B.SC-II SEMESTER

UNIT-1: ENZYMOLOGY AND METABOLISM

INTRODUCTION:

- Enzymes are biocatalysts—Catalysts of life
- A catalyst is defined as a substance that increases the velocity or the rate of a chemical reaction, without itself undergoing any change in the process
- Enzymes are biocatalysts that are synthesized by living cells.
- They are proteinaceous (exception is the ribozyme, which is an RNA), colloidal, and thermolabile (inactive at 0°C and destroyed at 100°C)
- They are specific in action, catalyze all biochemical reactions and are susceptible to many factors like temperature, pH, etc.
- Examples: urease, carbonic anhydrase, pepsin, rennin, etc.

  The word enzyme was used by Kuhne 1878, to indicate the catalysis taking place in the biological systems. lsolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol. Sumner first achieved the isolation and crystallization of the enzyme urease from jack bean and identified it as a protein.

1. Chemical Nature and Properties of Enzymes

- Each enzyme has its own tertiary structure and specific conformation, which is very essential for its catalytic activity
- Chemically, enzymes may be divided into 2 categories

Simple protein enzymes

- These contain simple proteins only. E.g., urease, amylase, papain, etc.

Complex protein enzymes

- These contain conjugated proteins, i.e., they have a protein part called apoenzyme and a non-protein part called prosthetic group, associated with the protein unit
- The 2 parts together constitute the holoenzyme. E.g., catalase, cytochrome c, etc.
  - Holoenzyme
    - The functional unit or the active structure of an enzyme (apoenzyme + prosthetic group) is called holoenzyme

Holoenzyme	→	Apoenzyme	+	Coenzyme
(Active enzyme)		(Protein part)		(Prostetic group)

- Apoenzyme
    - It is the protein part of the enzyme
    - It is the inactive form of the enzyme
    - It becomes functional only by associating itself with the prosthetic group

- Prosthetic group
    - A prosthetic group is that which is covalently bound to the apoenzyme
    - They do not dissociate from the protein part of the enzyme and repeatedly participate in enzyme-catalyzed reactions
    - E.g., FAD, FMN, TPP, PLP, biotin, etc.
- Cofactor
    - They are mainly inorganic metal complexes which are tightly bound to the enzyme
    - They are highly required for normal conformational structure and function of the enzyme
    - They act as donors or acceptors in oxidation and reduction reactions
    - E.g., Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, K^{+}, Fe^{2+}, Mn^{2+}, Mo^{2+}, etc.
- Coenzymes
    - They are also called co-substrate or second substrate
    - They are organic, metallo-organic, or inorganic substances which are thermostable and dialyzable, highly required for the normal functioning of the enzyme
    - They bind covalently or non-covalently to the apoenzyme
    - Reactions involving oxidoreductions, group transfers, isomerization, and covalent bond formation require coenzyme
    - Coenzymes account for 1% of the entire enzyme molecule
    - E.g., NAD, FAD, THF, CoA. FMN, TPP, etc.

Functions of Coenzymes

- Their function is usually to accept atoms or groups from a substrate and to transfer them to other molecules
- They are less specific than enzymes and the same coenzyme can act as such in a number of different reactions
- Coenzymes are also attached to the protein at different but adjacent site, so as to be in a position to accept the atoms or groups, which are removed from the substrate

Nicotinamide adenine dinucleotide (**NAD**) and **nicotinamide adenine dinucleotide phosphate** (**NADP**) coenzymes are based on a common structure consisting of the base adenine, two ribose sugars linked by phosphate groups and a nicotinamide ring. NADP differs from NAD in having an additional phosphate group attached to one of the ribose sugars. These two coenzymes share a common function as they both act as carriers of electrons and are involved in **oxidation–reduction reactions**. NADis more commonly used in **catabolic** (breakdown) reactions, whilst NADP is used in **anabolic** (biosynthetic) reactions. The reactive part of both molecules is the **nicotinamide ring** which exists in either a reduced or an oxidized form, and so acts to accept or donate electrons in an enzymic reaction. The reaction also involves the transfer of protons, according to the equation:

$$NAD^+ + H^+ + 2e <.....>NADH$$

Flavin adenine dinucleotide (**FAD**) and **flavin mononucleotide** (**FMN**) are also carriers of electrons and have related chemical structures. Both of these coenzymes consist of a **flavine mononucleotide unit** which contains the reactive site. FAD has an additional sugar group and an adenine base which complete its structure. FAD and FMN react with two protons, as well as two electrons, in alternating between the reduced and oxidized state:

