Chapter two: Biochemical Reactions

2.1. ENZYMES

1.1. Introduction to enzyme

❑Catalyst

- ✓ substance that increase rates of a reactions.
- ✓ does not effect equilibrium.
- ✓ remain unchanged in overall process.
- ✓ reactants bind to catalyst, products are released.
- ✓ Catalyst could be

  * Chemical catalyst

  * Biological catalyst

Cont……..

- ✓ Catalysts increase product formations by:

A. Lowering energy barrier (activation energy) for product formation.

B. Increases the favorable orientation of colliding reactant molecules for product formation to be successful (stabilize transition state intermediate).

cont,.

Free energy, G

$\Delta G^{\ddagger}$, Free energy of activation

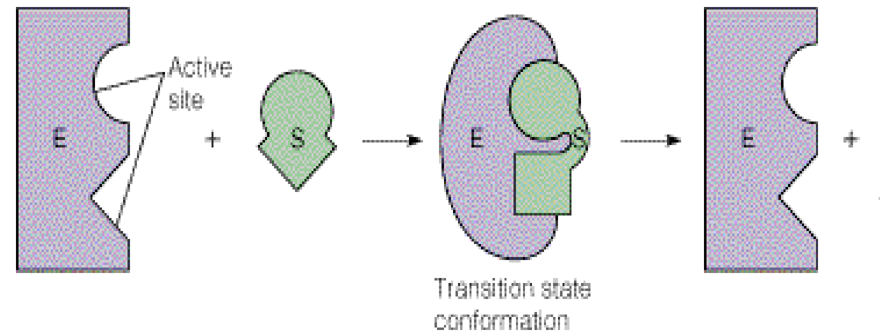
$\Delta G^{\ddagger}$, Energy of activation with enzymes

Glucose $+ 6O_2$

ΔG, Free energy released

$6CO_2 + 6H_2O$

Progress of reaction



Cont…….

❑ Biological catalysts: Enzymes

- The enormous variety of biochemical reactions that comprise life are nearly all mediated by a series of remarkable biological catalysts known as **enzymes-** are biological catalysts that increase the rates of biochemical reactions.
- Although enzymes are subject to the same laws of nature that govern the behavior of other substances, they differ from ordinary chemical catalysts in several important respects:

Cont……

A. Higher reaction rate

- **Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates!**
- **Urease is a good example:**
  - **Catalyzed rate: 3×10^{4}/sec**
  - **Uncatalyzed rate: 3×10^{-10}/sec**
  - **Ratio is 1×10^{14} !**

B. Milder Reaction conditions

- Enzymatically catalyzed reactions occur under relatively mild conditions: temperatures below 100°C, atmospheric pressure, and nearly neutral pH's. In contrast, efficient chemical catalysis often requires elevated temperatures and pressures as well as extremes of pH.

Cont.

C. Greater Reaction Specificity

- Enzymes generally exhibit specificity for substrates and may be of the following categories:

a. Absolute specificity: some enzyme act on only one substrate.

b. Broad specificity: some enzymes act on a range of substrates related to each other. For example, carboxypeptidase acts on protein chain.

c. Group specificity : some enzymes have a preference for a specific organic group on the substrate molecule. For example, alcohol dehydrogenase acts on alcohol.

Cont……

d. Stereo specificity : some enzymes show optical specificity, i.e, they descriminate between their optical isomers, which are related substrates. For example, L-amino acid oxidase acts on an L-isomer and not on D-isomer.

D. Capacity For Control

- This related with the mechanism how enzyme works.

Enzyme Classification

1. Oxidoreductases = catalyze oxidation-reduction reactions (NADH)
2. Transferases = catalyze transfer of functional groups from one molecule to another.
3. Hydrolases = catalyze hydrolytic cleavage
4. Lyases = catalyze removal of a group from or addition of a group to a double bond, or other cleavages involving electron rearrangement.
5. Isomerases = catalyze intramolecular rearrangement.
6. Ligases = catalyze reactions in which two molecules are joined.

Enzymes named for the substrates and type of reaction

catalases
dehydrogenases
hydrogenases
hydroxylases
oxidases
oxygenases
peroxidases
reductases

Class 2: Transferases
acyltransferase
glucosyltransferase
kinases
methyltransferase
phosphomutases
phosphoryltransferase
transaldolase
transmutases

Class 3: Hydrolases
amidases
deaminases
esterases
glycosidases
phosphatases
phospholipidases
ribonucleases
thiolases

aldolases
decarboxylases
dehydratases
hydratases
lyases
synthases

Class 5: Isomerases
epimerases
isomerases
some mutases
racemases

Class 6: Ligases
carboxylases
synthetases

Concepts of active site

- The active site of an enzyme is the center of catalytic activity.
- There are one or more regions on the enzyme molecule where the substrate can bind. These are composed of aas where the substrate attachment is possible and location of the site is always with reference to the shape of the enzyme molecule.
- If the shape of the enzyme molecule is altered, the active site is also displaced, blocking the catalytic function.

Enzymatic Reaction Mechanism

The Mechanism of Enzymatic Action

1. The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the active site.
2. A temporary intermediate compound forms, called an enzyme-substrate complex.
3. The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.

Cont……….

4. The transformed substrate molecules—the products of the reaction—are released from the enzyme molecule because they no longer fit in the active site of the enzyme.
5. The unchanged enzyme is now free to react with other substrate molecules.

2.1. Enzymatic Catalysis

❑ Catalytic Mechanisms

✓ Enzymes use several types of catalytic mechanisms, including:

*acid-base catalysis

*covalent catalysis

*metal ion catalysis

* catalysis by proximate and orientation effects

* catalysis by preferential binding of the transition state

✓ Acid-base catalysis-

Enzyme Kinetics:Introduction

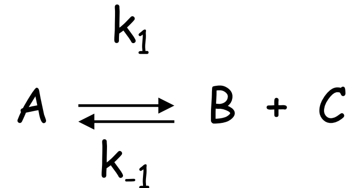
❑ What is kinetics?

- Is the study of the rate and mechanism in the reactor.
- Gives us a quantitative description of how fast chemical reactions occur, and the factors affecting these rate.
- Identifies “rate-limiting“ step.
- **Why Kinetics?**

✓ In chemical reaction engineering (CRE), the information obtained from kinetics is a means to determine something about the reactor: size, flow and thermal configuration, product distribution, etc.

Rate constants and reaction order

Rate constant (k) measures how rapidly a rxn occurs



Rate (v, velocity) = (rate constant) (concentration of reactants)

$v = k_1 [A]$
1st order rxn (rate dependent on concentration of 1 reactant)

$v = k_{-1}[B][C]$
2nd order rxn (rate dependent on concentration of 2 reactants)

Zero order rxn (rate is independent of reactant concentration)

Enzyme Kinetics

- Michaelis-Menten kinetics or saturation kinetics which was first developed by V.C.R. Henri in 1902 and developed by L. Michaelis and M.L. Menten in 1913.

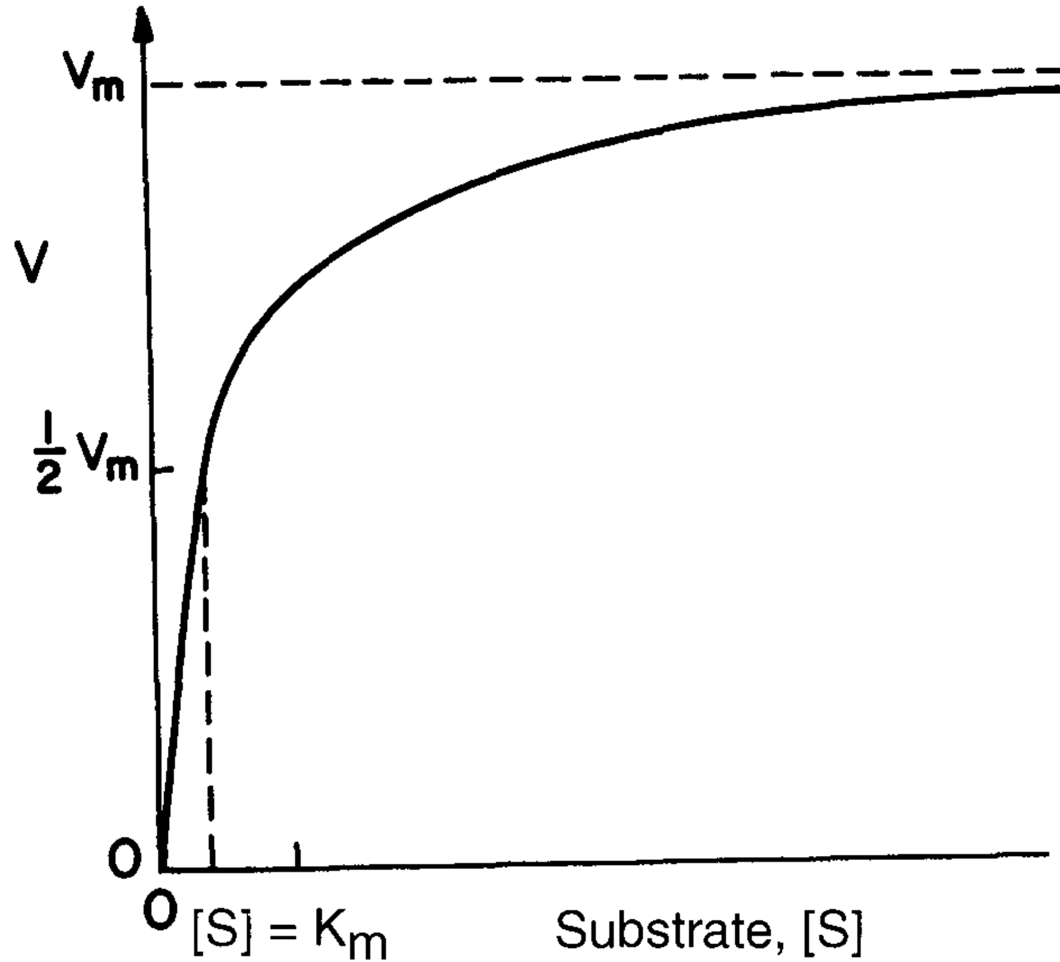
- This model is based on data from batch reactors with constant liquid volume.

  - Initial substrate, $[S_0]$ and enzyme $[E_0]$ concentrations are known.

  - An enzyme solution has a fixed number of active sites to which substrate can bind.

  - At high substrate concentrations, all these sites may be occupied by substrates or the enzyme is saturated.

Saturation Enzyme Kinetics



From the curve graph……

- Reaction rate is influenced by
- ✓ Substrate concentrations
- ✓ Enzyme concentrations
- Rate (v, velocity) = (rate constant) (concentration of reactants).
- Based on these conditions we can conclude the following points

A. Reaction rate is proportional to substrate concentrations, i.e, 1st order rxn (rate dependent on concentration of 1 reactant).

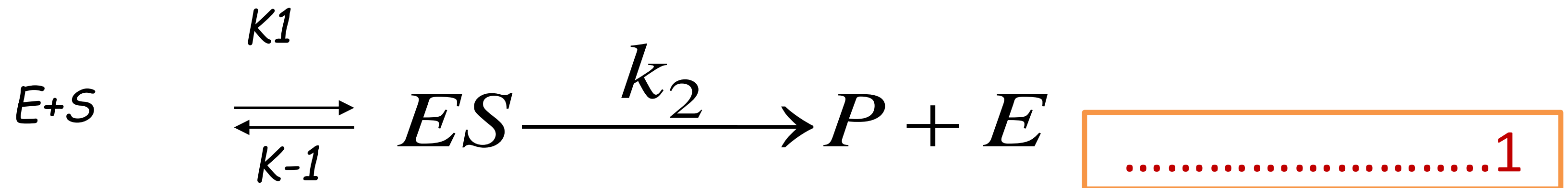
Cont…..

B. The reaction rate does not depend on substrate concentration when the substrate concentration is high, i.e, Zero order rxn (rate is independent of reactant concentration).

C. The maximum reaction rate Vmax is proportional to the enzyme concentration within the range of the enzyme tested.

M-M Enzyme Kinetics

- Saturation kinetics can be obtained from a simple reaction scheme that involves a reversible step for enzyme-substrate complex formation and a dissociation step of the ES complex.



$$ES \xrightarrow{k_2} P + E \quad \text{..........................1}$$

where the rate of **product** formation v (moles/l-s, g/l-min) is

$$v = \frac{d[P]}{dt} = k_2 [ES] \quad \text{.................................2}$$

K_i is the **respective** reaction rate constant.

Enzyme Kinetics

The rate of variation of ES complex is

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

Since the enzyme is not consumed, the conservation equation on the enzyme yields

$$[E] = [E_0] - [ES]$$

.........................3

Enzyme Kinetics

Velocity

$$v = \frac{d[P]}{dt} = k_2[ES]$$

Recall equation 2

How to use independent variable [S] to represent v?

Enzyme Kinetics

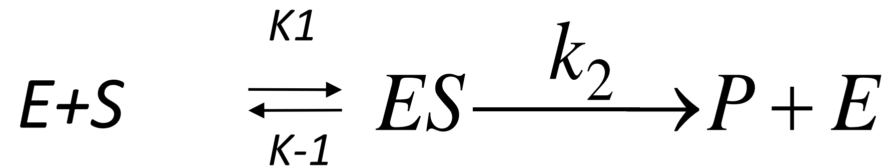
At this point, an assumption is required to achieve an analytical solution.

- Michaelis - Menten Approach.
  - -The rapid equilibrium assumption
  - - The slowest step determines the rate.
- Briggs and Haldane Approach.
  - -The quasi-steady-state assumption

Michaelis - Menten Approach

The rapid equilibrium assumption:

- Assumes a rapid equilibrium between the enzyme and substrate to form an [ES] complex.



$$ES \xrightarrow{k_2} P + E$$

$$k_1[E][S] = k_{-1}[ES]$$

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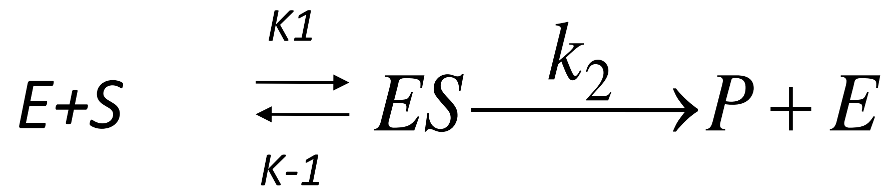
Michaelis - Menten Approach

The equilibrium constant $K_m^{'}$ can be expressed by the following equation in a dilute system.



$$K_m^{'} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$ ………………..5

Michaelis - Menten Approach

Then rearrange the above equation,

$$[ES] == \frac{[E][S]}{K_m^{'}}$$ 6

Substituting [E] in the above equation with enzyme mass conservation equation (3),

$$[E] = [E_0] - [ES]$$

yields,

$$[ES] == \frac{([E_0]-[ES])[S]}{K_m^{'}}$$ 7

Michaelis - Menten Approach

[ES] can be expressed in terms of [S], rearranging eq(7) and yields,

$$[ES] == \frac{[E_0][S]}{K_m^{'} + [S]} \qquad \text{....................8}$$

Then the rate of production formation v can be expressed in terms of [S],

$$v = \frac{d[P]}{dt} = k_2 [ES] = \frac{k_2 [E_0][S]}{K_m^{'} + [S]} = \frac{V_m [S]}{K_m^{'} + [S]} \qquad \text{.......9}$$

Where $V_m = k_2 [E_0]$

represents the maximum forward rate of reaction (e.g.moles/L-min).

Michaelis - Menten Approach

$K_m^{'}$ - is often called the Michaelis-Menten constant, mol/L, mg/L.

The prime reminds us that it was derived by assuming rapid equilibrium in the step of enzyme-substrate complex formation.

- Low value indicates high affinity of enzyme to the substrate.

-It corresponds to the substrate concentration, giving the ____Half maximum reaction velocity.

$$K_m^{'} = \frac{k_{-1}}{k_1} \qquad v = \frac{1}{2}V_m = \frac{V_m[S]}{K_m^{'} + [S]}$$

Re-arrange the above equation,

$K_m^{'} = [S]$ When $v = \frac{1}{2}V_m$

Michaelis - Menten Approach

- Vm is maximum forward velocity (e.g.mol/L-s)

$$V_m = k_2 [E_0]$$

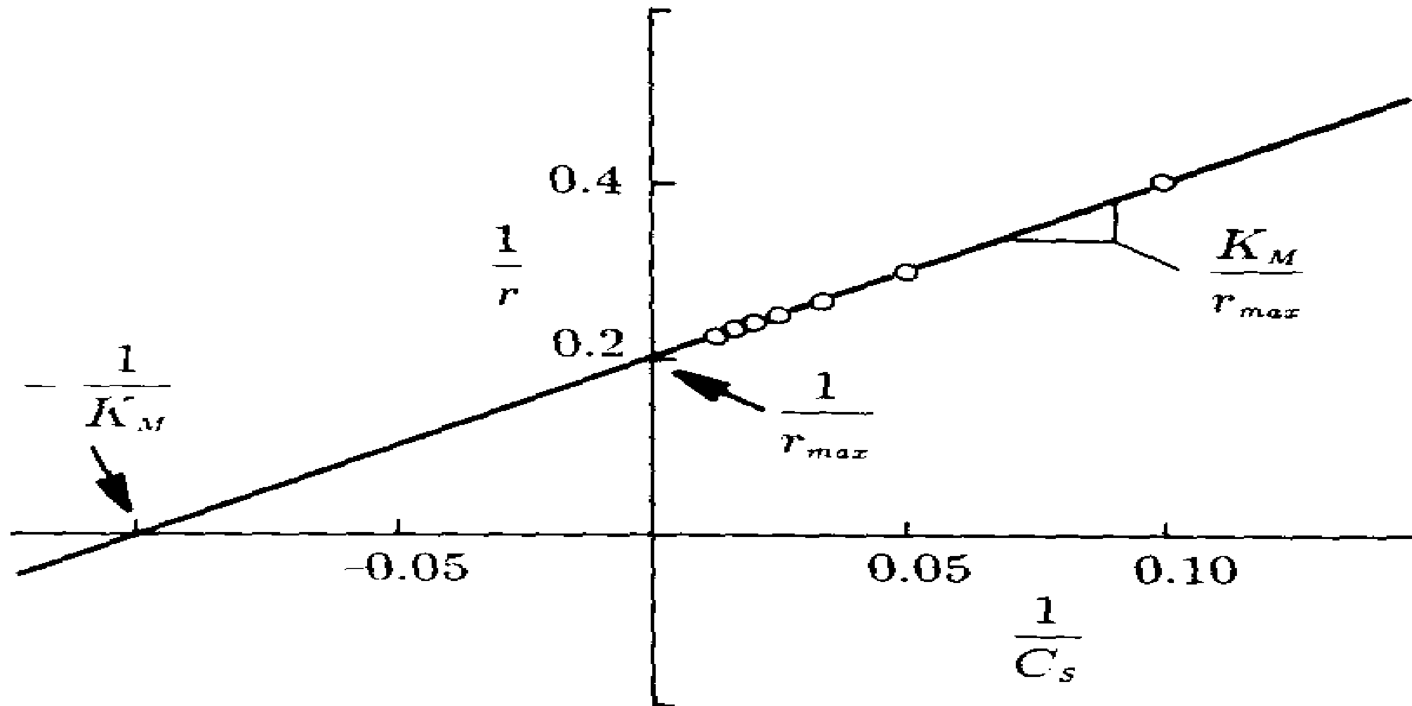
- It <u>increases</u> with initial enzyme concentration.
- It is determined by the rate constant k_2 of the product formation and the initial enzyme concentration.

Lineweaver-Burk Plot (Double-Reciprocal Plot)

$$v = \frac{V_m [S]}{K_m + [S]}$$

Linearizing it in double-reciprocal form:

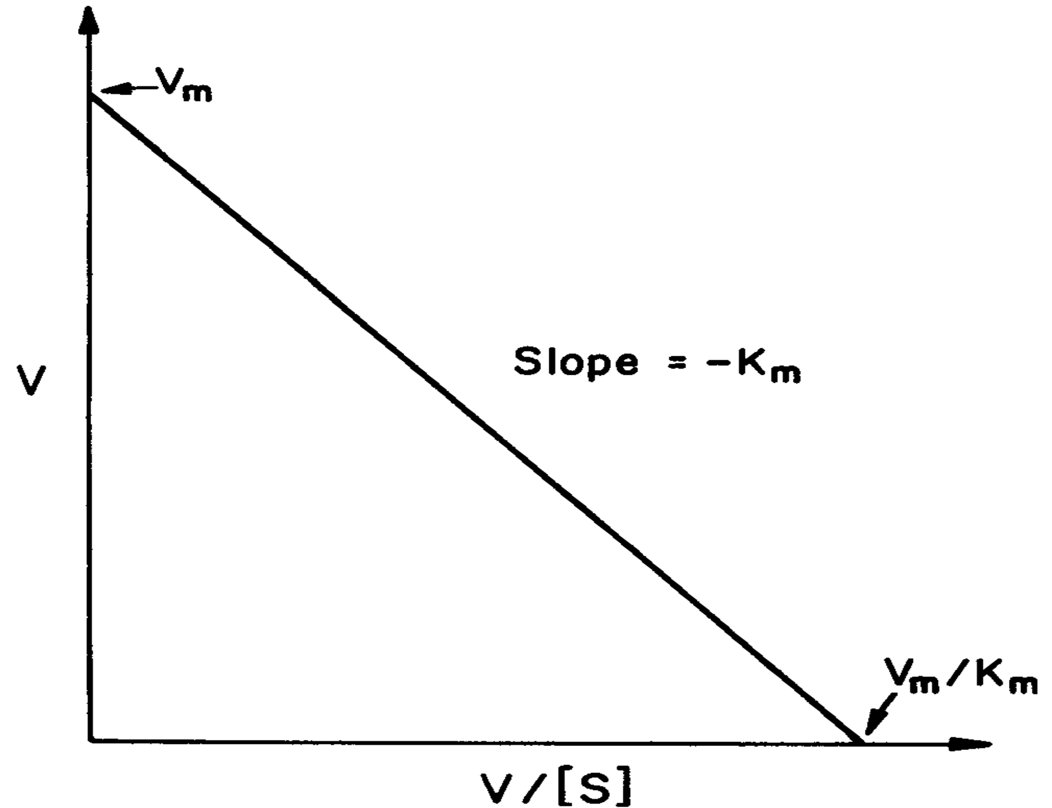
$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} \frac{1}{S}$$



- slope = Km/Vm
- y-intercept= 1/Vm.
- More often used as it shows the independent variable [S] and dependent variable v.
- 1/v approaches infinity as [S] decreases

Eadie-Hofstee Plot

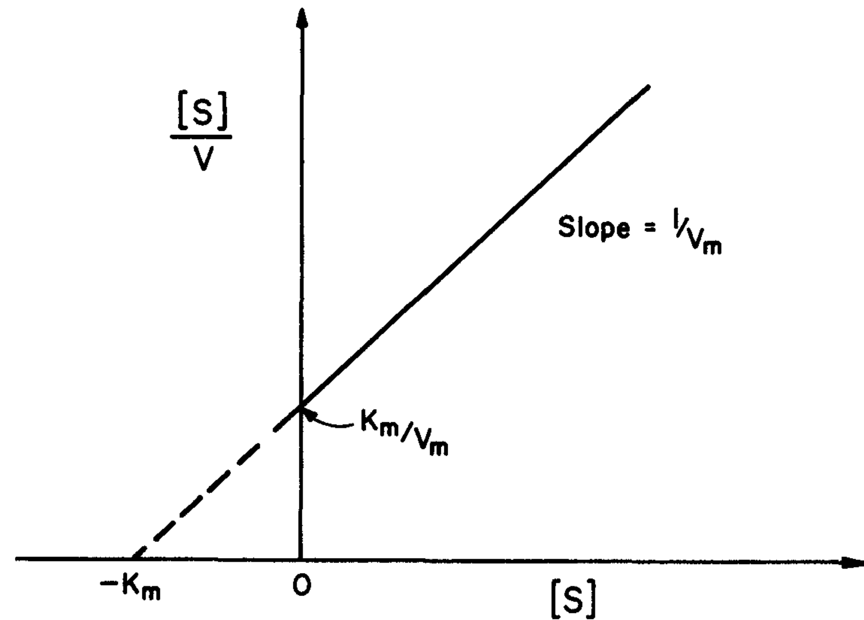
$$v = V_m - K_m \frac{v}{[S]}$$



- slope = –Km
- y-axis intercept = Vm.

