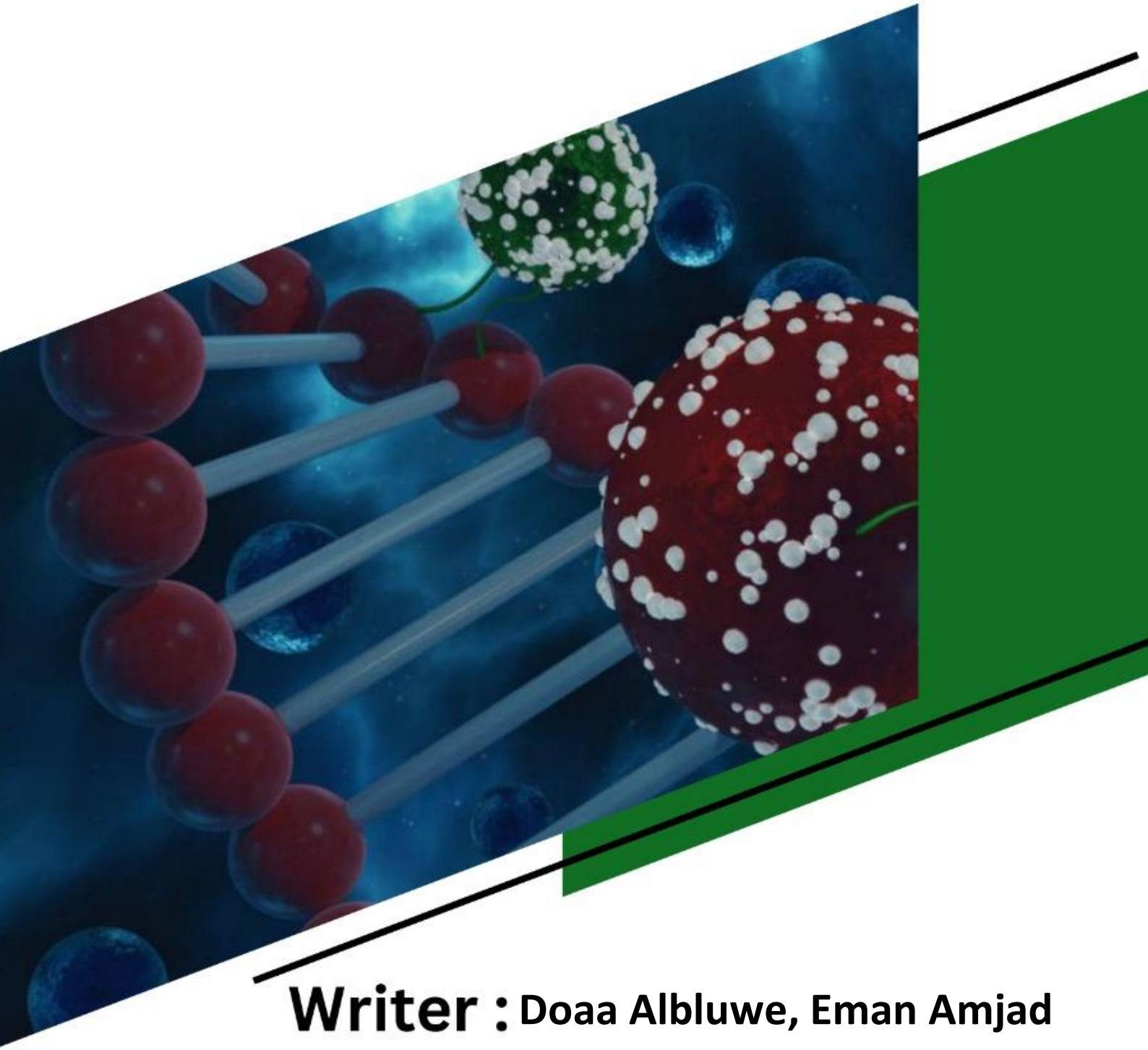
Doctor 021

METABOLISM

Sheet no. 15



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In the previous lecture:

Gluconeogenesis is synthesis of glucose molecules from non-carbohydrate precursors (lactate, glycerol, glucogenic amino acids " amino acids when degraded it produce pyruvate or kreps cycle intermediate").