$$FAD + 2H^+ + 2e <.....> FADH2$$

Some common coenzymes, their vitamin precursors and deficiency diseases

Coenzyme	Precursor	Deficiency disease
Coenzyme A	Pantothenic acid	Dermatitis
FAD, FMN	Riboflavin (vitamin B_2)	Growth retardation
NAD^+, $NADP^+$	Niacin	Pellagra
Thiamine pyrophosphate	Thiamine (vitamin B_1)	Beriberi
Tetrahydrofolate	Folic acid	Anemia
Deoxyadenosyl cobalamin	Cobalamin (vitamin B_{12})	Pernicious anemia
Cosubstrate in the hydroxylation of proline in collagen	Vitamin C (ascorbic acid)	Scurvy
Pyridoxal phosphate	Pyridoxine (vitamin B_6)	Dermatitis

2. Active Site

- It is also called the catalytic site or the substrate site
- It is the site at which the substrate binds to an enzyme
- It consists of specific amino acids or groups, which contributes for specificity of the enzyme for a substrate
- They are involved in the formation and breakage of bonds and are known as the catalytic groups

Common features of the active site

1. It occupies a relatively small portion of the enzyme molecule

- Most of the amino acid residue in the enzyme are *not* in contact with the substrate
- Nearly all enzymes are made up of more than 100 amino acid residues, which give them a mass of greater than 10 kD, and a diameter of more than 25 Å
- Only a fraction of the amino acids are involved in the active site formation

2. It is a 3-D entity

- The active site of an enzyme is not a point, a line or a plane
- It is an intricate 3-D form made up of groups that come from different parts of the linear amino acid sequence
- Residues far apart in the linear sequence ay interact more strongly than adjacent residues in the sequence
- For example, in lysozyme, the important groups in the active site are contributed by residues numbered 35, 52,62,63,and 101 in a linear sequence of 129 amino acids

3. Substrates are bound to the enzyme by weak bonds

- E–S complex have an equilibrium constant that range from 10^{-2} to 10^{-8} M, corresponding to free energy of interaction ranging from -3 to -12 kcal/mol
- These values can be compared with strengths of covalent bonds which are below -50 to -110 kcal/mol

4. Active sites are clefts or crevices

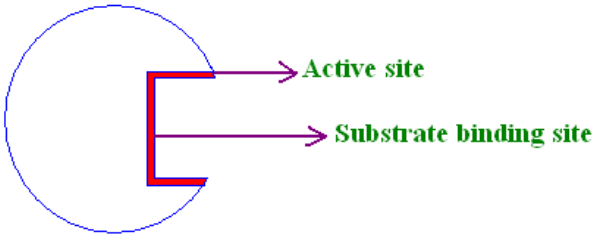
- In all enzymes, the substrate are bound to clefts or crevices from which water is usually excluded
- The cleft also has several polar residues that are essential for binding and catalysis

- The nonpolar characteristic regions of the cleft enhances the binding ot the substrate
- The cleft creates a microenvironment in which certain polar residues acquire special properties essential for their catalytic role

5. Specificity of binding depends on precisely defined arrangement of atoms in an active site

- The substrate must have matching shape to fit into the site (Fischer's model)
- The active site of some enzymes are not rigid and the active site is thus modified by the binding of the substrate
- The active site has a shape complementary to that of the substrate only after the substrate is bound (Koshland's model)

- The side chain groups like –COOH, –NH2, –CH2OH, etc.. serve as catalytic groups in the active site
- The crevice creates a microenvironment in which certain polar residues acquire special properties essential for catalysis
- The following figure illustrates the same:



- There must be at least 3 different points of interaction between the enzyme and the substrate
- These interactions can have either a binding or a catalytic function
- Binding sites link to specific groups in the substrate, ensuring that the enzyme and substrate molecules are held in a fixed orientation with respect to each other with the reacting group or groups in the vicinity of catalytic sites
- Consider the following 3-point interaction:

- Here, sites A″ and A‴ might represent binding sites of R″ and R‴
- A′ is the catalytic site for a reaction involving R′
- Thus, even if R′ and R″ are identical, the asymmetry of the E–S complex means that only R′ can react, providing binding site
- A‴ is specific for R‴ and R″ can never undergo reaction as it is not brought to the vicinity of A′ site even when R′ binds to A″ site

3. ENZYME NOMENCLATURE and CLASSIFICATION:

The International Union of Biochemistry (lUB) appointed an EnzymeC ommissionin 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since 1964, the IUB system of enzyme classification has been in force. Enzymes are divided into six major classes. Each class on its own represents the general type of reaction brought about by the enzymes of that class.