Hanes-Woolf (Langmuir) Plot

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m}[S]$$



- the slope is =1/Vm
- y-axis intercept is Km/Vm
- better fit: even weighting of the data

Summary on M-M Parameters

[S]/V = Km/Vmax + [S]/Vmax — Langmuir Plot

1/V = 1/Vmax + Km/Vmax[S] — Lineweaver-Burk Plot

V = Vmax -Kmax V/[S] — Eadie –Hofdtee Plot

Cs =[s] r= v rmax = vmax

- [s]/v vs[s]---------Langmuir plot

-slope =1/Vmax

-Inter = Km/Vmax

- 1/V Vs 1/[s]-------Lineweaver-Burk plot

-slope =Km/Vmax

-Inter = 1/Vmax

- V Vs V/[S]----

Eadie-Hofstee

-Slope = Vmax

-Slope = Km

Evaluation of kinetic parameters

Example1: Here is substrate converted to product in the presence of enzyme.

[S], mM	V, mmol/sec	1/V	[S]/V	V/[S]	1/[S]
1	2.5	0.4	0.4	2.5	1
2	4.0	0.25	0.5	2.0	0.5
5	6.3	0.158	0.793	1.26	0.2
10	7.6	0.1315	1.315	0.76	0.1
20	9.0	0.111	2.222	0.45	0.05

First calculate variables that used for plotting. Variables with orange color should be calculated

Using this table, evaluate Km and Vmax by employing:

1. Langmuir plot
2. Lineweave-Burk plot
3. Eadie-Hofstee plot

Example 1

The following table shows the value of Km and Vmax calculated using the above information.Y=A+BX, A= Y-int. and B=slope

Plotting	Km	Vmax
Langmuir	3.274	10.393
Lineweaver-Burk	3.069	10.165
Eadie-Hofstee	3.091	10.189

N.B. please cross check the values using your calculator

Step:

1. Change mode----REG(3)-----Lin(1)

2.State clear----shift Mode ----Stat cl(1)

Now ready for calculation

3. Enter the data corresponding to each variables to be plotted.

4. .continue in such away!

Quiz 2(10%)

1. From a series of batch runs with a constant enzyme concentration, the following initial rate data were obtained as a function of initial substrate concentration.

substrate con. Mmol/L	Initial rxn rate mmol/Lmin
1	0.20
2	0.22
3	0.30
5	0.45
7	0.41
10	0.50

Evaluate the M-M kinetic parameters, Km and Vm by employing

A. Langmuir plot
B. Lineweaver-Burk plot
C. Edie-Hofstee
D. Compare KM of Langmuir plot and Lineweaver –Burk plot. What do you conclude from the value

Metabolic Pathways

Introduction to Metabolism

Figure 18.3

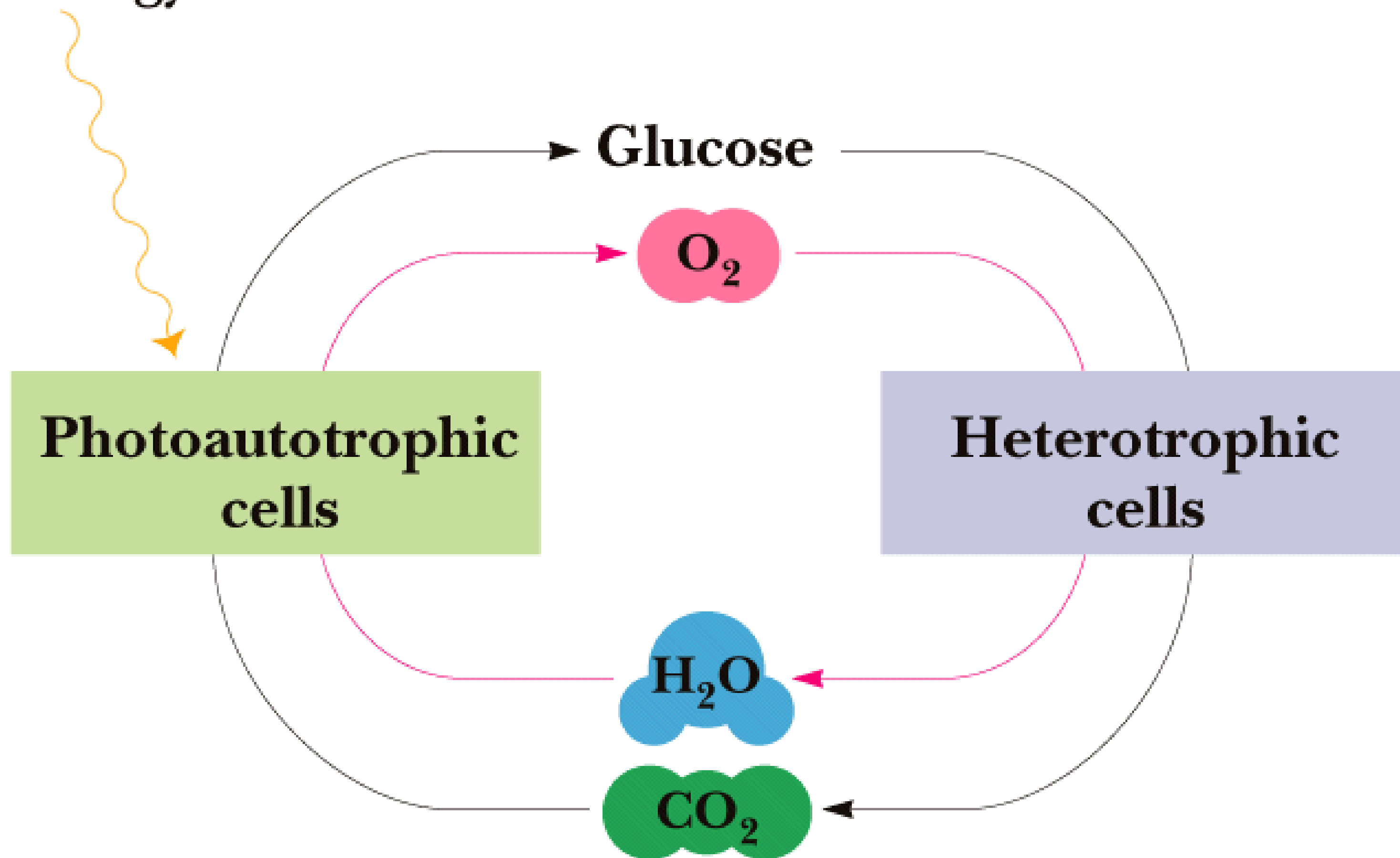


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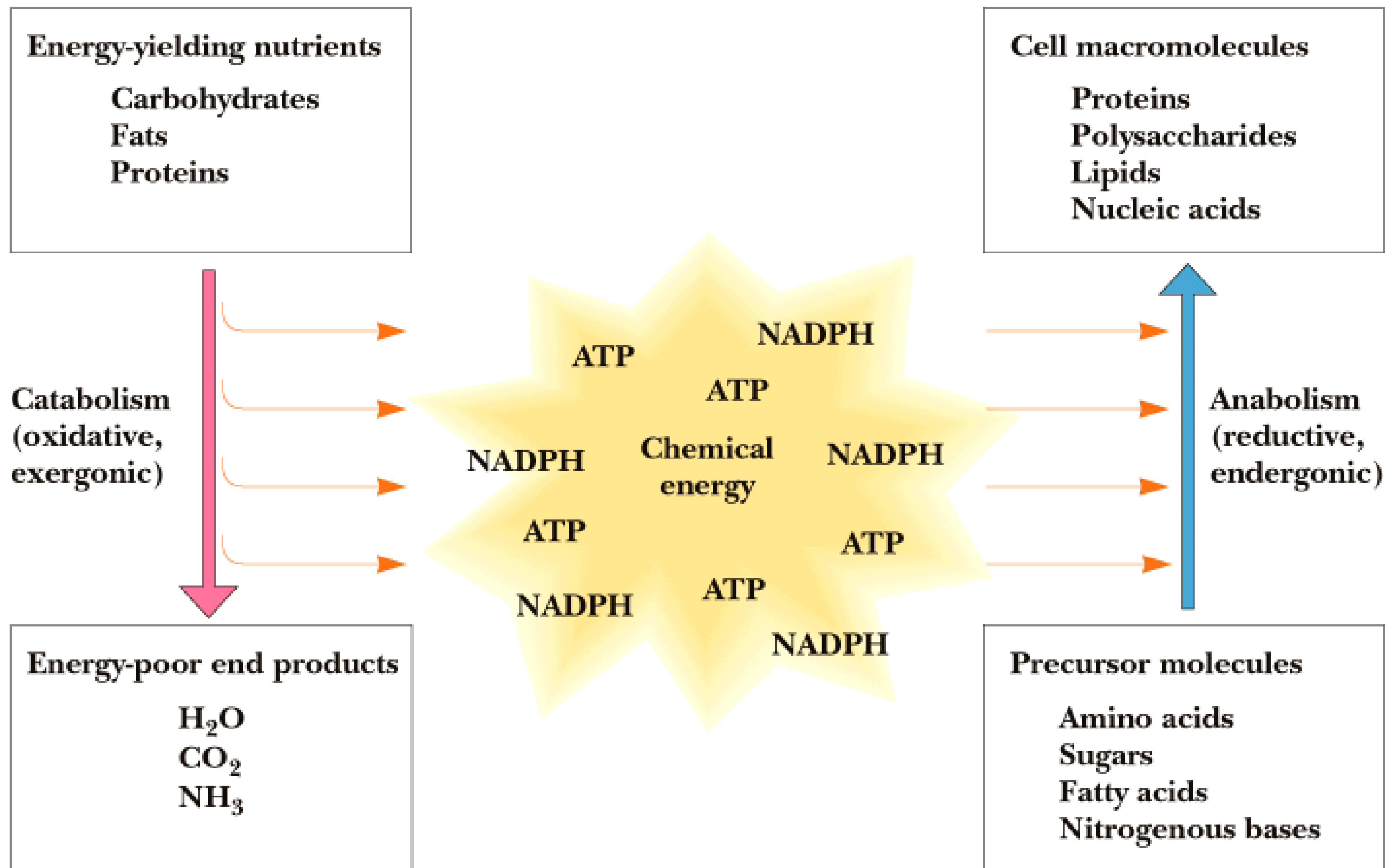
Figure 18.4



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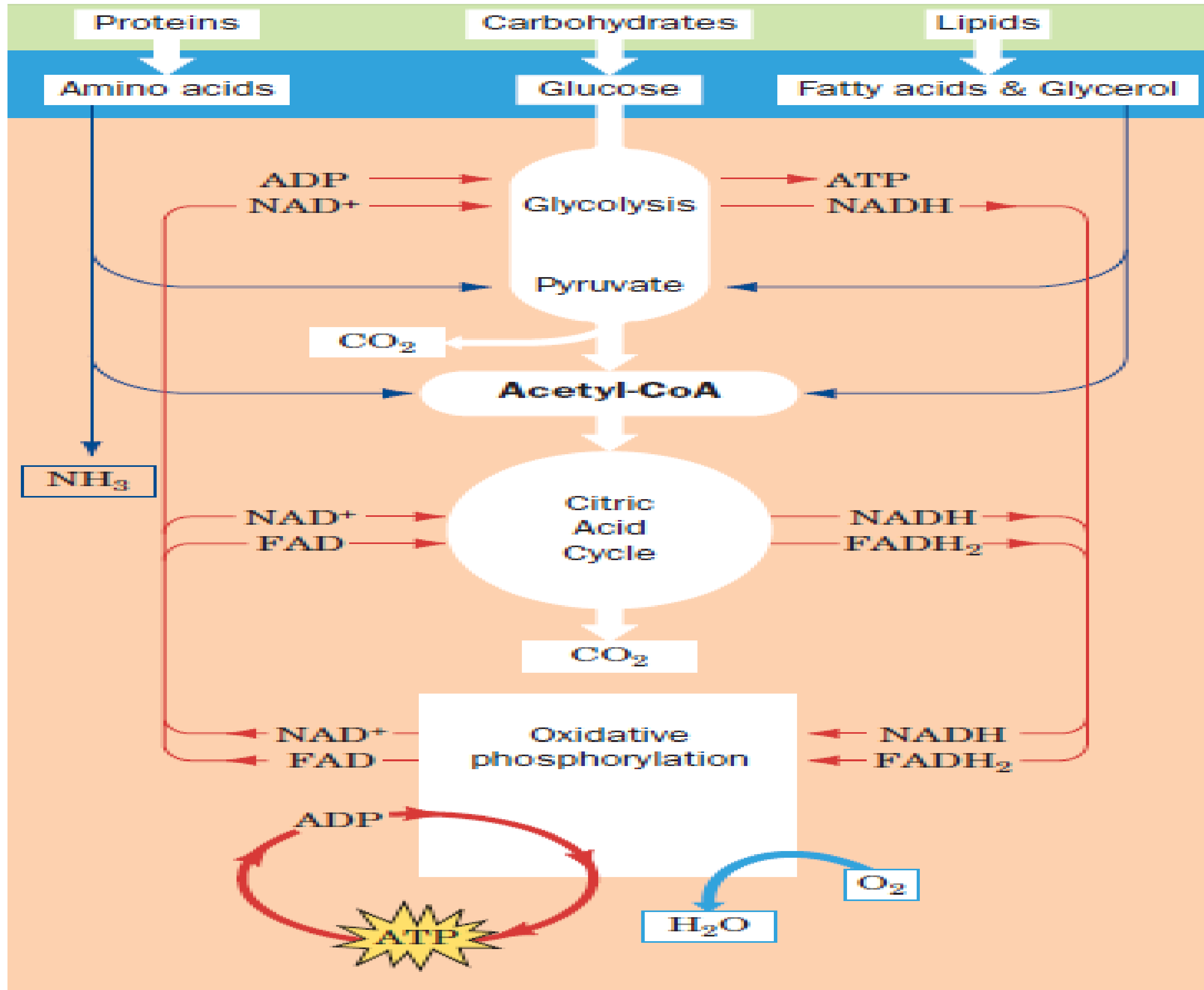
Flavin adenine dinucleotide, or **FADH2**, is a redox cofactor that is created during the Krebs cycle and utilized during the **last part of respiration,** the electron transport chain.

Nicotinamide adenine dinucleotide, or **NADH**, is a similar compound used more actively in the electron transport chain as well

Cont……….

- **Metabolism** is the overall process through which living systems acquire and utilize the free energy they need to carry out their various functions. They do so by coupling the exergonic reactions(**reaction** where energy is released), of nutrient oxidation to the endergonic processes (**reaction** (such as photosynthesis) is a **reaction** that requires energy to be driven), required to maintain the living state such as the performance of mechanical work, the active transport of molecules against concentration gradients, and the biosynthesis of complex molecules.

Cont……….



Three principal characteristics of metabolic pathways stem from their function of generating products for use by the cell:

1. Metabolic Path ways are irreversible

2. They have an exergonic step that serve as the 1st committed step and ensures irreversibility.

3. Catabolic and Anabolic pathway involving the interconversion of two metabolites differ in key exergonic reactions

Carbohydrate Metabolism

❑Glucose Catabolism

ATP can produce from d/t Metabolic pathways

- ✓Cellular Respiration (+O2)
  - * Glycolysis
  - * Krebs Cycle
  - * ETC
- ✓Fermentation
- ✓Photosynthesis

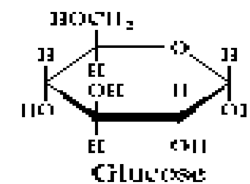
Glycolysis

- Anaerobic process
- Converts hexose to two pyruvates
- Generates 2 ATP and 2 NADH
- For certain cells in the brain and eye, glycolysis is the only ATP generating pathway

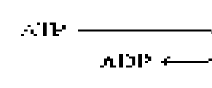
Glycolysis

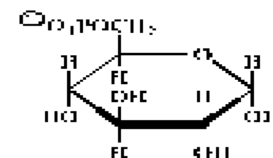
- Essentially all cells carry out glycolysis
- Ten reactions - same in all cells - but rates differ
- Two phases:
  - First phase converts glucose to two G-3-P
  - Second phase produces two pyruvates
- Products are pyruvate, ATP and NADH
- Three possible fates for pyruvate

Phase I: Cleavage of 1 hexose to 2 triose

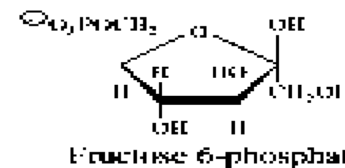


Transfer of a phosphoryl group from ATP to glucose — ① Hexokinase, glucokinase

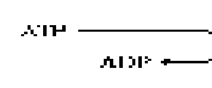


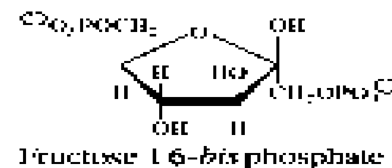


Isomerization — ② Glucose 6-phosphate isomerase

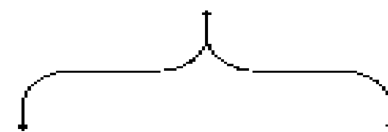


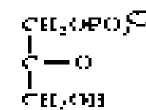
Transfer of a second phosphoryl group from ATP to fructose 6-phosphate — ③ Phosphofructokinase-1

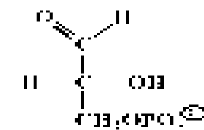




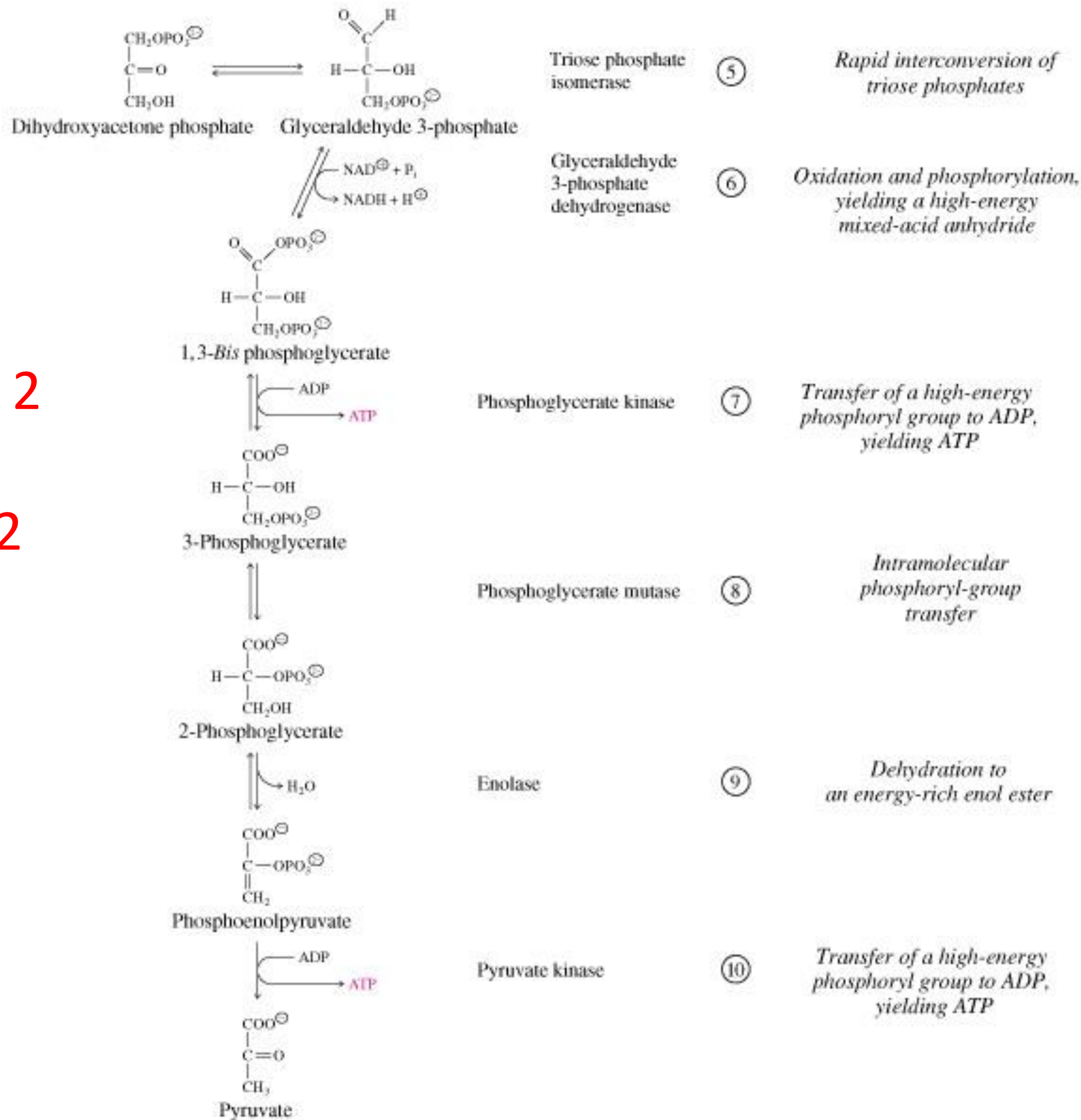
C-3—C-4 bond cleavage, yielding two triose phosphates — ④ Aldolase