It is the opposite of the glycolysis except in the three irreversible steps, it needs different enzymes:

Glucokinase or hexokinase ------ glucose 6- phosphatase (in single step)

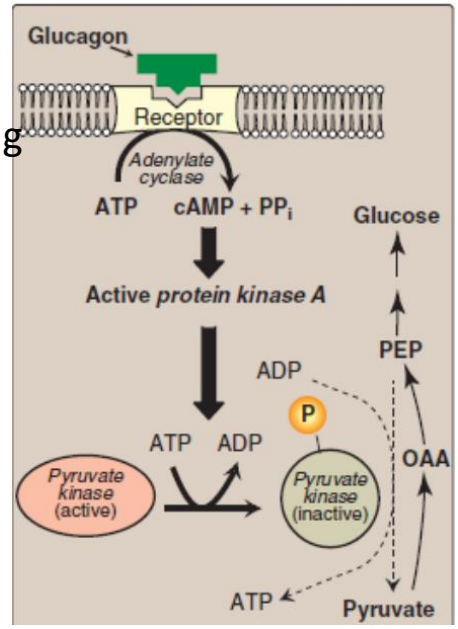
Phosphofructokinase ----- phosphatase (in single step)

Pyruvate kinase ----- pyruvate carboxylase / PEP carboxykinase (two steps)

This process is active under fasting condition which means that **GLUCAGON** is in high levels.

Sooo, let's start our lecture…

REGULATION OF GLUCONEOGENESIS



Glucagon binds to its GPCR, and this activates

Adenylyl cyclase converting ATP to cAMP→activating

Protein kinase A.

STOP, lets remember the targets of this enzyme.

1. Bifunctional enzyme (glycolysis)
2. Glycogen phosphorylase kinase
3. Glycogen synthase
4. Pyruvate kinase

Phosphorylation of pyruvate kinase inhibits it, means inhibition of glycolysis in case of gluconeogenesis, we want to build glucose not to degrade it in fasting condition.

- **regulation Mainly by:**

1. **The circulating level of glucagon**

   - **Glucagon lowers the level of fructose 2,6-bisphosphate** (which is activator of glycolysis)**, resulting in activation of fructose 1,6-bisphosphatase and inhibition of phosphofructokinase 1 (PFK1)**

- **Inhibition of pyruvate kinase**
- **Glucagon increases the transcription of the gene for PEP-carboxykinase** (converts oxaloacetate to phosphoenolpyruvate "PEP")

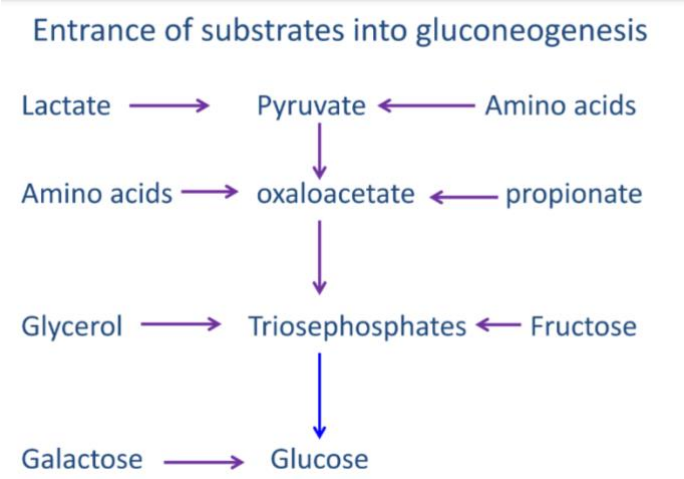
2. The availability of gluconeogenic substrates (the amount of raw materials)

3. Slow adaptive changes in enzyme activity due to an alteration in the rate of enzyme synthesis or degradation, or both

The concentration of enzyme, determined by the rate of expression to its gene, the stability of the mRNA (degraded or not), the rate of synthesis of the enzyme of this mRNA and the rate of degradation of the enzyme.
This can be applied in all pathways.

Different precursors enter the Gluconeogenesis process at different stages.

remember this? →



if pyruvate produced of alanine metabolism it will start from the first step.

An amino acid produce oxa. It will start from the second step.