1. **Oxidoreductases** : Enzymes involved in oxidation and reduction reactions are known as oxidoreductases.
    - Oxidation is carried out by the removal of electrons from a specific group of substrate or by the addition of oxygen to the specific group
    - Reduction is the opposite change
    - This class is divided into subclasses, according to electron donor or acceptor of the substrate
    - For example: Alcohol dehydrogenase, Lactate dehydrogenase, Oxalate oxidase

2. **Transferases**: Enzymes that catalyse the transfer of functional groups.
    - They catalyze reactions of the type $AX + B \Rightarrow BX + A$
    - Enzymes of this class transfer a particular group from one substrate to the other
    - These enzymes are further divided into subclass according to the specific group transferred by them
    - For example:Glucokinase, Transaldolase

3. **Hydrolases** : Enzymes that bring about hydrolysis of various compounds.
    - Enzymes which catalyze cleavage of bonds by the addition of water are called hydrolases
    - They catalyze reactions of the type: $A-X + H_2O \Rightarrow X-OH + HA$
    - They have different classes according to the bond hydrolyzed

- For example: Carboxyesterase, α-amylase, urease

4. **Lyases** : Enzymes specialised in the addition or removal of water, ammonia, CO2 etc.

- Lyases cleave a covalent bond of the substrate to convert it into more than one product, but the reaction does not involve any hydrolysis
- Their action frequently produce a double bond in one of the product
- They are divided into subclasses according to the atom connected to the bond
- They generally catalyze the breaking of C–C, C–S, and C–N bonds
- Examples: Pyruvate decarboxylase

5. **lsomerases**: Enzymes involved isomererization reactions.

- Isomerases convert their substrates to their isomers by intramolecular rearrangement
- They are divided into subclasses according to the type of isomerization
- Examples: Phosphoglycerate mutase

6. **ligases** : Enzymes catalyzing the synthetic reactions (Creek : ligate-to bind) where two Molecules are joined together and ATP is used.

- Ligases are enzymes that catalyze the formation of a bond between 2 substrates
- They catalyze the formation of bonds between C, O, N, and S coupled to hydrolysis of high energy compounds like ATP
- They are subdivided into classes according to the atom connected by the new bond
- Examples: Tyrosine t-RNA ligase; pyruvate carboxylase.

The word OTHLIL (first letter in each class) may be memorised to remember the six classes of enzymes in the correct order. Each class in turn is subdivided into manv sub-classes which are further divided. A four digit Enzyme Commission (E C.) number is assigned to each enzyme representing the class (first digit), sub-clas (second digit), sub-sub class (third digit) and the individual enzyme (fourth digit). Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalysed by the enzyme. Although the IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the trivial names, along with the E.C. numbers as and when needed, are commonly used and widely accepted.

- The nomenclature of the enzyme is done as follows:
    - The first part of the name gives the name of the substrate
    - The second part indicates the type of reaction catalyzed

- The third part is the suffix "*-ase*" indicating that it is an enzyme. However, some enzymes do not have this suffix (e.g., trypsin, pepsin, diastase, papain, etc..)

- Example:

ALCOHOL DEHYDROGENASE

In this enzyme, alcohol is substrate, the type of reaction is dehydrogenation and the suffix "*-ase*" is places at the end.