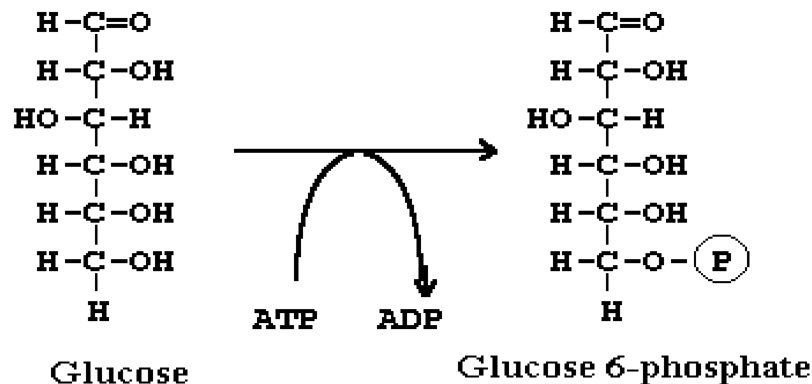


Phase II:
Generation of 2 ATPs,
2 NADH and 2 Pyruvates



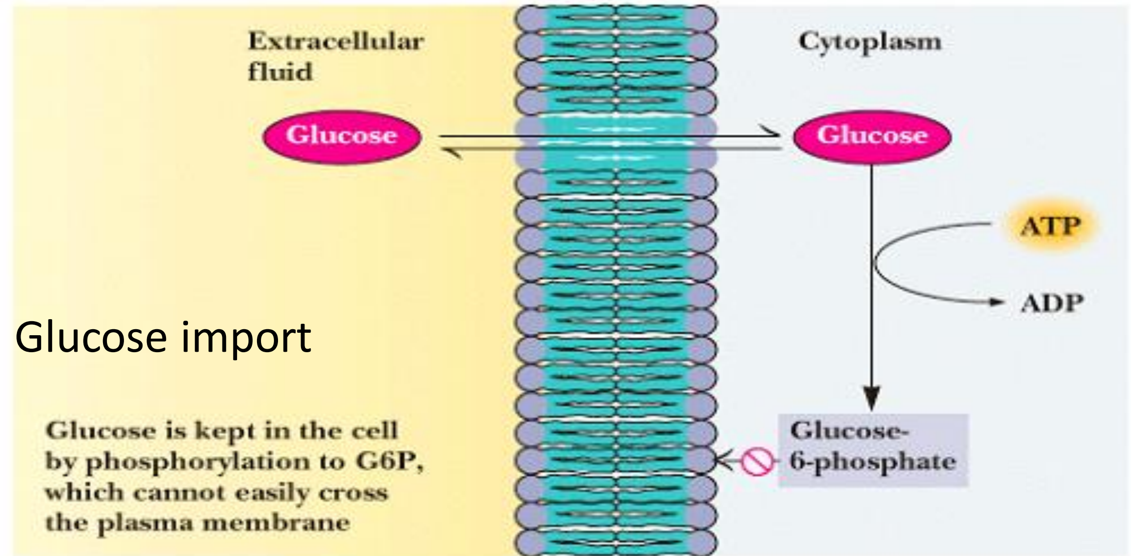
Rx1. Hexose Kinase

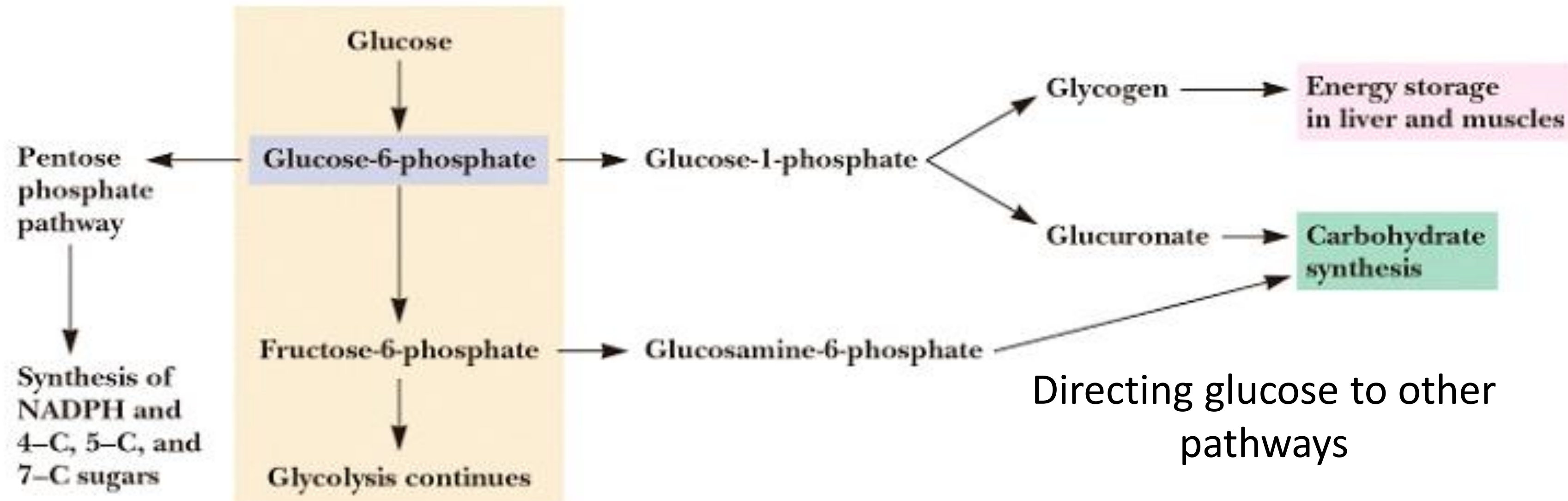
- ✓ 1st step in glycolysis; ΔG large, negative
- ✓ This is a priming reaction, (where a phosphate group is added to glucose using ATP.) - ATP is consumed here in order to get more later
- ✓ ATP makes the phosphorylation of glucose spontaneous



Hexokinase also functions in other processes

Not 1st committed step in glycolysis



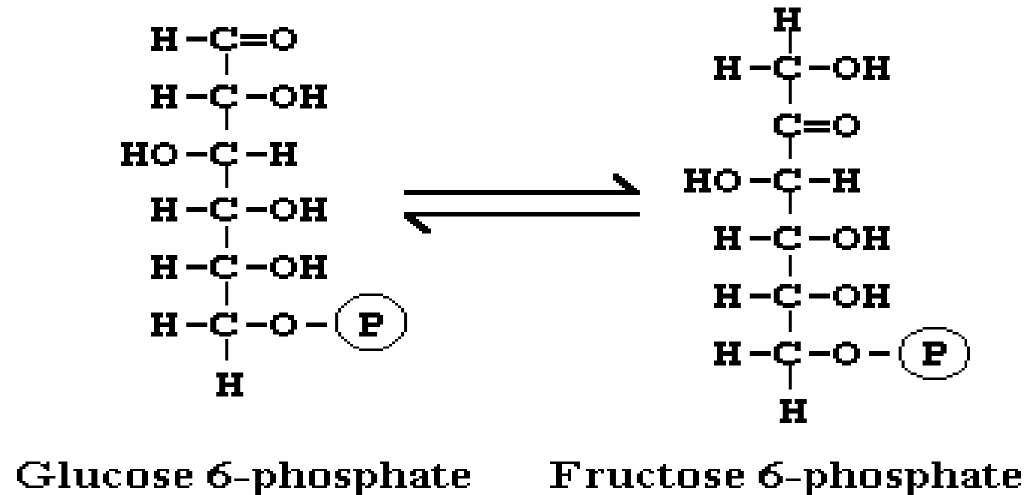


Different Hexokinase Isozymes

- Two major forms hexokinase (all cells) & glucokinase (liver)
- K_m for hexokinase is 10^{-6} to 10^{-4} M; cell has 4 X 10^{-3} M glucose
- K_m for glucokinase is 10^{-2} M only turns on when cell is rich in glucose
- Glucokinase functions when glucose levels are high to sequester glucose in the liver.
- Hexokinase is regulated - allosterically inhibited by (product) glucose-6-P

Rx 2: Phosphoglucoisomerase

- Uses open chain structure as substrate
- Near-equilibrium rxn (reversible)
- Enzyme is highly stereospecific (doesn't work with epimers of glucose-6-phosphate

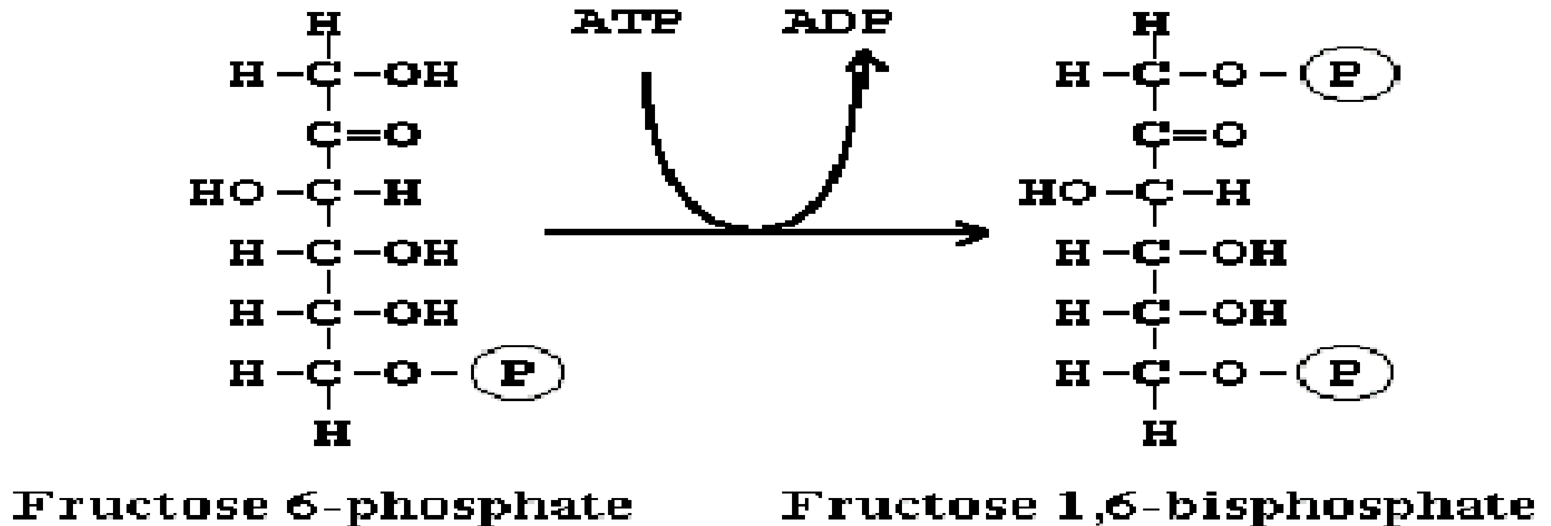


Cont….

- Why does this reaction occur?
  - next step (phosphorylation at *C*-1) would be tough for hemiacetal -OH, but easy for primary -OH
  - isomerization activates *C*-3 for cleavage in aldolase reaction

Rx 3: Phosphofructokinase

- *PFK is the committed step in glycolysis!*
- ✓ The second priming reaction of glycolysis
- ✓ Committed step and large, -ΔG – means PFK is highly regulated
- ✓ β-D-fructose-6-phosphate is substrate for rxn

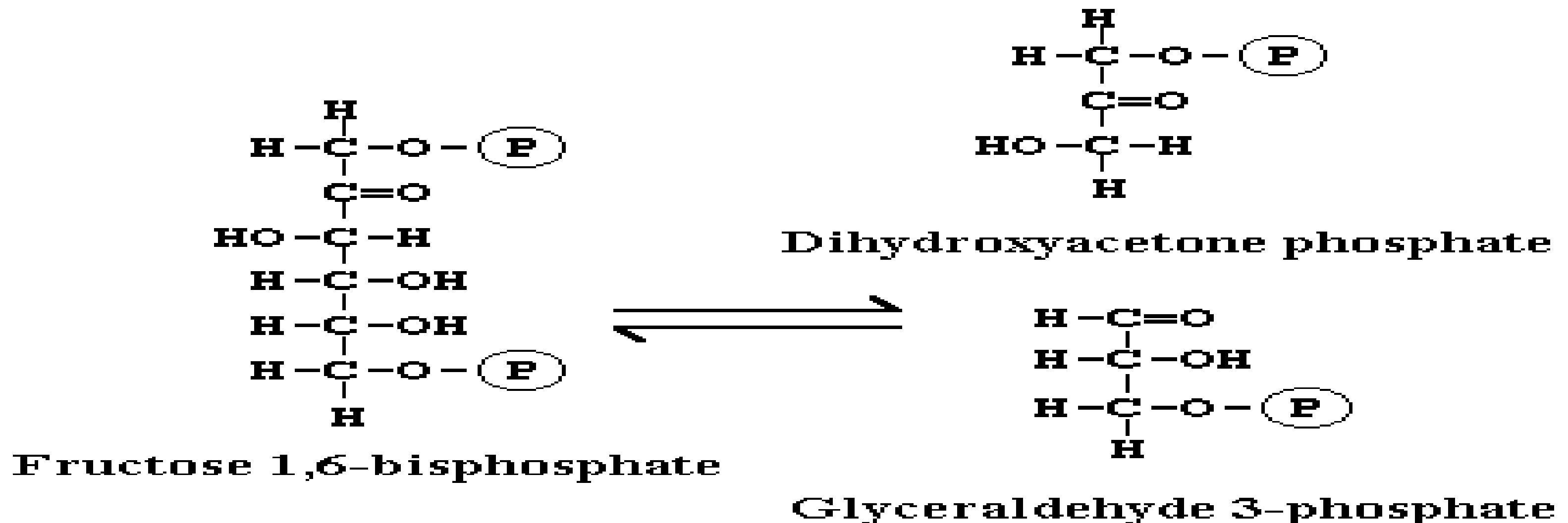


❑Phosphofructokinase is highly regulated

- ❑Citrate is also an allosteric inhibitor
- ❑Fructose-2,6-bisphosphate is allosteric activator
- ❑PFK increases activity when energy status is low
- ❑PFK decreases activity when energy status is high

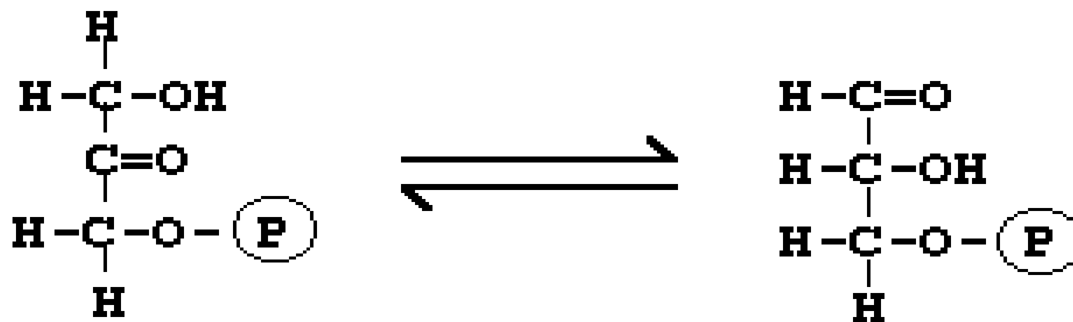
Rx 4: Aldolase

- ✓ Hexose cleaved to form two trioses
- ✓ C1 thru C3 of F1,6-BP -> DHAP
- ✓ C4 thru C6 -> G-3-P
- ✓ Near-equilibrium rxn
- ✓ Position of carbonyl group determines which bond cleaved.
- ✓ If Glucose-6 -P was the substrate would end up with 2 carbon and 4 carbon product



Rx 5: Triose Phosphate Isomerase (TPI)

- ✓ Near equilibrium rxn
- ✓ Conversion of DHAP to G-3-P by TPI maintains steady state [G-3-P]
- ✓ Triose phosphate isomerase is a near-perfect enzyme (Kcat/Km near diffusion limit



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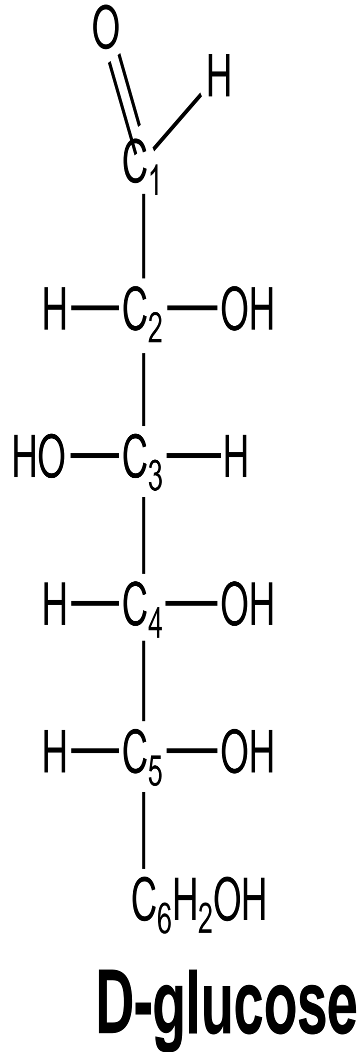
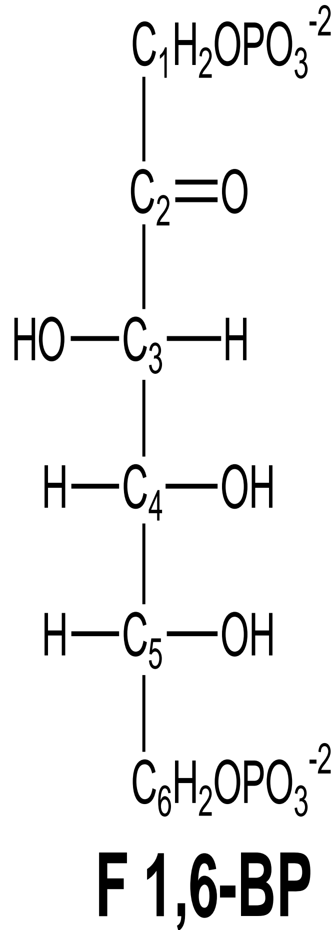
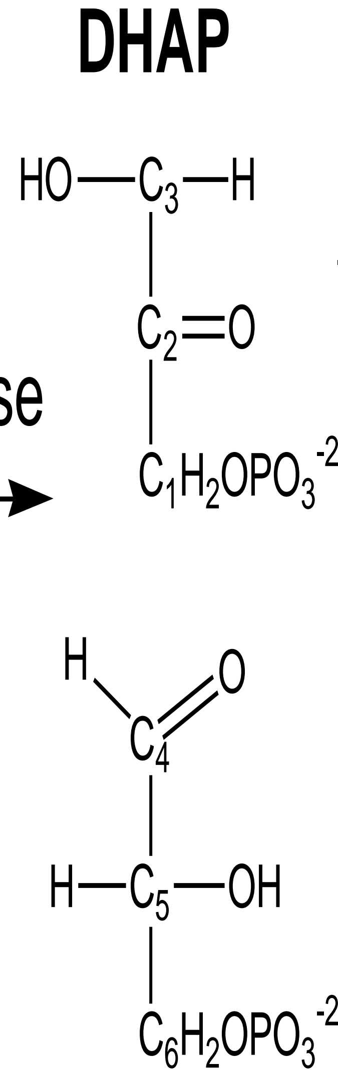


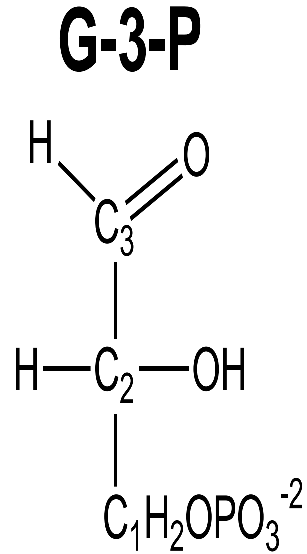


Aldolase



G-3-P

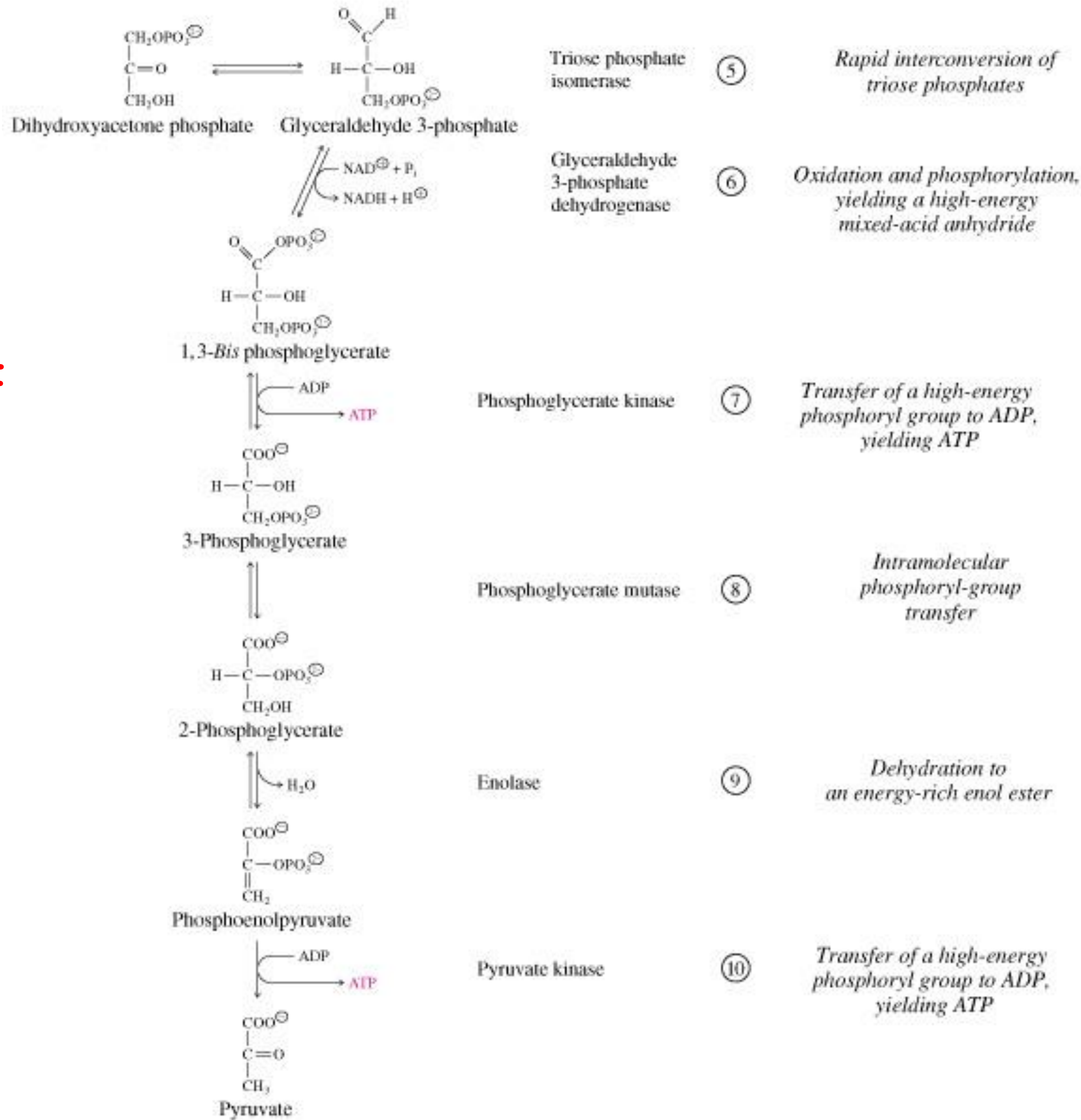
TPI



❑Glycolysis - Second Phase

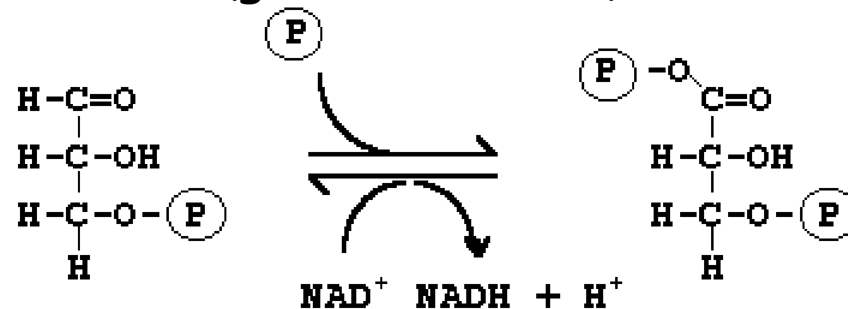
- *Metabolic energy produces 4 ATP*
- ✓Net ATP yield for glycolysis is two ATP
- ✓Second phase involves two very high energy phosphate intermediates
  - 1,3 BPG
  - Phosphoenolpyruvate