If an amino acid produces succinyl coA, it will proceed in krep's cycle to produce Oxaloacetate and continue from the second step.

Glycerol oxidized to dihydroxyacetone-p and this converts to glyceraldehyde 3-p and continue in the process 😊

that's it for GLUCONEOGENESIS

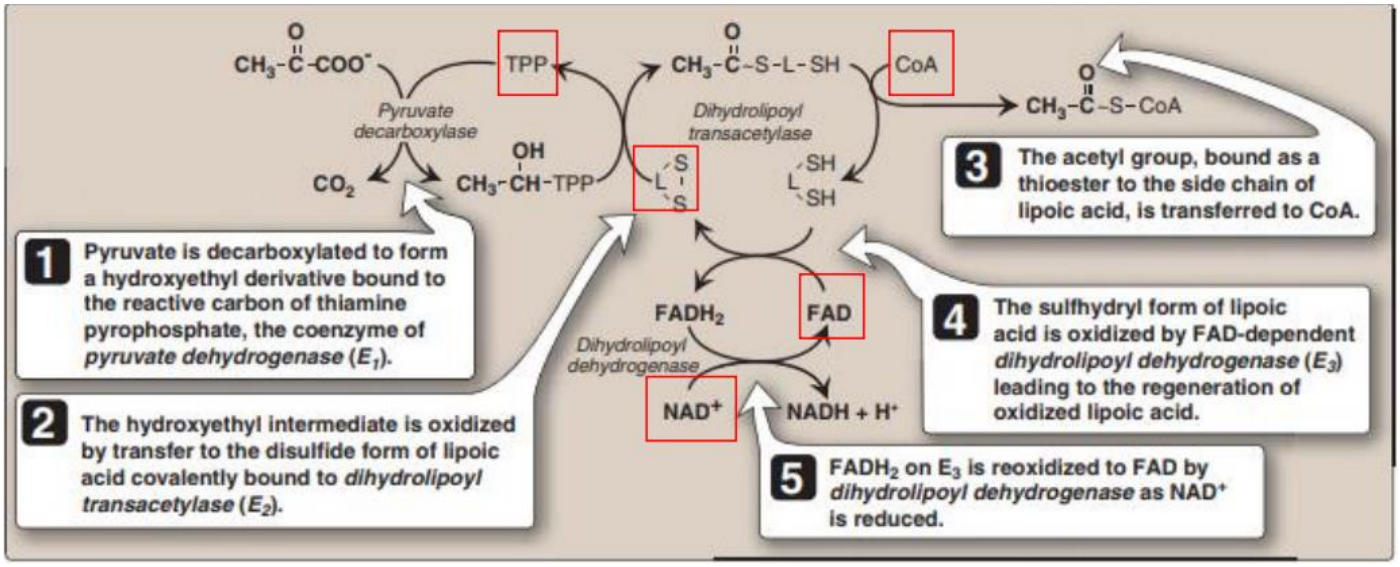
AFTER GLYCOLYSIS: FROM PYRUVATE TO ACETYLCOA

Pyruvate 3 carbons	acetyl coA, 2 carbons coA (S → attachment point)

Oxidative decarboxylation of pyruvate

- **Pyruvate is produced in the cytosol and needs to be transported to the mitochondria by a specific pyruvate transporter**
- **Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase (PDH) complex, which is a multienzyme complex made of 3 enzymes, E1 (decarboxylase), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3)**

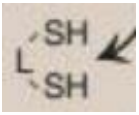
read the steps guys. 😊



E1: co-enzyme is thiamine pyrophosphate

E2: co-enzyme is lipoic acid and coA

E3: co-enzyme is FAD

Disulfide form of lipoic acid  sulfhydryl form of lipoic acid 

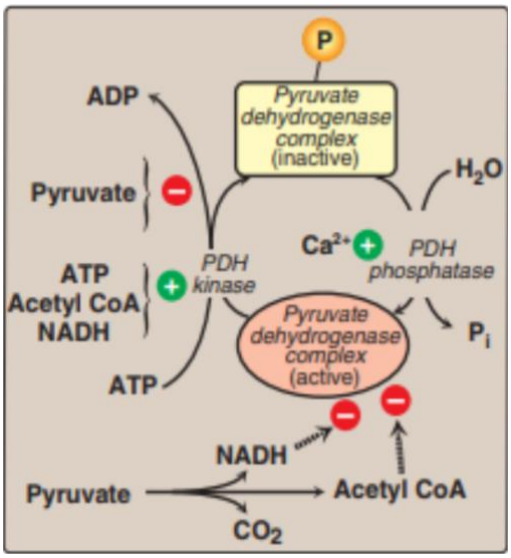
E2 does two functions: dehydrogenation of the substrate, and trans it to the coA.