Classification of enzymes

*Enzyme class with examples**	*Reaction catalysed*
1. **Oxidoreductases**	
Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. **Transferases**	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. **Hydrolases**	
Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. **Lyases**	
Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition ⟶ Elimination $A - B + \dot{X} - \dot{Y} \longrightarrow A\dot{X} - B\dot{Y}$
5. **Isomerases**	
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. **Ligases**	
Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ADP + Pi]{ATP} A - B$

**For one enzyme in each class, systematic name along with E.C. number is given in the brackets.*

4. Enzyme activity, units of measurement and factors affecting enzyme reaction:

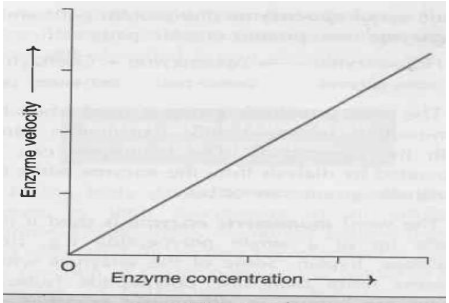
Enzyme activity is commonly expressed by the initial rate (*V*0) of the reaction being catalyzed. The units of *V*0 are mol min_1, which can also be represented by the enzyme unit (U) or the katal (kat), where 1 μmol/mi =1 U=16.67 nanokat. The term activity (or total activity) refers to the total units of enzyme in a sample, whereas specific activity is the number of units per milligram of protein (units/mg).

The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity. The rate of an enzyme-catalyzed reaction is often called its velocity. Enzyme velocities are normally reported as values at time zero (**initial velocity**, symbol*V*0; μmol min_1), since the rate is fastest at the point where no product is yet present. This is because the substrate concentration is greatest before any substrate has been transformed to product, because enzymes may be subject to **feedback inhibition** by their own products and/or because with a reversible

reaction the products will fuel the reverse reaction. The important factors that influence the velocity of the enzyme reaction are discussed hereunder:

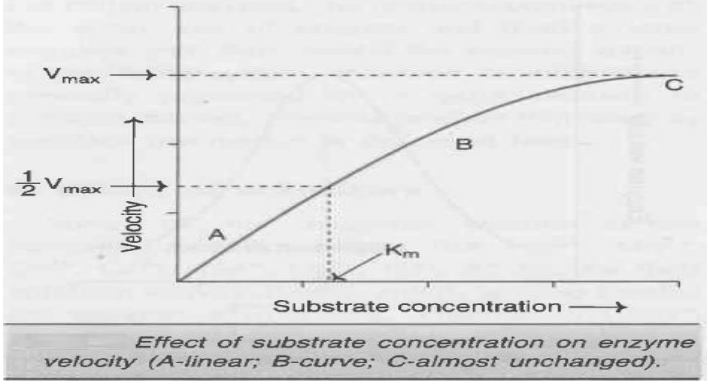
1.Concentration of enzyme:

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis o f diseases. By using a known volume of serum, and keeping all the other factors (substrate, pH, temperature etc.) at the optimum level, the enzyme could be assayed in the laboratory.



Effect of enzyme concentration on enzyme velociy.

2. **Concentration of substrate:** Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration.Three distinct phases of the reaction are observed in the graph (A-linear;B-curve; C-almost unchanged).

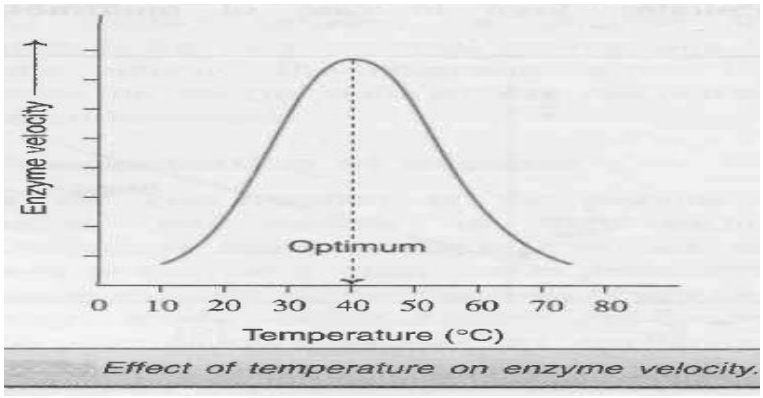


Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

3. **Effect of temperature:**

   Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed. Temperature coefficient or Q_{10} is defined as increase in enzyme velocity when the temperature is increased by 10"C. For a majority of enzymes, Q_{10} is 2 between 0^0C and 40^oC. increase in temperature results in higher activation energy of the molecules and more molecuar (enzyme and substrate) collision and interaction for the reaction to proceed faster. The optimum temperature for most of the enzymes is between 40^0C-45^0C. However, a few enzymes

active even at 100^0C. In general, when the enzymes are exposed to a temperature above 50^0C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70^0C).