Phase II: Generation of 2 ATPs, 2 NADH and 2 Pyruvates



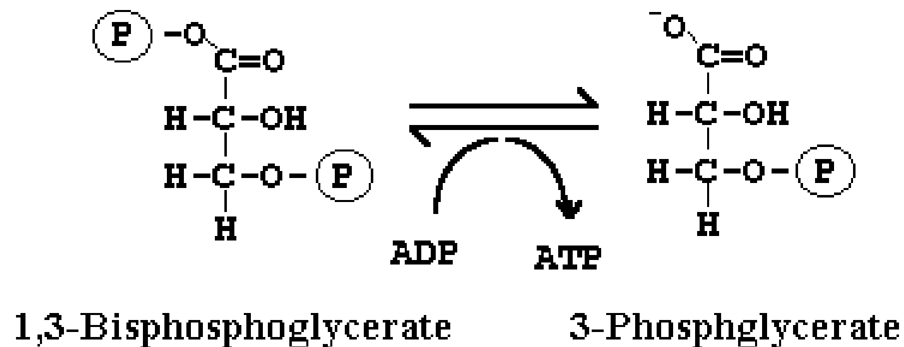
Rx 6: Glyceraldehyde-3P-Dehydrogenase

- G3P is oxidized and phosphorylated to 1,3-BPG
- Near equilibrium rxn
- Pi is used as phosphate donor
- C1 phosphoryl group has high group transfer potential, used to phosphorylate ADP to ATP in next step of glycolysis
- Arsenate can replace phosphate in rxn (results in lower ATP)
- NADH generated in this reaction is reoxidized by respiratory electron transport chain (generates ATP)



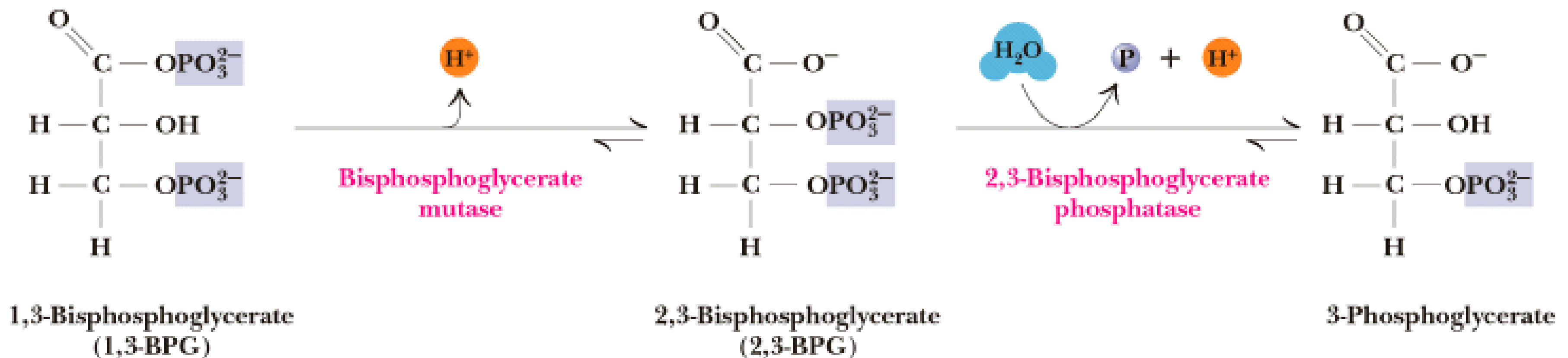
Rx 7: Phosphoglycerate Kinase (PGK)

- ATP synthesis from a high-energy phosphate
- This is referred to as "substrate-level phosphorylation"
- Although has large negative $DG^{o'}$ (-18 kJ/mole) because PGK operates at equilibrium in vivo, the overall DG is 0.1 Kj/mole and is a near-equilibrium rxn.
- 2,3-BPG (for hemoglobin) is made by circumventing the PGK reaction



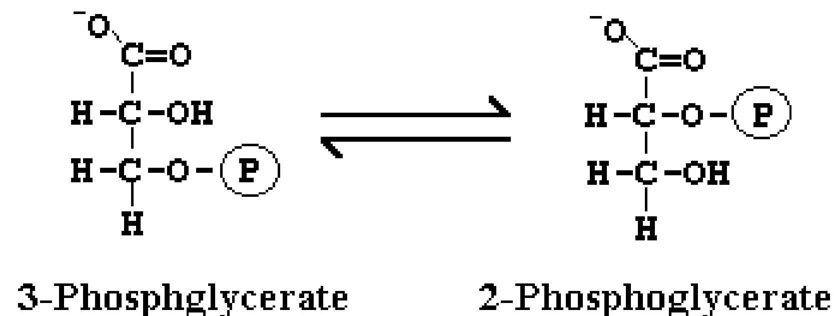
2,3-BPG (for hemoglobin) is made by circumventing the PGK

- 2,3-BPG acts to maintain Hb in low oxygen affinity form
- RBC contain high levels of 2,3 BPG (4 to 5 mM)



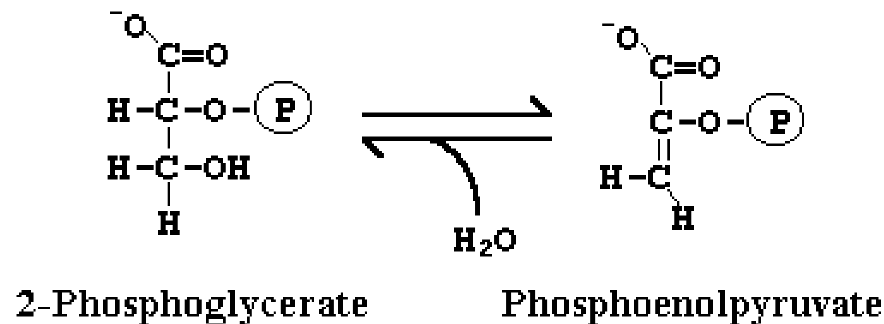
Rx 8: Phosphoglycerate Mutase

- Phosphoryl group moves from C-3 to C-2
- Mutases are isomerases that transfer phosphates from one hydroxyl to another
- Involves phosphate-histidine intermediate



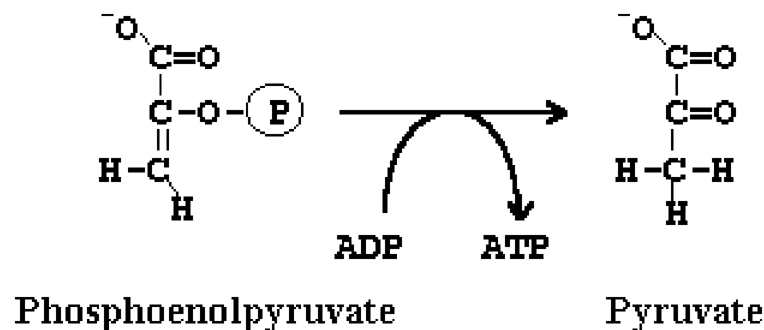
Rx 9: Enolase

- Near equilibrium rxn
- "Energy content" of 2-PG and PEP are similar
- Enolase just rearranges to a form from which more energy can be released in hydrolysis
- Requires Mg^{2+} for activity, one bings Carboxyl group of substrate the other involved in catalysis.



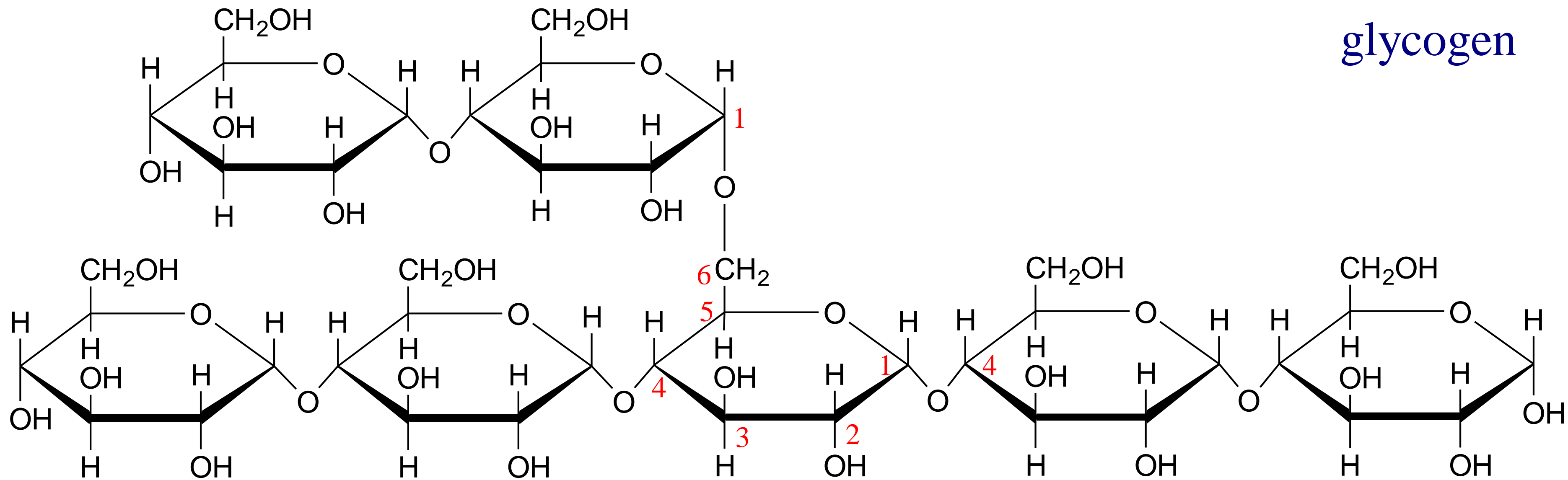
Rx 10: Pyruvate Kinase

- Substrate level phosphorylation generates second ATP
- Large, negative G - regulation!
- Allosterically activated by AMP, F-1,6-bisP
- Allosterically inhibited by ATP and acetyl-CoA



Glycogen Metabolism

- Glycogenolysis is a catabolic process; the breakdown of glycogen to glucose units.
- In liver - The synthesis and breakdown of glycogen is regulated to maintain blood glucose levels.
- In muscle - The synthesis and breakdown of glycogen is regulated to meet the energy requirements of the muscle cell.



Glycogen is a polymer of **glucose** residues linked by

- α(1→4) glycosidic bonds, mainly
- α(1→6) glycosidic bonds, at branch points.

Glycogen chains & branches are longer than shown.

Glucose is stored as glycogen predominantly in **liver** and **muscle** cells.

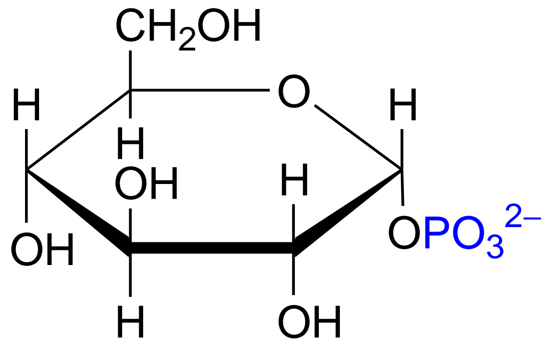
Cont……….

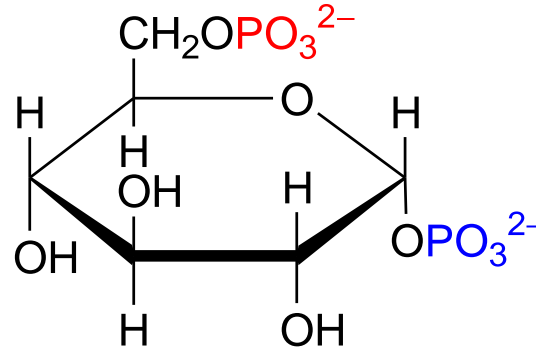
- Glycogen breakdown (glycogenolysis) utilizes three enzymes:

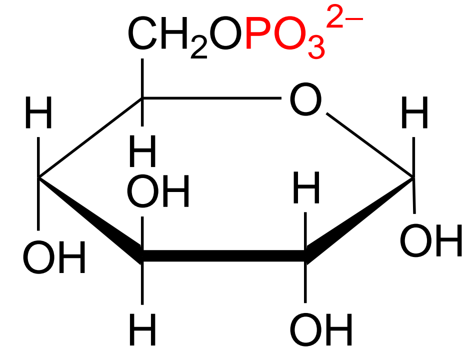
(a) Glycogen phosphorylase, which catalyzes the phosphorolysis of the glucose residues at the non-reducing ends of glycogen to yield glucose-1-phosphate (G1P).

(b) Glycogen debranching enzyme, which transfers a tri- or tetrasaccharide and hydrolyzes the $\alpha(1\rightarrow6)$ linkage at branch points.

(c) Phosphoglucomutase, which converts G1P to G6P.







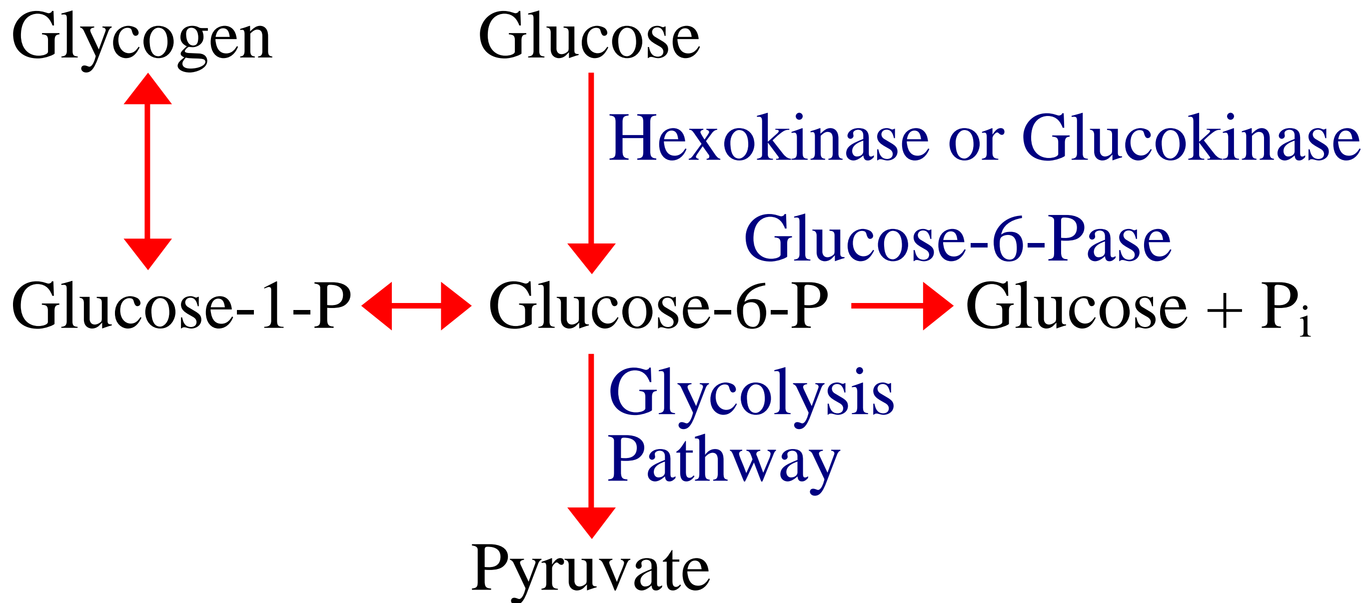
- **Phosphoglucomutase** catalyzes the reversible reaction:

glucose-1-phosphate ←→ glucose-6-phosphate

A **serine OH** at the active site donates & accepts P_i.

The bisphosphate is not released.

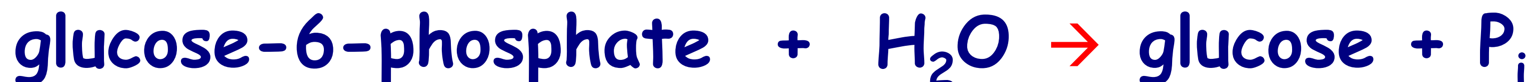
Phosphoglycerate Mutase has a similar mechanism, but instead uses His for P_i transfer.



Glucose metabolism in liver.

Glucose-6-phosphate may enter Glycolysis or (mainly in liver) be dephosphorylated for release to the blood.

Liver **Glucose-6-phosphatase** catalyzes the following, essential to the liver's role in maintaining blood glucose:



Most other tissues lack this enzyme.

Glycogen Synthesis

- Glycogen synthesis requires three enzymes to covert G1P to glycogen.

1. **Uridine diphosphate (UDP)** -glucose pyrophosphorylase .

- catalyzes the transfer of UMP from UTP to the phosphate group of G1P to form UDP-glucose and PPi. *PPi is eventually hydrolyzed to Pi by inorganic* pyrophosphatase, which provides the exergonic push for this reaction.

Cont……….

2.Glycogen Synthase

- catalyzes a transfer reaction in which the glucosyl residue of UDP– glucose is added to the nonreducing end of glycogen through an α(1→4) bond. Glycogen synthase can only extend a pre-existing α(1→4)-linked chain. The glycogen molecule originates through the action of the protein glycogenin, which assembles a seven-residue glycogen "primer" for glycogen synthase to act on

Cont….

3.Glycogen branching enzyme

- transfers a seven-residue segment from the end of an α(1→4)- linked glucan chain to the C6-hydroxyl group of a glucosyl residue on the same chain or another chain, thereby forming an α(1→6)-linked branch.