E3 step is for recycle the co-enzymes (lipoic acid and FAD) from the reduced state to oxidized state

REGULATION OF PDH COMPLEX

This complex is highly regulated by many ways:

- **Allosteric regulators**
  1) NADH (Kreps won't work so no need to acetyl coA)
  2) Acetyl coA (it makes sense, it is product of This enzyme)
- **Enzymes**



The complex also contains two tightly bound regulatory enzymes, PDH kinase and PDH phosphatase.

Phosphorylation of PDH inactivates it,

Mains PDH kinase is active when we want to inhibit the PDH.

The allosteric regulators of the enzymes that regulate the PDH enzyme 🥹:

PDH kinase: here we go again, NADH and Acetyl coA activate kinase, as a result this will inhibit the PDH.

ATP activate kinase to inhibit PDH cause no need to run kreps cycle.

Pyruvate inhibits kinase to activate PDH, so the pyruvate will not accumulate.

Read it again 🤝

PDH phosphatase:

Ca ions increased in active state and higher requirement of energy, as a result they will activate the PDH phosphatase and this activates the PDH → kreps cycle runs.

CLINICAL APPLICATION: PDH DEFICIENCY

- **Rare deficiency in the E1 component of the PDH complex.**
- **However, the most common biochemical cause of congenital lactic acidosis.**
- **This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA** (which is needed to TCA cycle) **causing pyruvate to be shunted to lactic acid via lactate dehydrogenase.**
- **Affected tissues: brain, relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis.**
- **Symptoms are variable and include neurodegeneration, muscle spasticity and, in the neonatal onset form, early death.**
- **X-linked dominant.** (on X chromosome, dominant: one copy of the gene is enough , which is problem 😢)
- **No proven treatment.**
- **Dietary restriction of carbohydrate and supplementation with TPP (thiamine pyrophosphate) may reduce symptoms in select patients.**

+ Keto diet (lack carbs), can bring acetyl coA with no need to this reaction, the tissues that depend on glucose , the gluconeogenesis will resolve this.

