic alcohol use	Increased risk of hepatic injury in chronic alcoholics

[a] Including the second-generation sulfonylurea cefotetan.
APAP: acetaminophen; CNS: central nervous system; GI: gastrointestinal; NSAIDs: nonsteroidal anti-inflammatory drugs.
Source: References 7-11, 14-18, 23-27, 29-31.

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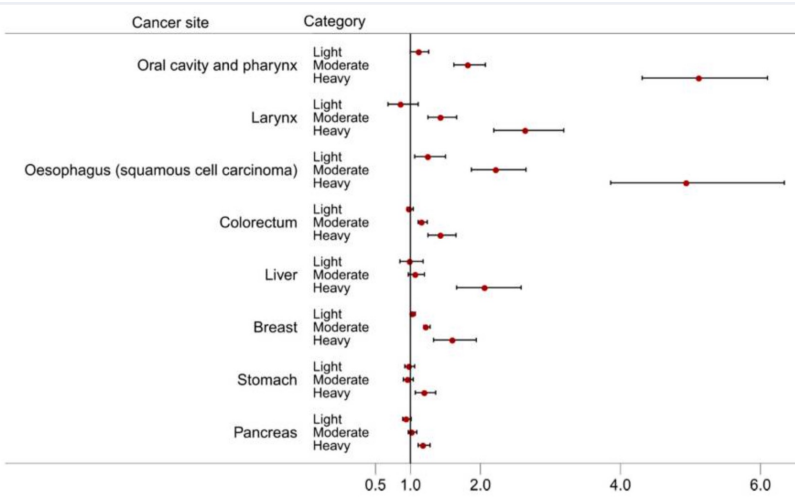
Alcohol-Related Cancer

Approximately 4% of cancers worldwide are caused by alcohol consumption ~ 740,000 global deaths in 2020.



The dose-response relationship for the risk of cancer at different sites by three level of alcohol intake: light (up to 12.5 g/day), moderate (12.5 to 50 g/day), and heavy (more than 50 g/day).

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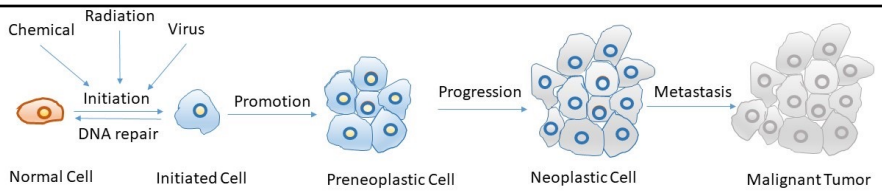
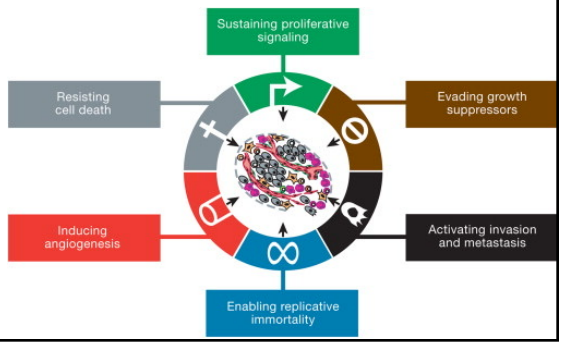
What is cancer?



Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.

- Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.
- Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors.
- Cancer at the simplest, are normal cells who's genes critical for controlled growth and movement have been mutated. Some genes are "activated" others what slow cell growth and movement are "inactivated" by these mutations.



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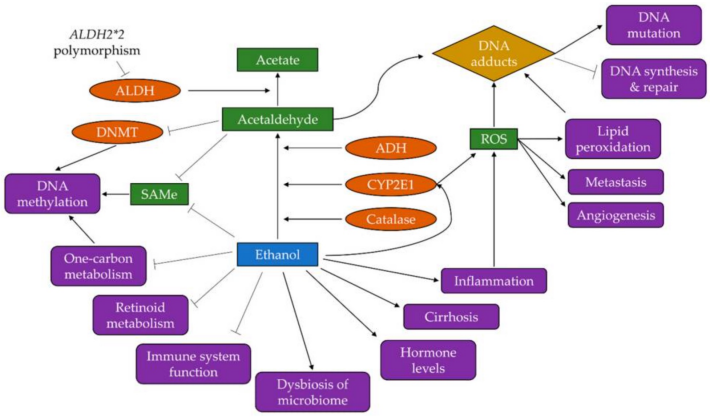
Alcohol-Related Mechanism of Cancer

- Genetic variations of ALDH2*2 (E487K) with almost no activity, is associated with a high incidence of liver cancer in heavy drinkers.
- ALD-associated cancer is high in populations with the disease who drink.
- Acetaldehyde and ROS combine to produce DNA adducts that lead to DNA double-strand breaks, sister chromatid exchanges and DNA cross-links
- Decreased methylation of DNA can activate some cancer causing genes



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