Citric Acid Cycle

- This process converts :

Pyruvate ⟶ NADH/FADH2

- ✓ Also called Tricarboxylic Acid Cycle (TCA) or Krebs Cycle. Three names for the same thing.
- ✓ Cellular respiration and intermediates for biosynthesis.
- ✓ Conversion of pyruvate to activated acetate
- ✓ Reactions of the citric acid cycle
- ✓ Conversion of acetate to carbohydrate precursors in the glyoxylate cycle

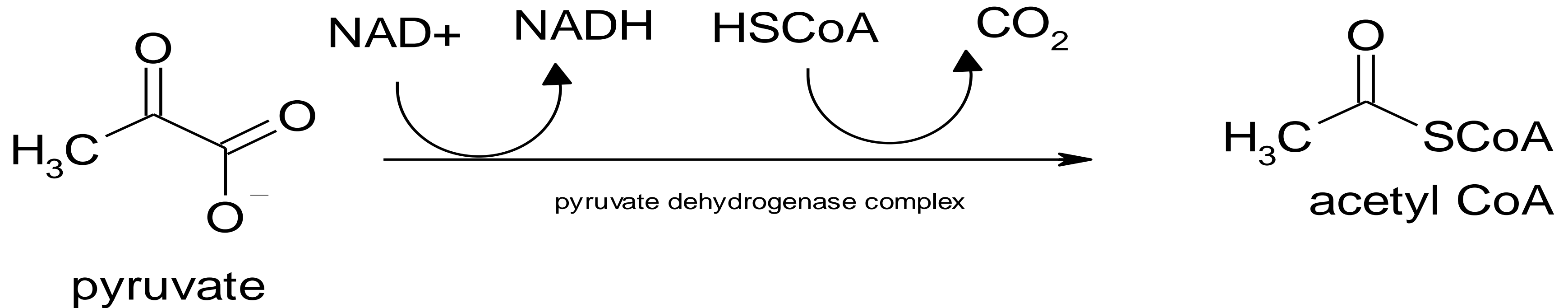
Overall goal

- Makes ATP
- Makes NADH
- Makes $FADH_2$
- Requires some carbohydrate to run
- Watch for reaction coupling

Geography

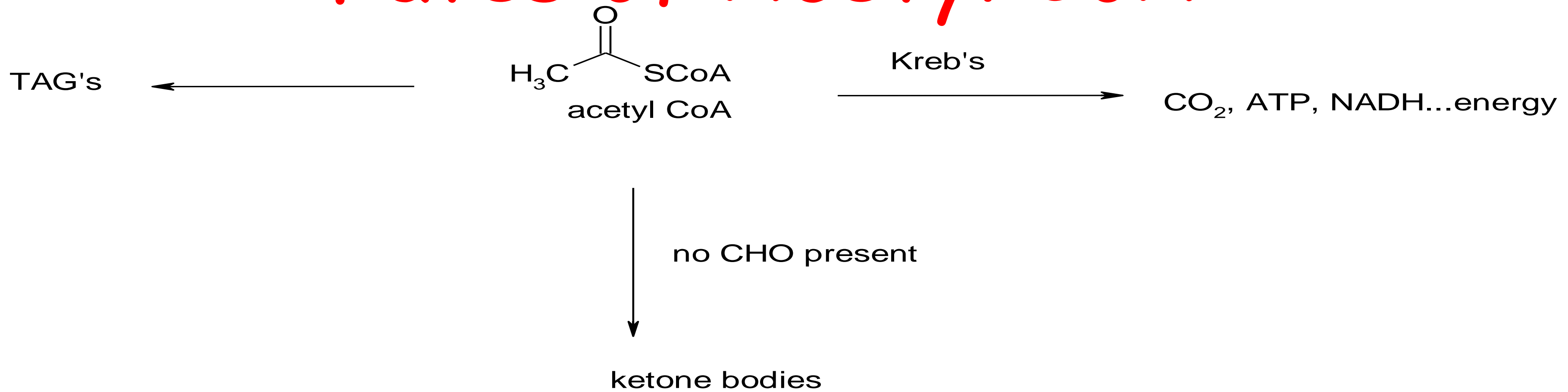
- Glycolysis in the cytosol
- Krebs in mitochondrial matrix
- Mitochondrion
  - Outer membrane very permeable
    - Space between membranes called intermembrane space (clever huh!)
  - Inner membrane (cristae)
    - Permeable to pyruvate,
    - Impermeable to fatty acids, NAD, etc
  - Matrix is inside inner membrane

Conversion of pyruvate to Acetyl CoA



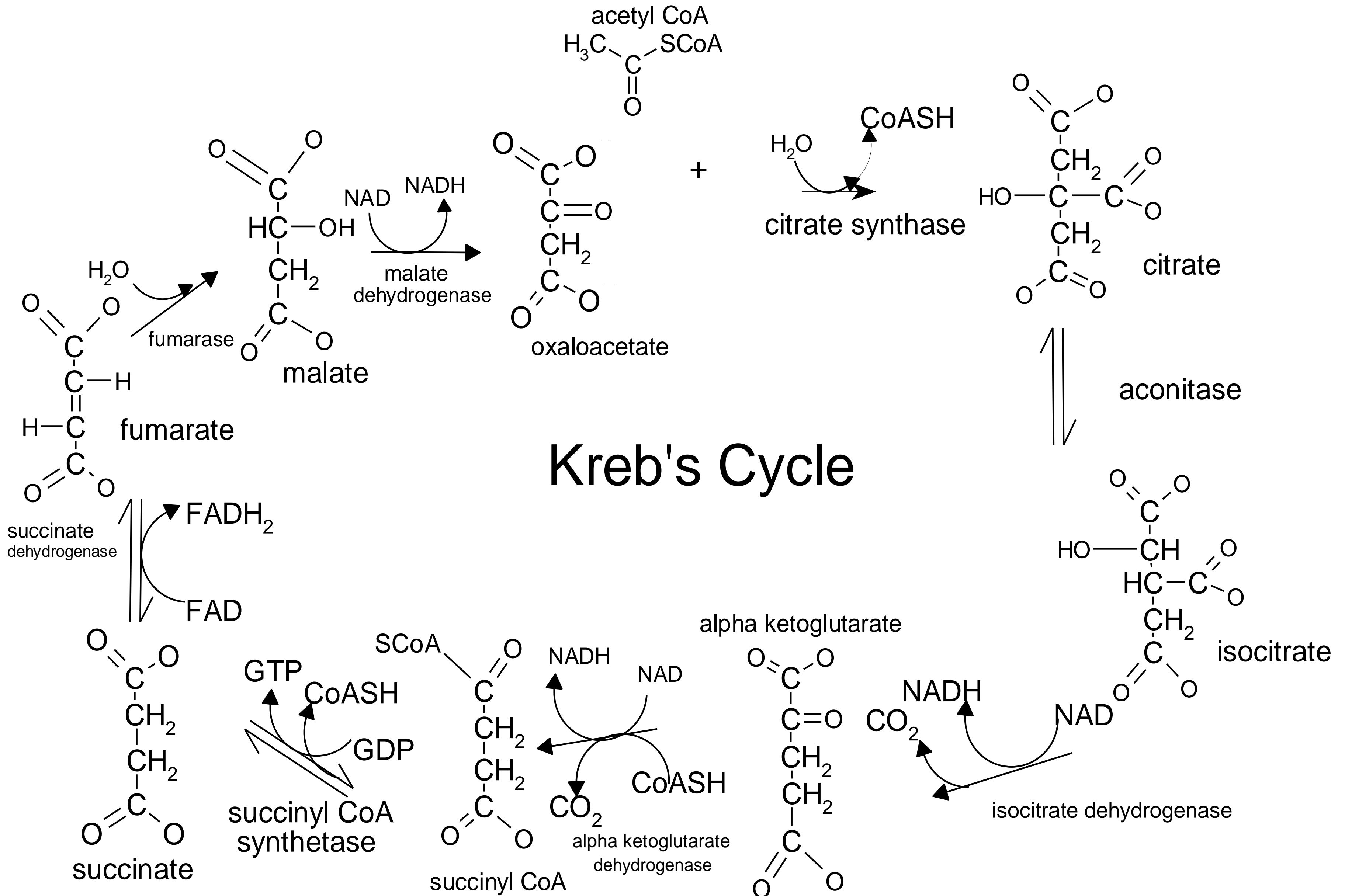
- ✓ 2 per glucose (all of Kreb's)
- ✓ Oxidative decarboxylation
- ✓ Makes NADH
- ✓ -33.4kJ

Fates of Acetyl CoA



- In the presence of CHO an using energy
  - Metabolized to CO_2, NADH, $FADH_2$,GTP and, ultimately, ATP
- If energy not being used (Lots of ATP present)
  - Made into fat
- If energy being used, but no CHO present
  - Starvation
  - Forms ketone bodies (see fat metabolism slides)
  - Danger!

Cont...



Net From Kreb's

- Oxidative process
  - 3 NADH
  - $FADH_2$
  - GTP
- X 2 per glucose
  - 6 NADH
  - 2 $FADH_2$
  - 2 GTP
- All ultimately turned into ATP (oxidative phosphorylation…later)

Total Energy per glucose

- Cytosol
  - Glycolysis
    - 2 NADH
    - 2 ATP
- Mitochondrion
  - Pyruvate dehydrogenase
    - 2 NADH
- Krebs
  - 6 NADH
  - 2 $FADH_2$
  - 2 GTP

Total Energy/glucose

- In mitochondrion:
  - Each NADH makes 2.5 ATP
  - Each $FADH_2$ makes 1.5 ATP
  - GTP makes ATP
- So…
  - From in mitochondrion
    - 8 NADH X 2.5 ATP/NADH = 20 ATP
    - 2 $FADH_2$ X 1.5 ATP/$FADH_2$= 3 ATP
    - 2 GTP X 1 ATP / GTP = <u>2 ATP</u>
    - TOTAL in mitochondrion 25 ATP

Total Energy/ glucose

- Cytosol
  - 2 ATP
  - 2 NADH
    - NADH can't get into mitochondrion
    - In eukaryotes two pathways,
      - transferred to $FADH_2$
        - get 1.5 ATP/ $FADH_2$
      - Or transferred to NADH
        - Get 2.5 ATP/ NADH
      - (Not a problem in prokaryotes (why?))
  - 2 NADH X 1.5 ATP = 3 ATP
  - Or 2 NADH X 2.5 ATP = 5 ATP
    - \+ =2 ATP
    - Total 3+ 2 or 5 + 2 so either 5 or 7

ATP/glucose

- Eukaryotes
  - Mitochondrial: 25 ATP
  - Cytosolic: 5 or 7 ATP
  - Total 30 or 32 ATP/glucose
  - $30 \text{ ATP} \times \frac{7.3 \text{kcal}}{\text{ATP}} \times 4.18 \frac{\text{kJ}}{\text{kcal}} = 915 \text{ kJ}$

If 32 ATP = 976 kJ

- Prokaryotes
  - $32 \text{ ATP} \times \frac{7.3 \text{kcal}}{\text{ATP}} \times 4.18 \frac{\text{kJ}}{\text{kcal}} = 976 \text{ kJ}$

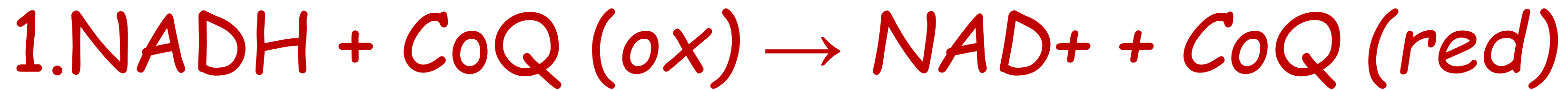
Electron Transport Chain (ETC) And Oxidative phosphorylation

In an electron transfer reaction, electrons flow from a substance with a lower reduction potential to a substance with a higher reduction potential. The standard reduction potential, $E^{o\prime}$, is a measure of a substance's affinity for electrons. For a redox reaction, $\Delta\mathscr{E}^{o\prime} = \mathscr{E}^{o\prime}_{(e-acceptor)} - \mathscr{E}^{o\prime}_{(e-donor)}$. When $\Delta\mathscr{E}^{o\prime}$ is positive, the reaction is spontaneous, since $\Delta G^{o\prime} = -n\mathscr{F}\Delta\mathscr{E}^{o\prime}$, where n is the number of electrons transported and F is the faraday ($96{,}485\ J\cdot V^{-1}\cdot mol^{-1}$). The transfer of electrons from NADH to O_2 ($\Delta\mathscr{E}^{o\prime} = 1.13$ V and $\Delta G^{o\prime} = -218\ kJ\cdot mol^{-1}$) provides enough free energy to synthesize three ATP molecules.

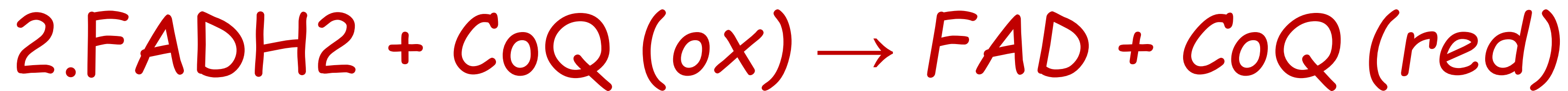
Cont…….

- Four large protein complexes in the inner mitochondrial membrane are involved in transferring electrons from reduced coenzymes to O2. Complexes I and II transfer electrons to the lipid-soluble electron carrier ubiquinone (coenzyme Q or CoQ), which transfers electrons to Complex III. From there, electrons pass to cytochrome *c*, a *peripheral* membrane protein with a heme prosthetic group, which transfers electrons to Complex IV. The reactions of Complexes I–IV are as follows:

Cont……

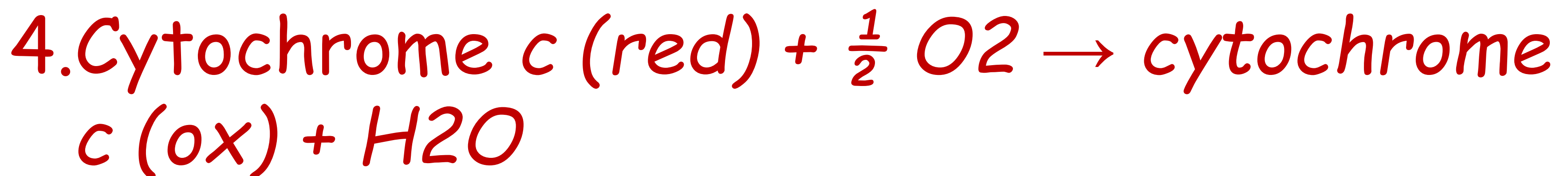


ΔE °' = 0.360 V and ΔG°' = –69.5 kJ·mol–1



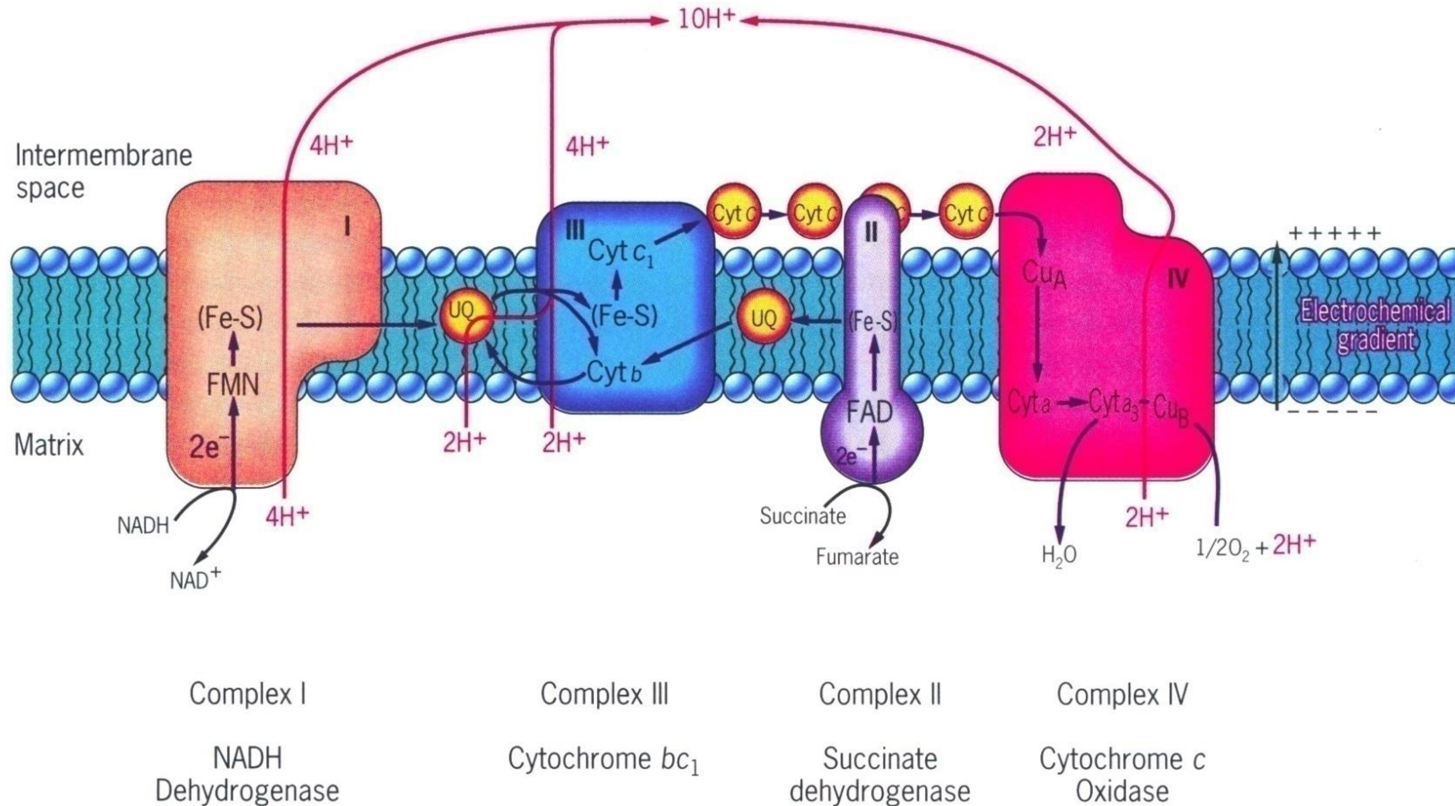
ΔE °' = 0.085 V and ΔG°' = –16.4 kJ·mol–1

ΔE °' = 0.190 V and ΔG°' = –36.7 kJ·mol–1



ΔE °' = 0.580 V and ΔG°' = –112 kJ·mol–1

Electron Transport Chain



During electron transport, energy released is used to transport H^+ across the inner mitochondrial membrane to create an electrochemical gradient

Cont..

- Complex I is an enormous protein complex containing flavin mononucleotide (FMN, which is FAD minus its AMP group) and multiple iron-sulfur clusters (which are one electron carriers).
- Here, 2e- donated by NADH are transferred to CoQ (coenzyme Q, Q-ubiquinone). So as these e- transferred, 4H+ (protons) are translocated from the outer membrane to the inner membrane of mitochondria via proton wire.

Cont………..

- Complex II has succinate dehydrogenase enzyme Transfers e- from the succinate to FAD and then to CoQ. In this cmplx no trnalocation of proton rather it's e-feeder to e-transport chain.
- Complex III (cytochrome c reductase) contains:

  - b type cytochrome
  - cytochrome c1
  - Iron-sulfur protein---contains 2Fe-2S cluster.

Cont……….

- e- flows from CoQ through cplx III follows cyclic path known as Q cycle. Here e- s are transferred from two QH2 to:

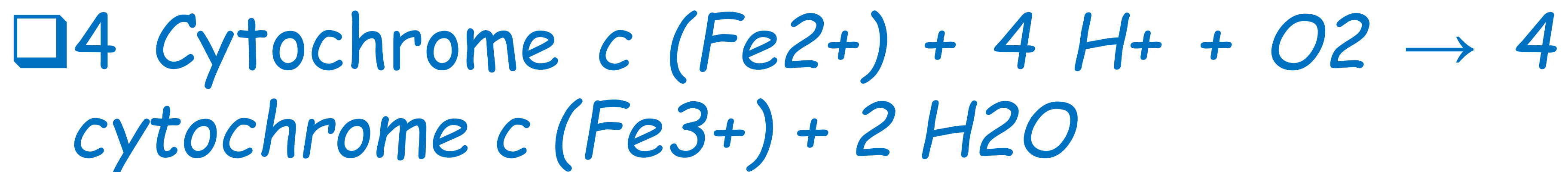
  -1st CoQ transfers 1e- from 1QH2 to Fe-S then to cyt c1 then to cyc c. The other 1e- transferred to cyc b then to the higher potential cytochrome.

  -2nd Another QH2 donates its electrons, one to the iron-sulfur protein and one to cytochrome bL (lower cytochrome b).
- This results the formation of 4protons on the outer membrane of mitochondria.

Cont……….

- Complex IV (cytochrome c oxidase) has four redox centers
  - *cytochrome a,
  - *cytochrome a3,
  - *CuA (which contains two Cu ions),
  - *CuB, and

  -it carries out the following reaction:

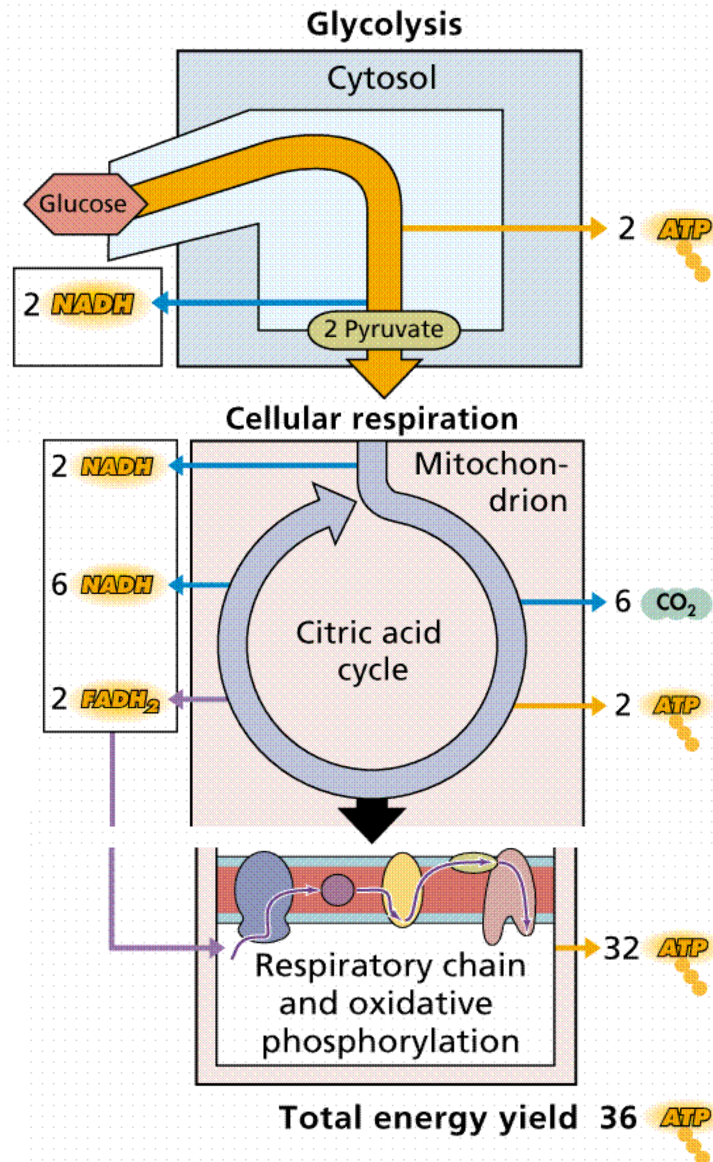


- 4 Cytochrome c (Fe^{2+}) + 4 H^+ + O_2 → 4 cytochrome c (Fe^{3+}) + 2 H_2O

Cont..........

- O2 reduction takes place at the *cytochrome a3–CuB binuclear complex*, *which mediates four* one-electron transfer reactions. Four protons are consumed in the production of H2O, and four additional proteins are pumped, most likely via a proton wire, from the matrix to the intermembrane space (two for each pair of electrons that enter the electron-transport chain).

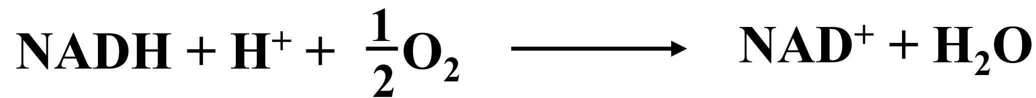
Step 3: Electron transport chain and oxidative phosphorylation



Oxidative Phosphorylation

Oxidative phosphorylation is the process by which the energy stored in NADH and $FADH_2$ is used to produce ATP.

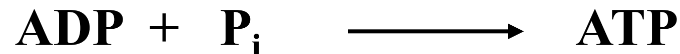
A. Oxidation step: electron transport chain



$$NADH + H^+ + \frac{1}{2}O_2 \longrightarrow NAD^+ + H_2O$$

$$FADH_2 + \frac{1}{2}O_2 \longrightarrow FAD + H_2O$$

B. Phosphorylation step



$$ADP + P_i \longrightarrow ATP$$

Lipids Metabolism

Points to be discussed:

- Fatty Acid Metabolism (Lipids → Acetyl-CoA)
- Ketone Bodies—The Fate of Unused Acetyl-CoA
- Fatty Acid Biosynthesis

Cont……..