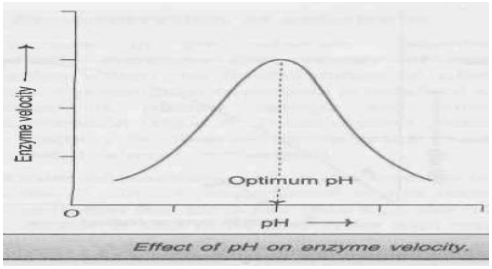


Effect of temperature on enzyme velocity.

4. **Effect of pH:**

lncrease in the hydrogen ion concentration (pH) considerably in fluencies the enzyme activity and a bell-shaped curve is normally obtained . Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive. Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8).

There are, however, many exceptional like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11). Enzymes from fungi and plants are most active in acidic pH (4-6). Hydrogen ions influence the enzyme activity by altering the ionic charge on the amino acids (particularly at the active site), substrate, ES complex etc.



Effect of pH on enzyme velocity.

5. **Effect of product concentration:**

The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. ln the living system, this type of inhibition is generally prevented by a quick removal of products formed.

6. **Effect of activators:**

Some of the enzymes require certain inorganic metallic cations like Mg2+, Mn2+, Zn2+, Ca2+, Co2+, Cu2+, Na+, K+ etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (C11 for amylase. Metals function as activators of enzyme velocity through various mechanisms combining with the substrate,

formation of ES-metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring metals for their activity are distinguished.

Metal-activated enzymes : The metal is not tightly held by the enzyme and can be exchanged easily with other ions e.g. ATPase and enolase with Mg2+.

Metalloenzymes : These enzymes hold the metals rather tightly which are not readily exchanged. e.g..

Phenol oxidase (copper);

Pyruvate oxidase (manganese);

Xanthine oxidase (molybdenum);

Cytochrome oxidase (iron and copper).

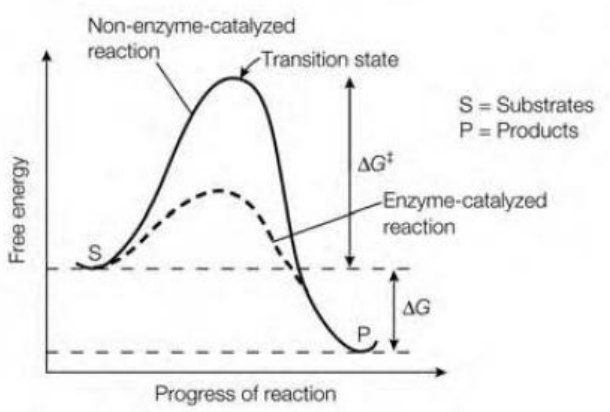
7. **Effect of time:** Under ideal and optimal conditions (like pH, temperature etc.), the time required for an enzyme reaction is less. Variations in the time of the reaction are generally related to the alterations in pH and temperature.
8. **Effect of light:** Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. e.g. UV rays inhibit salivary amylase activity.

5. Activation energy:

In all reactions there is an energy barrier that has to be overcome in order for the reaction to proceed. This is the energy needed to transform the substrate molecules into the transition state – an unstable chemical form part-way between the substrates and the products. The transition state has the highest free energy of any component in the reaction pathway. The Gibbs free energy of activation (ΔG‡) is equal to the difference in free energy between the transition state and the substrate. An enzyme works by stabilizing the transition state of a chemical reaction and decreasing ΔG ‡ .The enzyme does not alter the energy levels of the substrates or the products. Thus an enzyme increases the rate at which the reaction occurs, but has no effect on the overall change in energy of the reaction.

The change in Gibbs free energy (ΔG) dictates whether a reaction will be energetically favorable or not. The Figure shows an example where the overall energy change of the reaction makes it energetically favorable (i.e. the products are at a lower energy level than the substrates and ΔG is negative). It should be noted that ΔG is unrelated to ΔG‡. The ΔG of a reaction is independent of the path of the reaction, and it provides no information about the rate of a reaction since the rate of the reaction is governed by ΔG‡. A negative ΔG indicates that the reaction is thermodynamically favorable in the direction

indicated (i.e. that it is likely to occur without an input of energy), whereas a positive ΔG indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated. In biochemical systems, this input of energy is often achieved by coupling the energetically unfavorable reaction with a more energetically favorable one (coupled reactions).



The energy changes taking place during the course of a biochemical reaction.