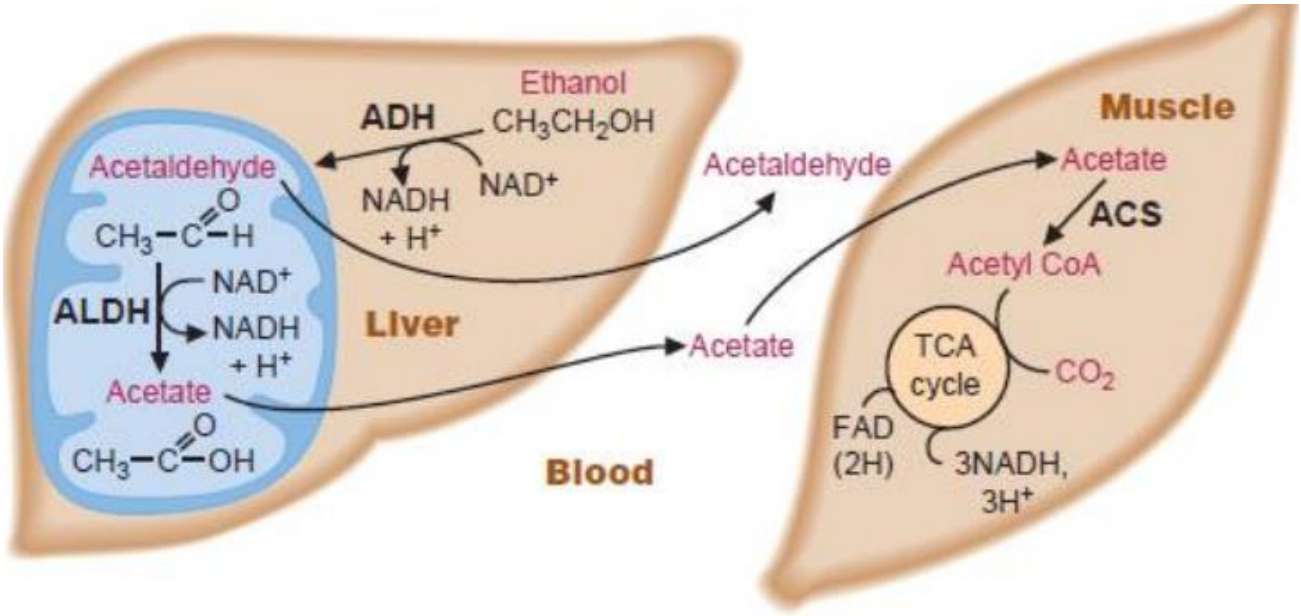
ALCOHOL METABOLISM

Alcohol metabolism means Ethanol metabolism specifically, which is 2 carbons and OH (هو الذي يُعتبر مادة مُسكِّرة من الكحول)

" كيف أشرب ما إن شربته أذهب عقلي و أضحك عليّ من هم أدنى مني"

- Alcohol's metabolism has 3 pathways, due to ethanol's small size. It could starts in stomach in early stage.
- The major site of metabolizing it is LIVER, consequently, people who drinks a lot of alcohol often get infected with liver cirrhosis.

METABOLISM OF ALCOHOL



- **When alcohol is ingested, a small amount is immediately metabolized in the stomach.**
- **Most of the remaining alcohol is subsequently absorbed from the gastrointestinal tract, primarily the stomach and upper small intestine.**
- Alcohol enters liver to hepatocytes, which is the functional cells.

Mechanism

1. First step happens in cytosol, Ethanol gets oxidized by Alcohol Dehydrogenase (ADH) enzyme, to form Acetaldehyde. NAD+ will be reduced to NADH.
2. Acetaldehyde (very harmful compound for our cells + مركبات لها رائحة) enters Mitochondria, get oxidized by Acetaldehyde Dehydrogenase (ALDH) enzyme. Also, reducing NAD+ to NADH. Which results in formation of carboxylic acid Acetate.

   Notice:

   For now NADH is increasing (2NADH is produced now), which indicates high level of energy that inhibitsTCA cycle; for this reason who drinks alcohol looks exhausted due to the lack of energy. This affects ATP levels.
3. Acetate is released from liver to circulation, muscles uptake it. ACS enzyme (Acetyl CoA Synthetase) converts Acetate to Acetyl CoA and enter Krebs cycle.

   Note:

   The energy that produced from TCA cycle now isn't enough at all, because we've lost a lot in the beginning;

   1) When Krebs cycle was inhibited

   2) In small intestines alcohol increase the permeability of intestinal cells, which excrete some bacterial endotoxins (bacteria waste) to circulation, then toxins go to the Liver, which stimulate immune response. And Cells while fighting toxins consume energy from hepatocytes.

   3) And this could damage the hepatocytes

   All these slowdown ATP generation

NADH/ NAD+ ratio

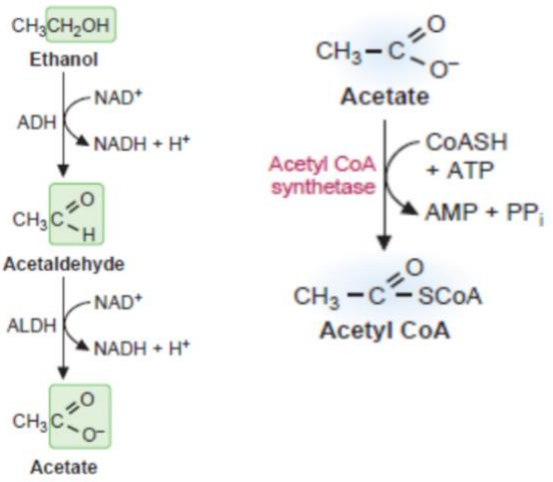
Most cells need the NAD+ state to function, so normally the NADH/NAD+ must be low. For example, for each 1000 NAD+ there is one NADH.