Lipids dissolve well in organic solvents but they are insoluble in water.

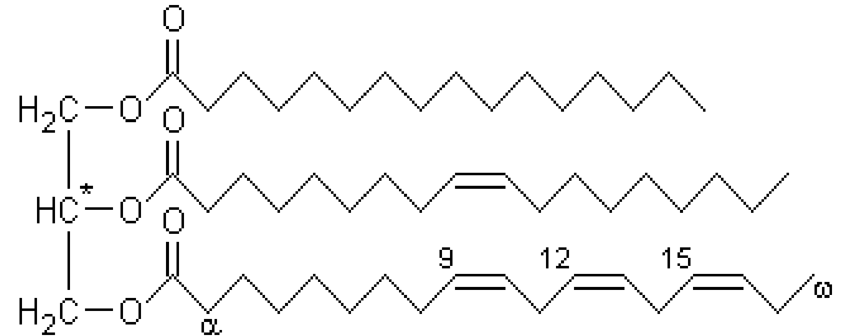
Biological roles of lipids:

- lipids are important source of energy - they serve as metabolic fuel
- amphipathic lipids are building blocks of cellular membranes
- some of them are substrates for synthesis of other compounds (eicosanoids, bile acids)
- lipids are excellent insulators

Classification of lipids

I. Simple lipids

- Triacylglycerols TAG (fats) ⟶
- Waxes



II. Complex lipids

- Phospholipids
- Sphingophospholipids
- Glycolipids

III. Isoprenoids and steroids

Isoprenoids: vitamins A, D, E, K

Steroids: sterols, bile acids, steroid hormones

Figure is found on http://en.wikipedia.org/wiki/Triacylglycerol

Degradation of fats in adipose tissue

Adipose tissue (fat cells) = fat storage

Degradation of TAG in adipose tissue (lipolysis) is catalyzed by **hormone sensitive lipase (HSL)**.

This enzyme is activated by **epinephrine** and **glucagon** and inhibited by **insulin**.

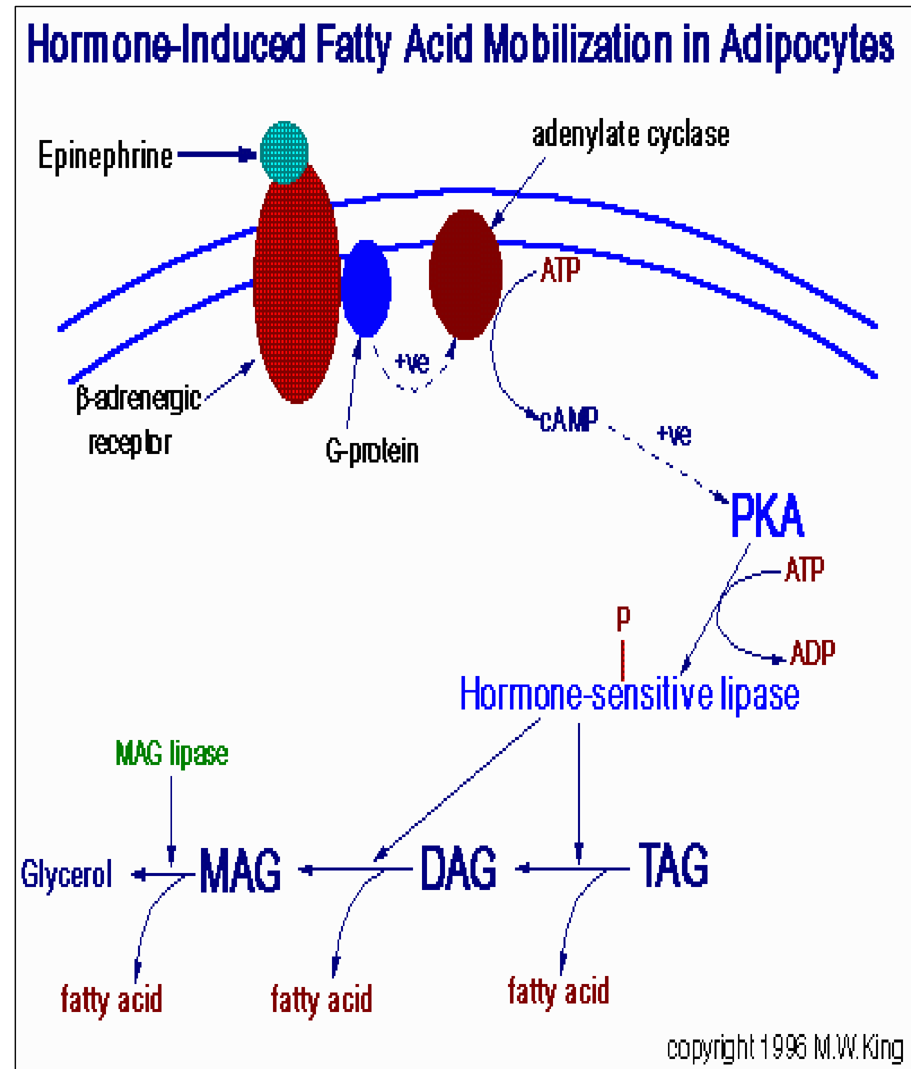


Figure is found on http://web.indstate.edu/thcme/mwking/fatty-acid-oxidation.html

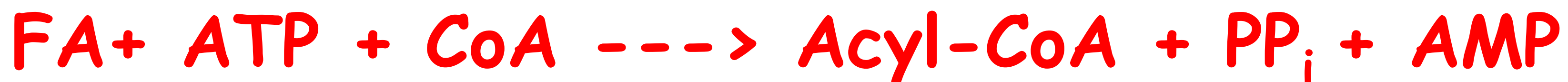
FA Metabolism

Tissues take up FA from the blood to rebuild fats or to obtain energy from their oxidation.

Metabolism of FA is especially intensive in the **liver**.

„Free“ fatty acids (FFA) are transferred with albumin in the blood.

FA in blood → enter to the cell → in the cytoplasm FA are converted to their CoA derivatives by enzyme **acyl-CoA-synthetase** (ATP is consumed) → **acyl-CoAs**



Transfer of acyl-CoAs from cytoplasm to the mit. matrix is performed by a **carnitine transporter**

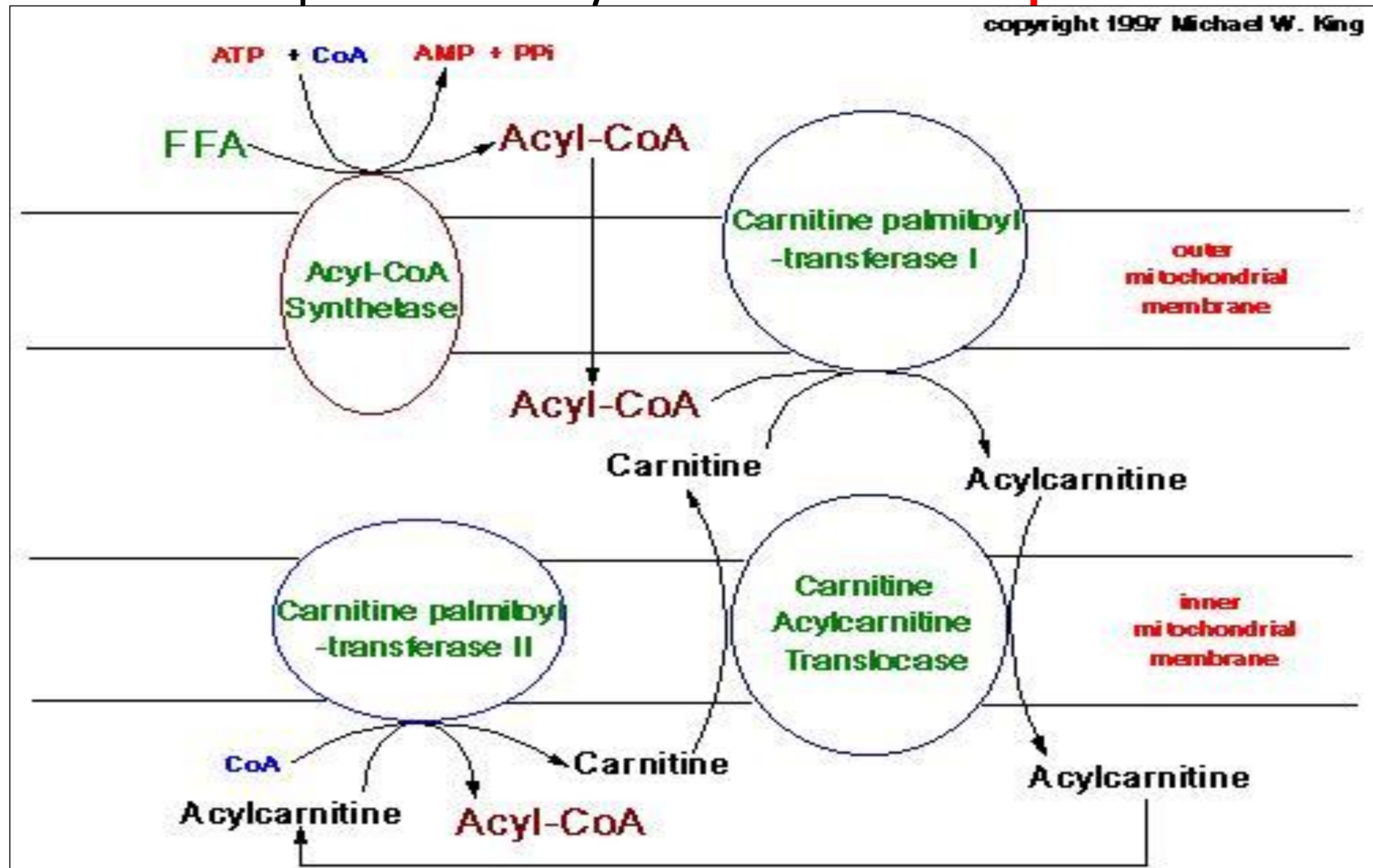


Figure is found on http://web.indstate.edu/thcme/mwking/fatty-acid-oxidation.html

β-oxidation of fatty acids

- **substrate**: acyl-CoA
- **product**: n acetyl-CoA, n NADH + H^+, n $FADH_2$
- **function**: gain of energy from fatty acids
- **subcelullar location**: matrix of mitochondria
- **organ location**: liver, skeletal muscles and other tissues with expection to CNS
- **regulatory enzyme**: carnitine acyltransferase I

- **Ketone bodies** are three chemicals that are produced when fatty acids are broken down in excess.
- Production of these compounds is called "ketogenesis", and this is necessary in small amounts.
- Ketone bodies are produced from acetyl-CoA, mainly in the mitochondrial matrix of liver cells when carbohydrates are so scarce that energy must be obtained from breaking down of fatty acids.

Synthesis of ketone bodies (ketogenesis)

- **substrate:** acetyl-CoA
- **product:** acetoacetate, 3-hydroxybutyrate, acetone
- **function:** energy substrate for extrahepatal tissues
- **subcelullar location:** matrix of mitochondria
- **organ location:** liver

Excessive production of ketone bodies is typical during starvation or diabetes mellitus:

↑ lipolysis → ↑ FA → β-oxidation of FA → excess of acetyl-CoA → ↑ ketogenesis

Use of ketone bodies by the extrahepatal tissues

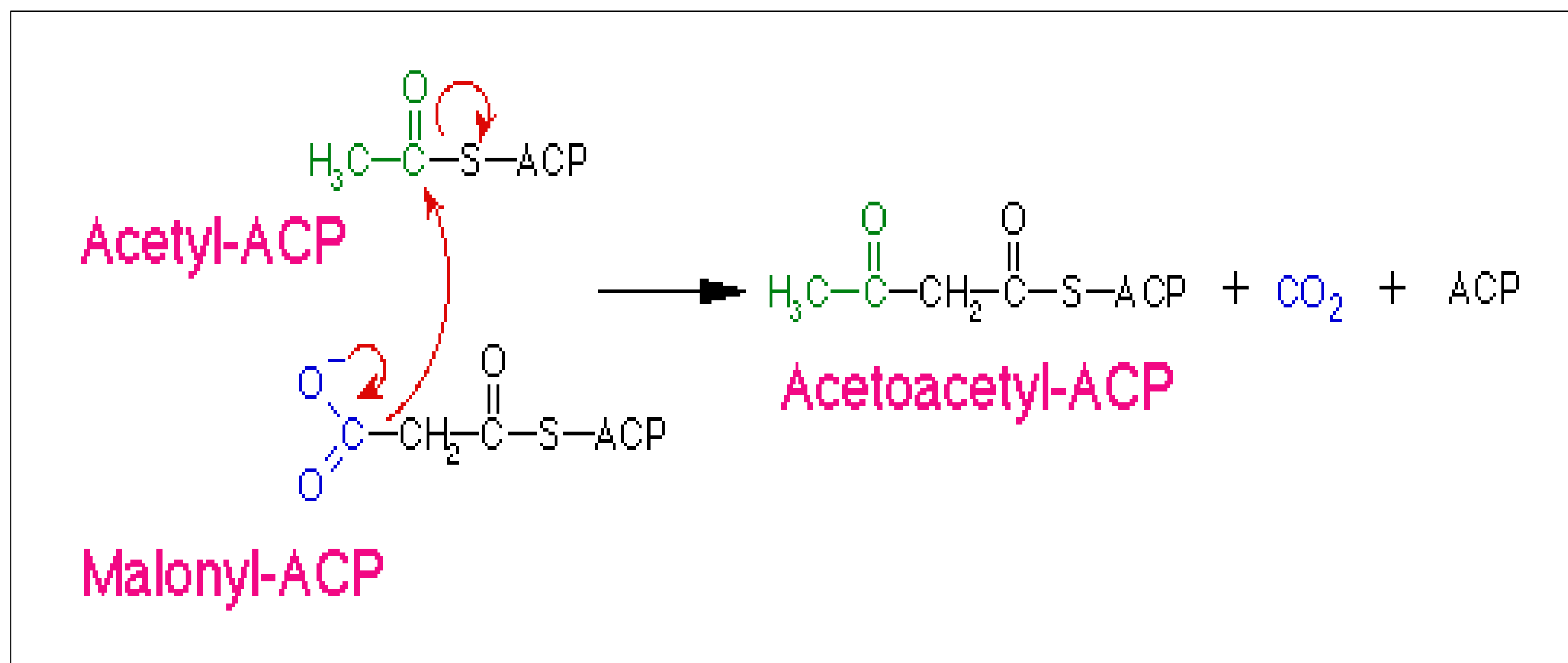
- acetoacetate and 3-hydroxybutyrate are reconverted to acetyl-CoA (→ citric acid cycle)
- is located in matrix of mitochondria of the peripheral tissues
- is significant in skeletal muscles, heart and also in the brain if lack of Glc occurs

Fatty acid Biosynthesis

- **substrate:** acetyl-CoA, NADPH + H^+
- **product:** palmitate (= endproduct of FA synthesis)
- **function:** de novo synthesis of FA which are stored as TAG
- **subcelullar location:** cytosol
- **organ location:** mainly liver and adipose tissue and also other tissues
- **regulatory enzyme:** acetyl-CoA carboxylase

The growing fatty acids are linked to a phosphopantetheine group of an **acyl carrier protein (ACP)** of FA synthase.

Acetyl-CoA is carboxylated by HCO_3^- to yield malonyl-CoA → condensation between the acetyl-ACP and the malonyl-ACP → **acetoacetyl-ACP** is formed.



Amino Acid Metabolism

Carbon skeleton

Points to be discused:

- ❑Deamination of amino acids
- ❑Elimination of Nitrogen-Urea cycle
- ❑Deaminated aas as metabolic fuels
- ❑Amino acid Biosynthesis

Amino Acid Carbon Skeletons

Amino acids, when deaminated, yield **a-keto acids** that, directly or via additional reactions, feed into major metabolic pathways (e.g., Krebs Cycle).

Amino acids are grouped into 2 classes, based on whether or not their carbon skeletons can be converted to glucose:

- **glucogenic**
- **ketogenic**.

Carbon skeletons of **glucogenic** amino acids are degraded to:

- **pyruvate**, or
- a **4-C or 5-C intermediate of Krebs Cycle**. These are precursors for gluconeogenesis.

Glucogenic amino acids are the major carbon source for **gluconeogenesis** when glucose levels are low.

They can also be catabolized for **energy**, or converted to glycogen or fatty acids for energy **storage**.

Carbon skeletons of **ketogenic** amino acids are degraded to:

- **acetyl-CoA**, or
- **acetoacetate**.

Acetyl CoA, & its precursor acetoacetate, cannot yield net production of oxaloacetate, the gluconeogenesis precursor.

For every 2-C acetyl residue entering Krebs Cycle, 2 C leave as CO_2.

Carbon skeletons of ketogenic amino acids can be catabolized for **energy** in Krebs Cycle, or converted to **ketone bodies** or **fatty acids**.

They **cannot be converted to glucose**.

GENERAL WAYS OF AMINO ACIDS METABOLISM

The fates of amino acids:

1) for protein synthesis;

2) for synthesis of other nitrogen containing compounds (creatine, purines, choline, pyrimidine);

3) as the source of energy;

4) for the gluconeogenesis.

The general ways of amino acids degradation:

- Deamination
- Transamination
- Decarboxilation

The major site of amino acid degradation - the liver.

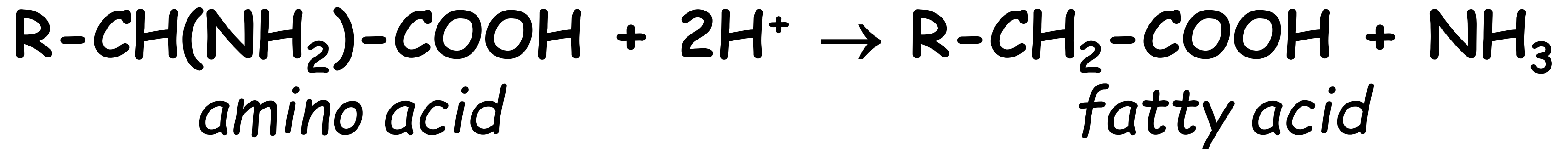
1. Deamination of amino acids

Deamination - ***elimination of amino group from amino acid with ammonia formation.***

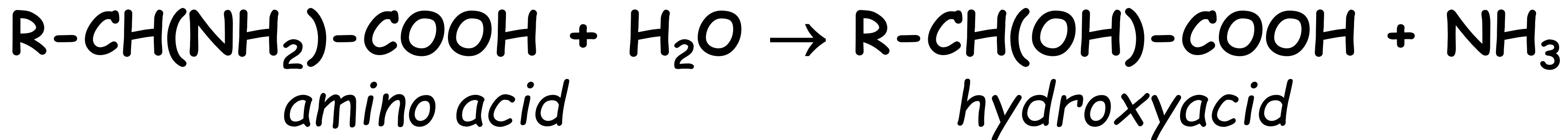
Four types of deamination:

- ***oxidative*** (the most important for higher animals),
- ***reduction,***
- ***hydrolytic, and***
- ***intramolecular***

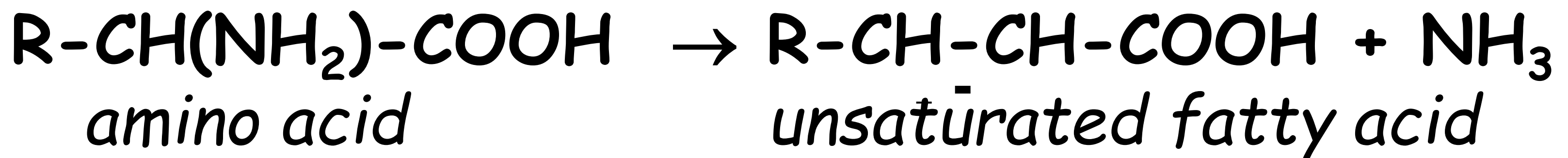
Reduction deamination:



Hydrolytic deamination:



Intramolecular deamination:

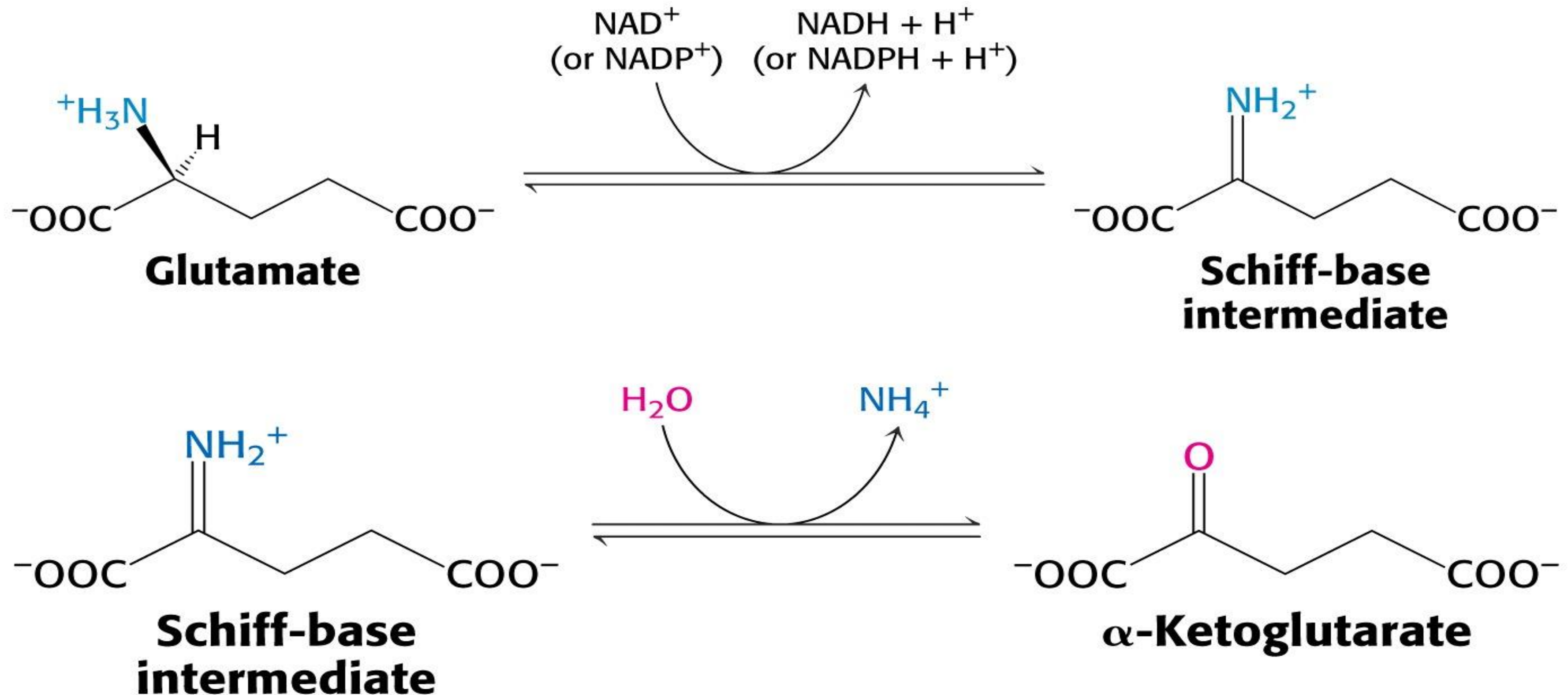


Oxidative deamination

L-Glutamate dehydrogenase plays a central role in amino acid deamination

In most organisms <u>glutamate is the only amino</u> acid that has active dehydrogenase

Present in both the cytosol and mitochondria of the liver



2. Transamination of amino acids

Transamination – ***transfer of an amino group from an α-amino acid to an α-keto acid (usually to α-ketoglutarate)***

Enzymes: ***aminotransferases (transaminases).***

α-amino acid α-keto acid

α-keto acid α-amino a

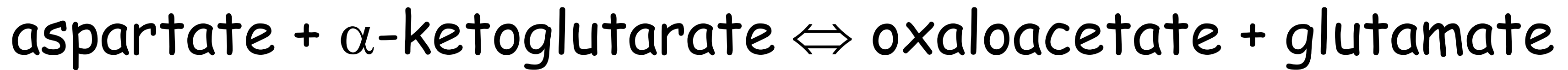
There are different transaminases

The most common:

alanine aminotransferase

alanine + α-ketoglutarate ⇔ pyruvate + glutamate

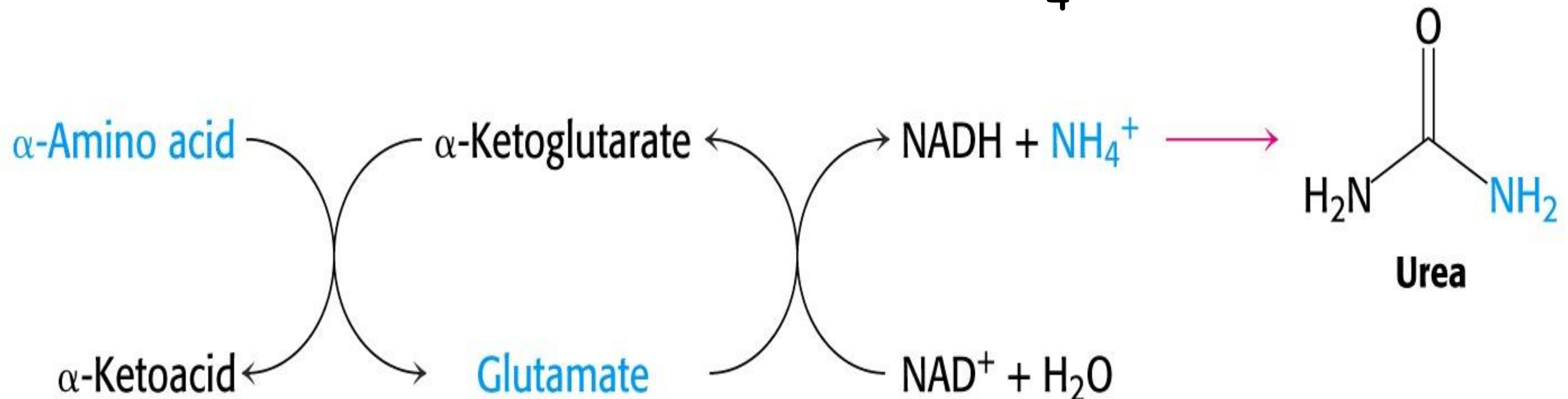
aspartate aminotransferase



aspartate + α-ketoglutarate ⇔ oxaloacetate + glutamate

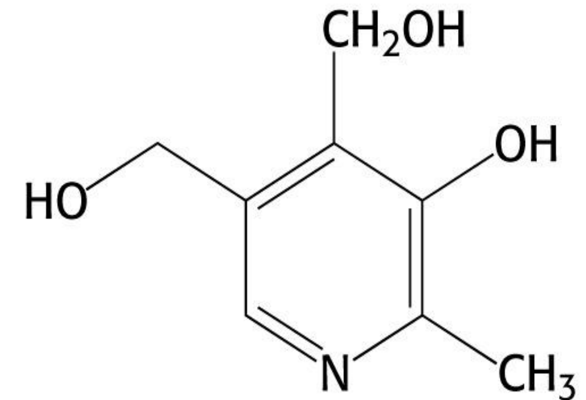
Aminotransferases **funnel *α*-amino groups from a variety of amino acids to α-ketoglutarate with glutamate formation**

Glutamate can be deaminated with NH_4^+ release



Mechanism of transamination

All aminotransferases require the prosthetic group ***pyridoxal phosphate (PLP)***, which is derived from ***pyridoxine (vitamin B_6)***.

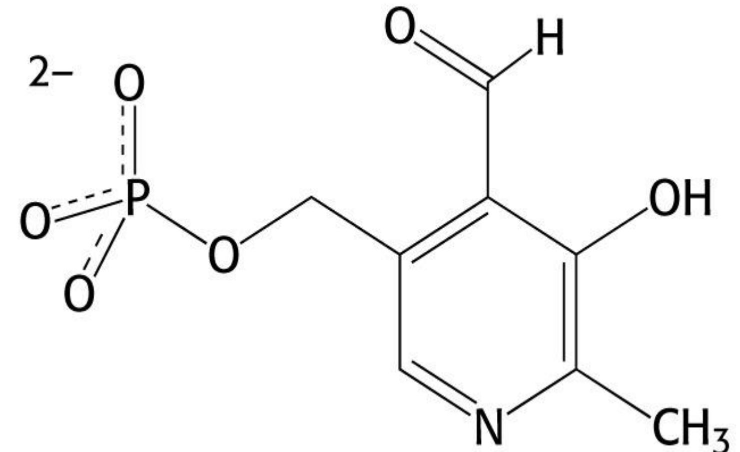


Pyridoxine (Vitamin B_6)

Ping-pong kinetic mechanism

First step: the amino group of amino acid is transferred to pyridoxal phosphate, forming pyridoxamine phosphate and releasing ketoacid.

Second step: α-ketoglutarate reacts with pyridoxamine phosphate forming glutamate



Pyridoxal phosphate (PLP)

Ping-pong kinetic mechanism of aspartate transaminase

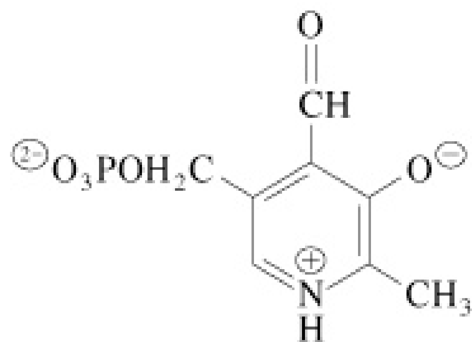
Aspartate + OH^{-} → Oxaloacetate

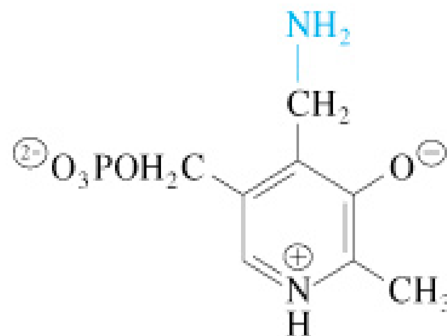
α-Ketoglutarate → Glutamate + OH^{-}

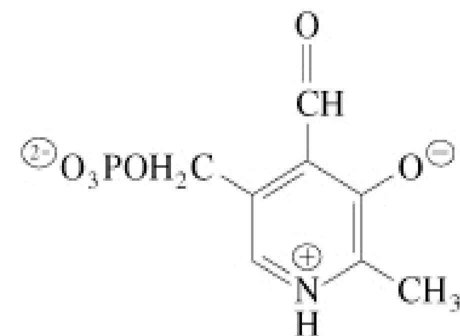
E-PLP

E-PMP

E-PLP

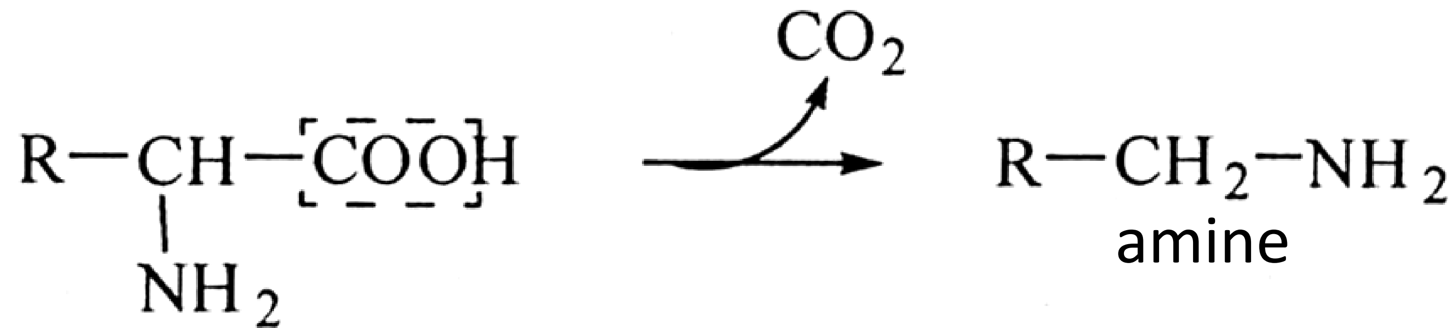






3. Decarboxylation of amino acids

Decarboxylation – removal of *carbon dioxide* from amino acid with formation of *amines*.

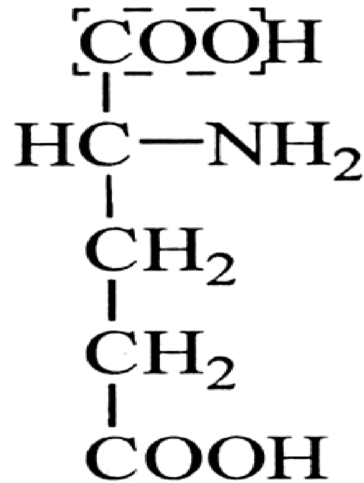


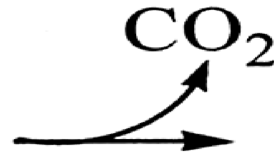
Usually amines have high physiological activity (hormones, neurotransmitters etc).

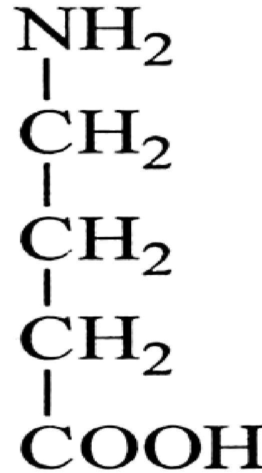
Enzyme: *decarboxylases*
Coenzyme – pyrydoxalphosphate

Significance of amino acid decarboxylation

1. Formation of physiologically active compounds

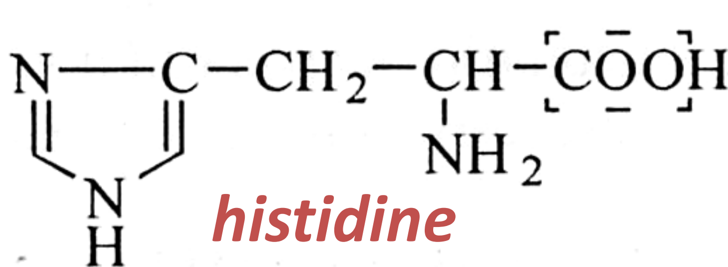




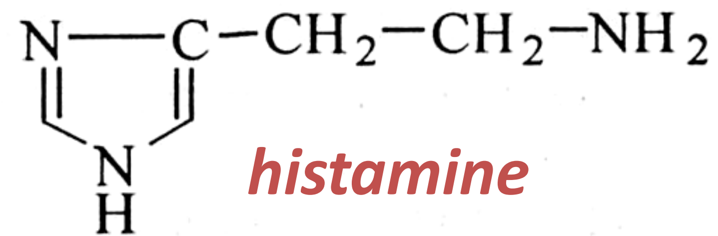


GABA – mediator of nervous system

glutamate *gamma-aminobutyric acid (GABA)*



histidine

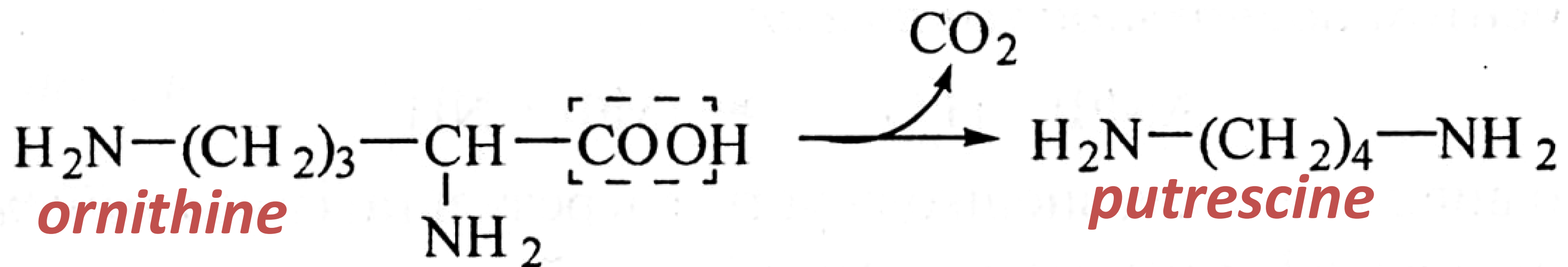


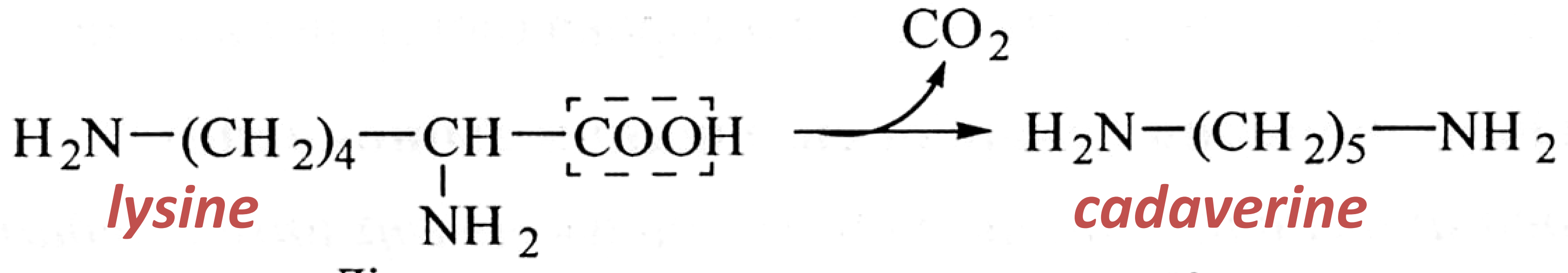
histamine

Histamine – mediator of inflammation, allergic reaction.

2. Catabolism of amino acids during the decay of proteins

Enzymes of microorganisms (in colon; dead organisms) decarboxylate amino acids with the formation of diamines.





Nitrogen removal from amino acids

Step 1: Remove amino group

Step 2: Take amino group to liver for nitrogen excretion

Step 3: Entry into mitochondria

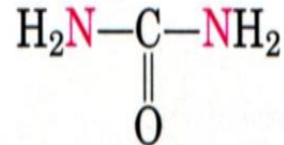
Step 4: Prepare nitrogen to enter urea cycle

Step 5: Urea cycle

Excretory forms of nitrogen

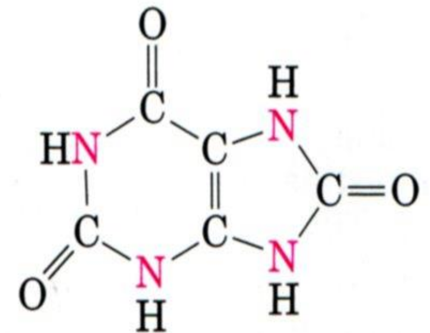
Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia



Urea

Ureotelic animals: many terrestrial vertebrates; also sharks



Uric acid

Uricotelic animals: birds, reptiles

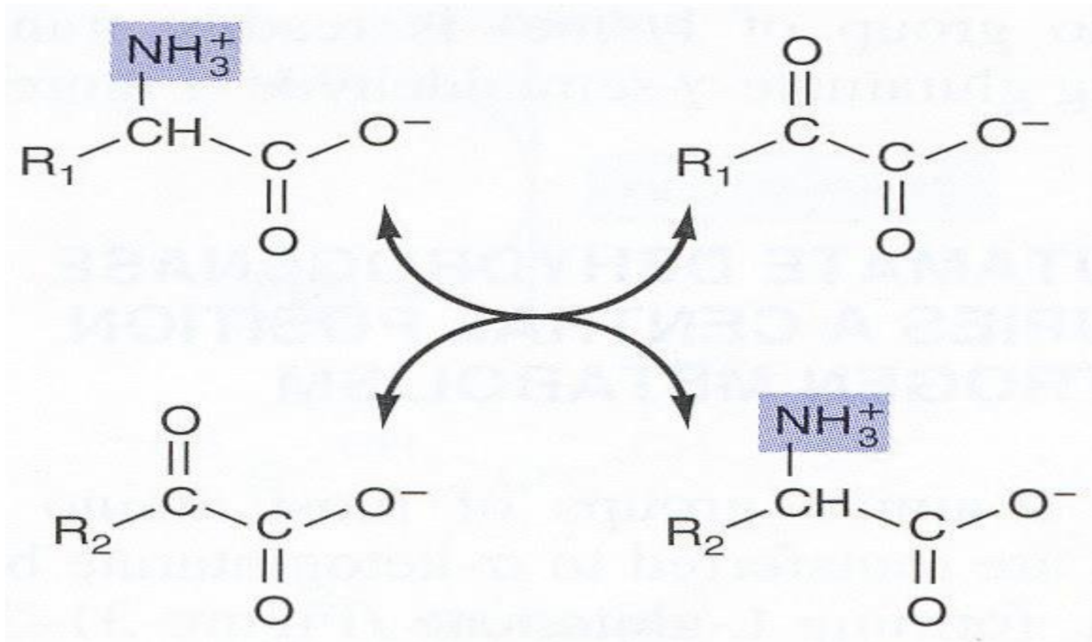
a) Excess NH_4^+ is excreted as ammonia (microbes, aquatic vertebrates or larvae of amphibia),

b) Urea (many terrestrial vertebrates)

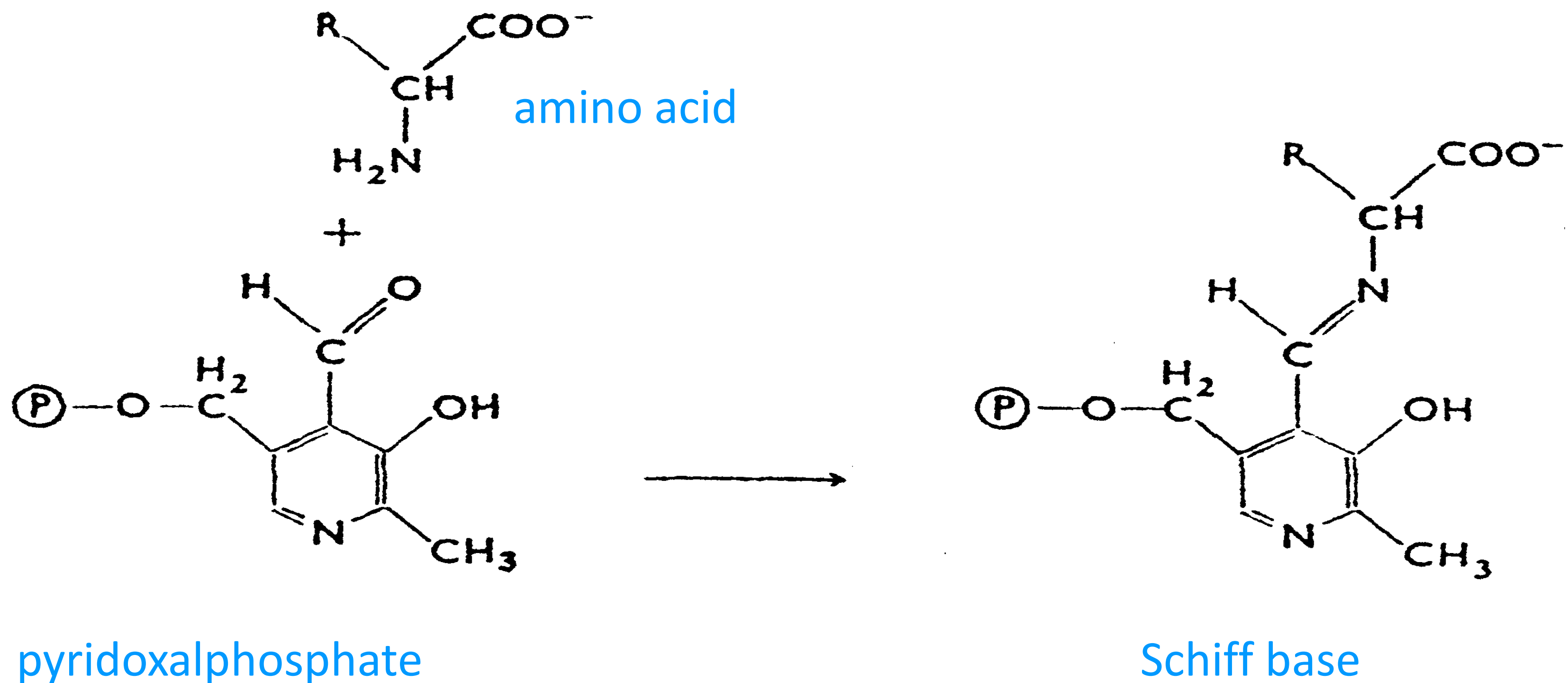
c) or uric acid (birds and terrestrial reptiles)

Step 1. Remove amino group

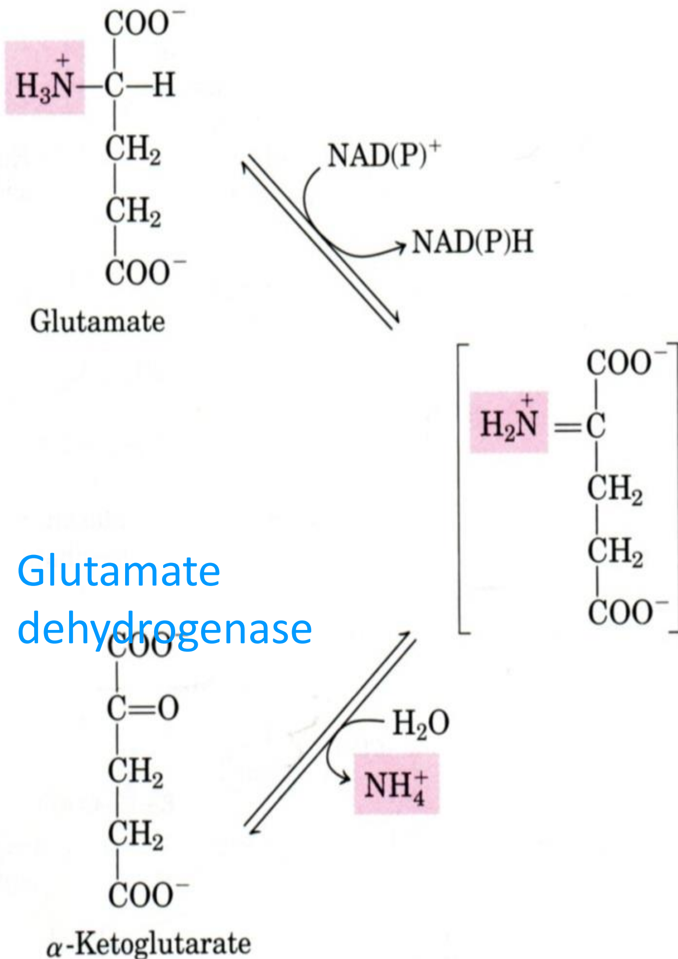
- ✓ Transfer of the amino group of an amino acid to an α-keto acid ⇒ the original *AA* is converted to the corresponding α-keto acid and vice versa:



✓ Transamination is catalyzed by transaminases (aminotransferases) that require participation of pyridoxalphosphate:



Step 2: Take amino group to liver for nitrogen excretion



Glutamate releases its amino group as ammonia in the liver.