What happens when a high amount of Ethanol is metabolized?

- **High NADH/NAD+**
- **Inhibition of Fatty acid oxidation, also energy get down.**
- **Inhibition of gluconeogenesis,** WHY? Lactate needs NAD+ to be converted to pyruvate

(Ans is from us; Dr.Diala said think about it)

- **Lactic acidosis**



Side note:

Why NADH isn't used directly in oxidative phosphorylation?

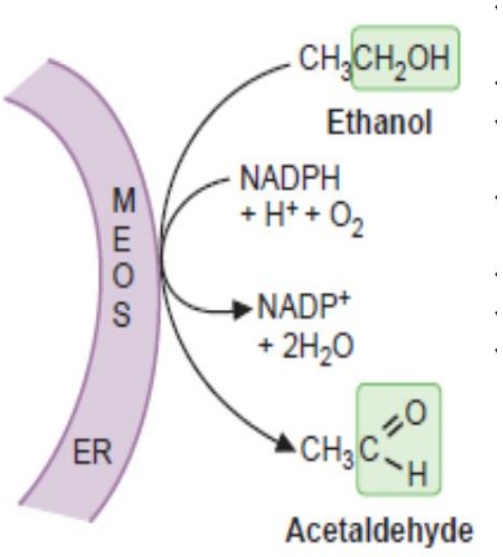
ANS. It needs final acceptor O_2, in immune response (due to endotoxins) Kupffer cells use O_2 massively which decreases its amount in blood and cells.

METABOLISM OF ALCOHOL

MEOS: Microsomal Ethanol Oxidizing System

2nd pathway

- **An alternative pathway for ethanol metabolism.** Take place in endoplasmic reticulum.
- **10-20% of the ingested ethanol in this pathway.**
- **Involves primarily the cytochrome P450 (CYP2E1).** Cytochrome is heme proteins, and CYP2E1 is one of them.



- **CYP2E1 is associated with NADPH-cytochrome P450 reductase in the ER**. 1) the functional protein P450-2E1 2)NADPH are used in this way.

1- CYP2E1 oxidizes ethanol to acetaldehyde

2- Oxidize NADPH to NADP+, and Trans the H^- & H^+ to form H_2O (here is reduction) at same time.

Note:

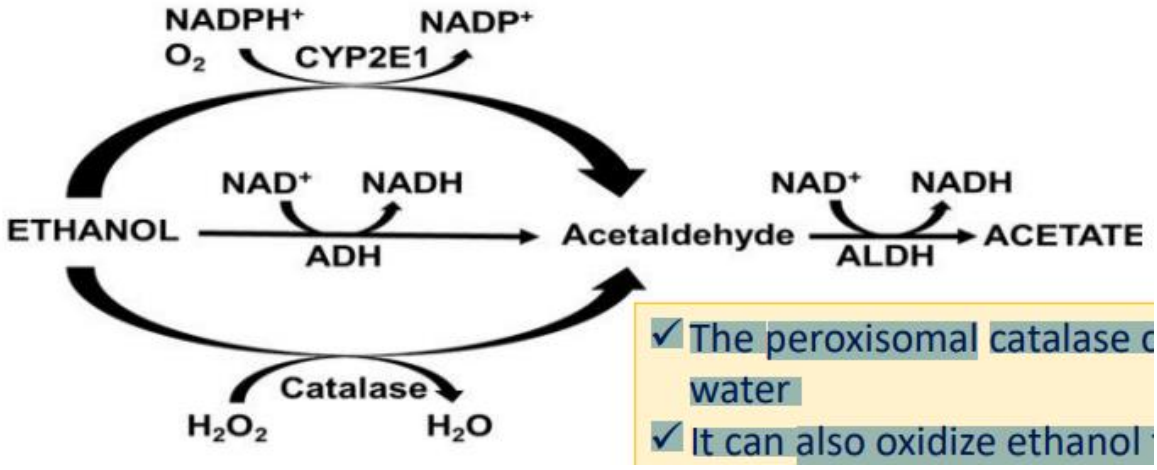
NADPH is in the opposite of NADH ratio, normally NADPH is higher than NADP+We need it in reduced form more. For example we need NADPH in fatty acid metabolism, so alcohol affect fatty acids pathways by increasing NADP+ (inhibition of fatty acids).