The amino groups from many of the a-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules.

The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor

Nitrogen carriers

1. Glutamate

   transferres one amino group WITHIN cells:

   Aminotransferase → makes glutamate from a-ketogluta-rate

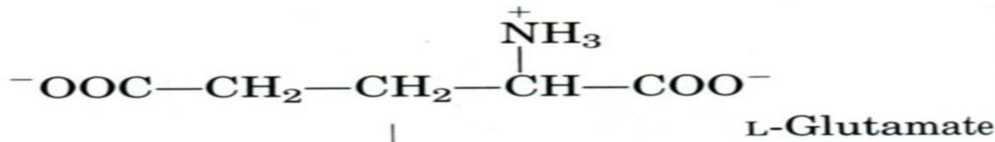
   Glutamate dehydrogenase → opposite

2. Glutamine

   transferres two amino group BETWEEN cells → releases its amino group in the liver

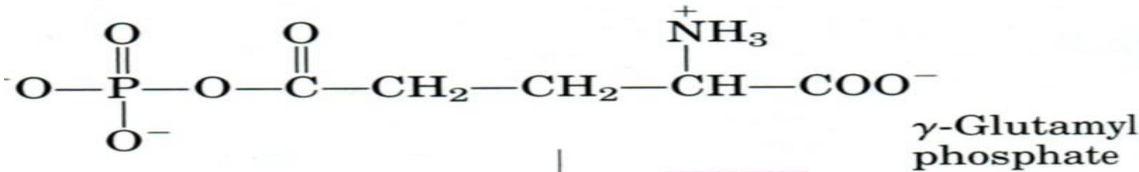
3. Alanine

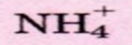
   transferres amino group from tissue (muscle) into the liver



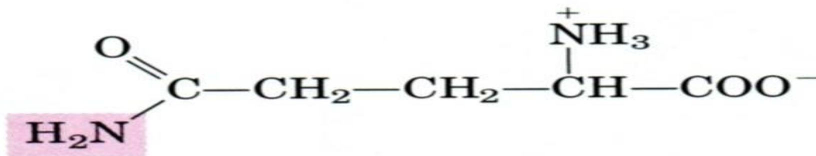
Move within cells

SynthAtase = ATP



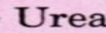


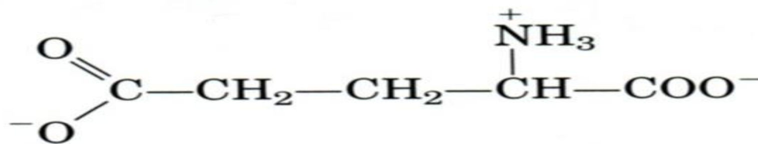
glutamine synthetase

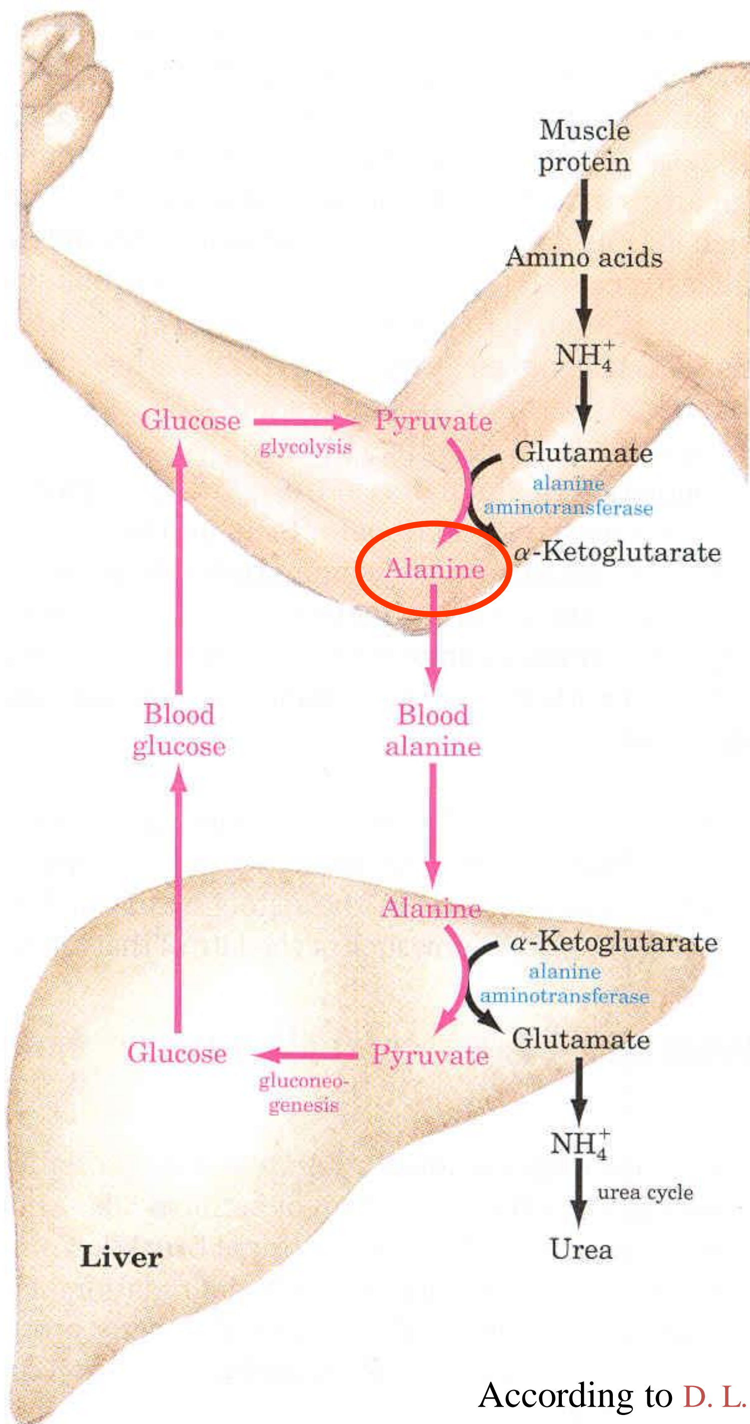


Move between cells

In liver







Glucose-alanine cycle

Alanine plays a special role in transporting amino groups to liver.

Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.

The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

According to D. L. Nelson, M. M. Cox :LEHNINGER. PRINCIPLES OF BIOCHEMISTRY Fifth edition

Urea cycle

- The **urea cycle** (also known as the ornithine **cycle**) is a **cycle** of biochemical reactions that produces **urea $(NH_2)_2CO$** from ammonia (NH_3). This **cycle** occurs in ureotelic organisms. The **urea cycle** converts highly toxic ammonia to **urea** for excretion
- A **ureotelic** organism excretes excess **nitrogen as urea**. Urea is less toxic and needs less water in comparison to Ammonia.
- **Ureotelic** organisms include cartilaginous fish, few bony fishes, adult amphibians and mammals including humans. The uricotelic organism excretes uric acid or its salts

Sources of ammonia for the urea cycle:

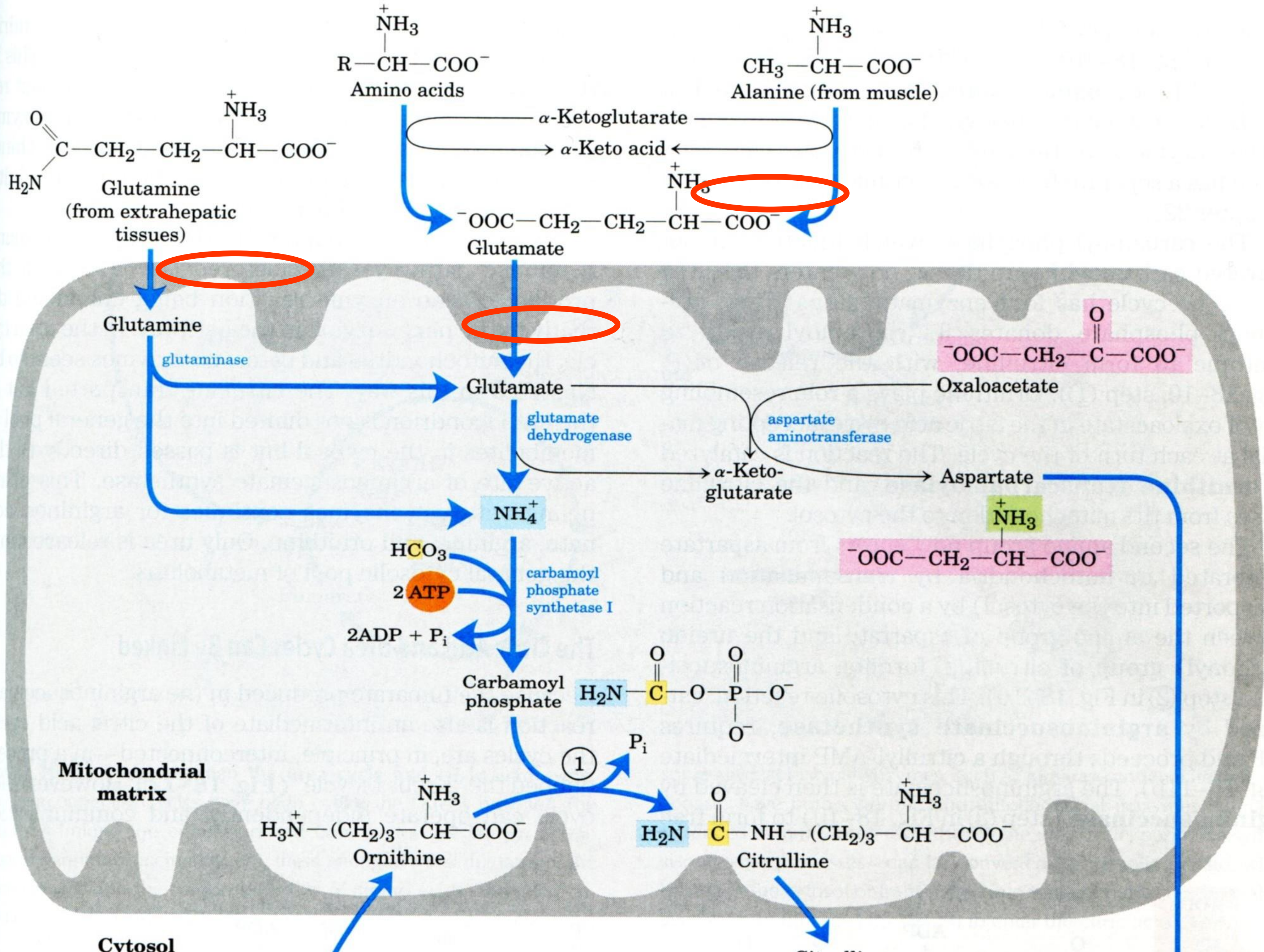
- Oxidative deamination of Glu, accumulated in the liver by the action of transaminases and glutaminase
- Glutaminase reaction releases NH_3 that enters the urea cycle in the liver (in the kidney, it is excreted into the urine)
- Catabolism of Ser, Thr, and His (nonoxidative deamination) also releases ammonia:

Serine - threonine dehydratase

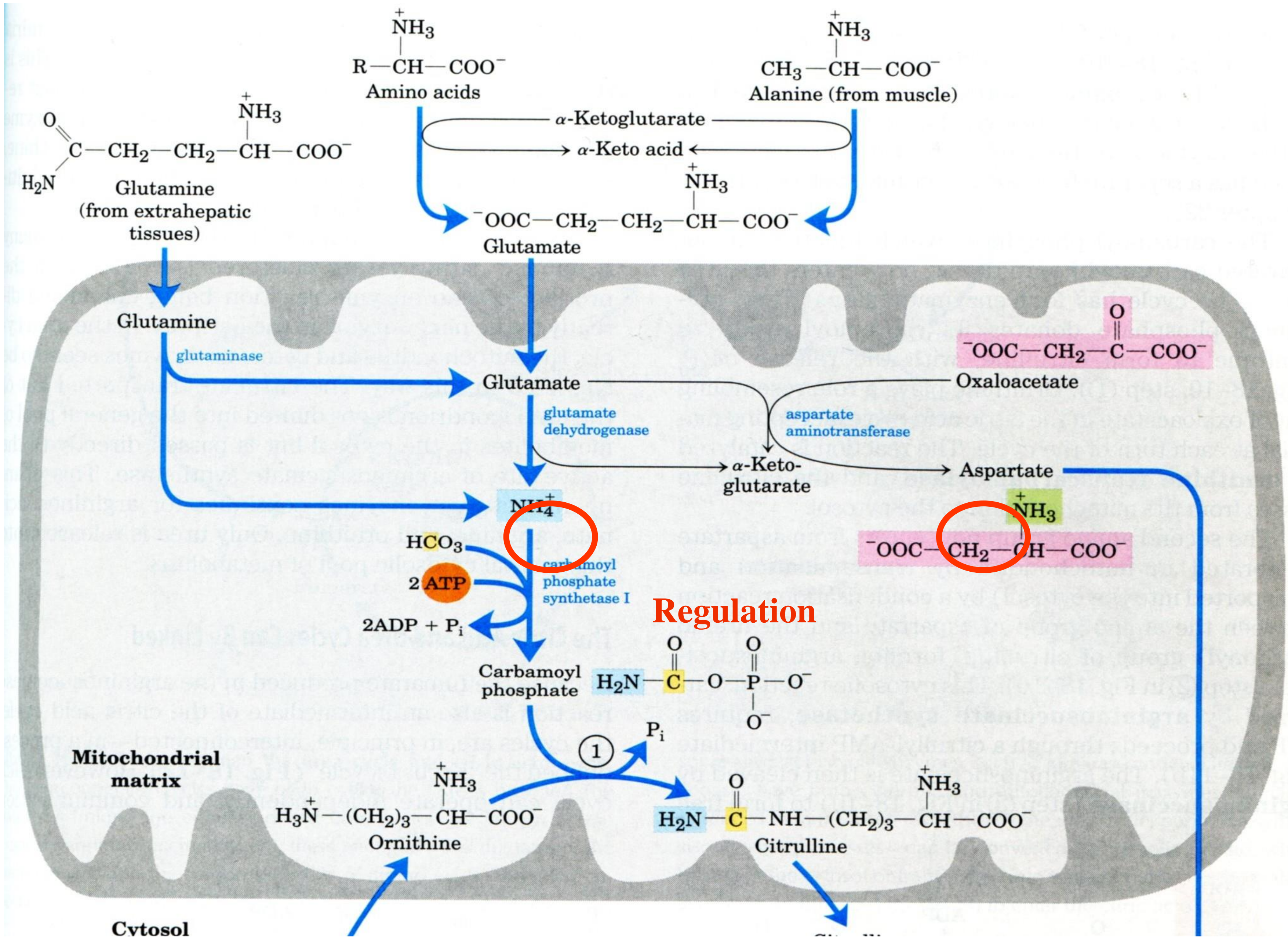
Serine →→ pyruvate + NH_4^+
Threonine →→ a-ketobutyrate + NH_4^+

- Bacteria in the gut also produce ammonia.

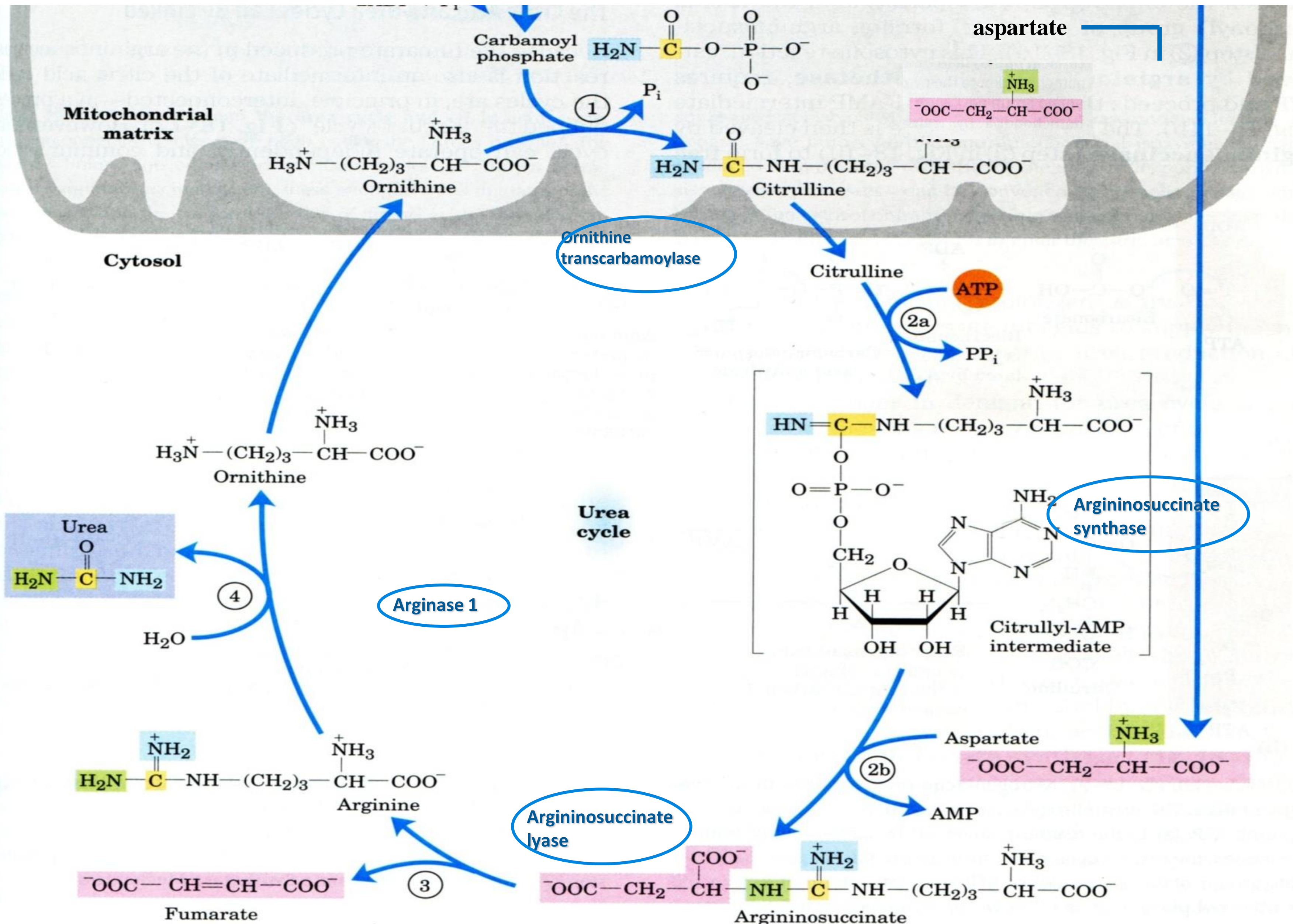
Step 3: entry of nitrogen to mitochondria



Step 4: prepare nitrogen to enter urea cycle



Step 5: Urea cycle



AAs Biosynthesis

- Amino acids that cannot be synthesized by mammals and that must therefore be obtained from their diet are called essential amino acids. Nonessential amino acids can be synthesized from common intermediates:

(a) Alanine, aspartate, and glutamate are formed by one-step transamination reactions. Asparagine and glutamine are formed by amidation of aspartate and glutamate. The activation of glutamate by glutamine synthetase, which occurs prior to its amidation, is a key regulatory point in bacterial nitrogen metabolism.

Cont…

(b) Glutamate gives rise to proline and arginine (which is also considered to be an essential amino acid because, when synthesized, it is largely degraded to urea).

(c) 3-Phosphoglycerate is the precursor of serine, which can be converted to cysteine and glycine. Glycine can also be produced from CO_2, NH_4^+, and N^5,N^{10}-methylene-THF

Cont...........

The essential amino acids can be categorized into four groups based on their synthetic pathways:

(a) The aspartate family. Aspartate serves as the precursor for the synthesis of lysine, threonine, and methionine. This pathway also produces homoserine and homocysteine.

(b) The pyruvate family. Pyruvate serves as a precursor for the synthesis of valine, leucine, and isoleucine.

(c) Aromatic amino acids. Phosphoenolpyruvate and erythrose-4-phosphate serve as the precursors for tyrosine, phenylalanine, and tryptophan. Serine is also required for the synthesis of tryptophan.

(d) Histidine. 5-Phosphoribosyl-a-pyrophosphate is a precursor for the synthesis of histidine.