- **Cytochrome P450 has High Km for ethanol** (low affinity) then it only work in case of large amount of alcohol (2nd pathway)
- **Inducible by ethanol**
- **CYP2E1 is a major contributor of oxidative stress in the hepatocytes by generating several reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), hydroxyethyl radical (HER·), hydroxyl radical (OH–) and superoxide (O2 –)** (another bad effect of alcohol!)

METABOLISM OF ALCOHOL-CATALASE

3rd pathway

- **The peroxisomal catalase converts H2O2 to oxygen and water.** Peroxisomal main function is to reduce oxidative stress.
- **It can also oxidize ethanol to acetaldehyde** (as same time of oxidation H_2O_2) by a catalase.
- **Is not a key pathway for ethanol elimination**
- **Catalase is ubiquitously expressed in almost all tissues**
- **Catalase is also expressed by colonic floras which may lead to acetaldehyde production in the lower gastrointestinal tract**
- **Catalase activity relies on the cellular level of H2O2**



ETHANOL METABOLISM APPLICATION

- **ADH has 5 classes or isoenzymes**
- **Different isoforms are expressed in**

  1) Different tissues such as liver, lung, stomach and esophagus.

  2) Varies in kinetic as speed in metabolism; For example Asian people get drunk by a little amount of alcohol (remember ALDH I isomer in biochemistry).
- **People with different races inherit different sets of ADH isoenzymes, for example African Americans have an isoform with a high maximal velocity resulting in fast ethanol metabolism**

DONE

و سبحان الله الي حرم علينا كل هالمصائب ❤️

ختمت الدكتورة بكلام جميل:

" عدا الناحية دينية, لا تخلي حالك عبد لإشي مثل السجائر والكحول ولا لظروف و أماكن معينة ولا ماركة و أزياء معينة, حتى الشاي و القهوة لا تقنع نفسك إنك ما بتصحى بدونهم, إذا بدك تصحى بتصحى بدونهم و بدون منبهات كمان"

Test bank:

Q1: An enzyme in liver which is part of both the glycolytic and gluconeogenic pathway is:

A) glucose 6-phosphatase

B) PEP carboxykinase

C) fructose 1,6-bisphosphatase

D) glucokinase

E) glyceraldehyde 3-phosphate dehydrogenase

Q2: which of the following is (are) unique reaction(s) for Gluconeogenesis:

A) Pyruvate to oxaloacetate

B) Glucose 6-phosphate to glucose

C) Fructose 1,6 bisphosphate to fructose 6-phosphate

D) All of the above

E) None of the above

Q3: One of the following is not a substrate for gluconeogenesis:

A) Succinate

B) Acetate

C) Glycerol

D) Glutamate

E) Malate

Q4: Which of the following is a not a common intermediate between glycolysis

and gluconeogenesis?

A) Glucose 6-phosphate

B) Phosphoenolpyruvate

C) Oxaloacetate

D) Fructose 1,6-bisphosphate

Q5: One of the following enzymes is common between gluconeogenesis and production of glucose from liver glycogen:

a) glucose 6- phosphatase.

b) phosphorylase.

c) phosphoglucose isomerase.

d) hexokinase.

e) fructose 2,6 bisphosphatase.

Q6: What are the main enzyme systems for catalyzing ethanol to acetaldehyde?

a) Alcohol dehydrogenase (ADH)
b) Catalase
c) Alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2EI), catalase
d) cytochrome P450 2E1 (CYP2EI)

Q7: Which of these equations showing the metabolism of alcohol is correct?

a) Alcohol -> Acetaldehyde -> Acetate -> Acetyl CoA
b) Alcohol -> Acetate -> Acetaldehyde -> Acetyl CoA
c) Alcohol -> Acetaldehyde -> Acetate -> Acetoacetate
d) Alcohol -> Acetate -> Acetaldehyde -> Acetate -> Acetone

Answers:

1- E
2- D
3- B
4- C
5- A
6- C
7- A

سَتَغمرنا السحائِبُ عن قريبٍ بِبُشرَى لا يُحاطُ بمُنتهاها ❀ ❤