6. Enzyme kinetics

The enzyme (E) and substrate(S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Here kl , k2 and k3 represent the velocity constants for the respective reactions, as indicated by arrows. Km or the Michaelis-Mentenc onstant(or Brigrs and Haldane's constant), is given by the formula:

$$K_m = \frac{k_2 + k_3}{k_1}$$

The following equation is obtained after suitable algebraic manipulation.

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad \text{equation (1)}$$

where v = Measured velocity,
V_{max} = Maximum velocity,
S = Substrate concentration,
K_m = Michaelis – Menten constant.

Let us assume that the measured velocity (v) is equal to $\frac{1}{2}V_{max}$. Then the equation (1) may be substituted as follows

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

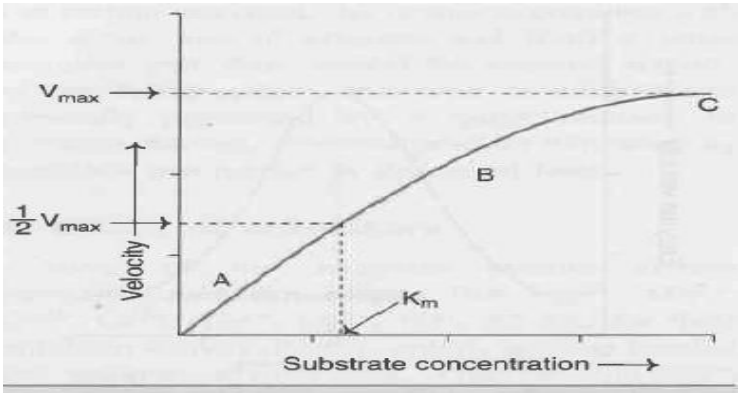
$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

K stands for a constant and m stands for Michaelis (in Km.). Km or Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/l) to produce half-maximum velocity in an enzyme catalysed reaction. It indicates that half of the enzyme molecules (i.e.50%) are bound with the substrate molecules when the substrate concentration equals the Km value which is a constant and a characteristic feature of a given enzyme (comparable to a thumb impression or signature). lt is a representative for measuring the strength of ES complex. A low Km value indicates a strong affinity between enzyme and substrate, whereas a high K. value reflects a weak affinity between them. For majority of enzymes, the Km values are in the range of 10^{-5} to 10^{-2} moles. lt may however, be noted that Km is not dependent on the concentration of enzyme.

Further, kcat is the turnover number or catalytic constant. where Vmax = **kcat**[E]t. **kcat** incorporates the rate constants for all the reactions between ES and E + P in a multistep enzymatic process. For a two-step reaction, **kcat** =k2. For more complex reactions, **kcat** depends on which steps in the process are rate-limiting. **kcat** gives a direct measure of the catalytic production of product under optimum conditions (saturated enzyme). The units of **kcat** are seconds^{-1}. The reciprocal of **kcat** can be thought of as the time required by an enzyme molecule to "turn over" one substrate molecule. Alternatively, **kcat** measures the number of substrate molecules turned over per enzyme molecule per second.



6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

Lineweaver-Burk double reciprocal plot :

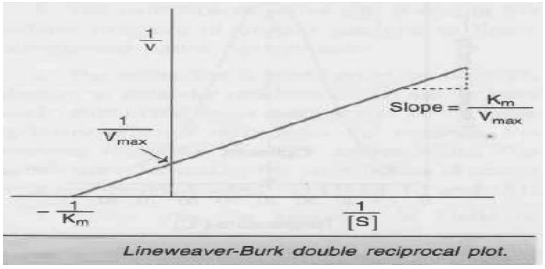
For the determination of K, value, the substrate saturation curve is not very accurate. By taking the reciprocals of the equation, a straight line graphic representation is obtained.

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

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

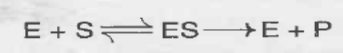
Therefore, a plot of the reciprocal of the velocity (1/v) Vs reciprocal of the substrate concentration gives a straight line. lt is much easier to calculate the Km from the intercept on x-axis which is -(l/Km). Further, the double reciprocal plot is useful in understanding the effect of various inhibitions.



Lineweaver-Burk double reciprocal plot.

7. Enzyme substrate complex formation

The prime requisite for enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme substrate complex (ES) which ultimately results in the product formation (P).



A few theories have been put forth to explain mechanism of enzyme-substrate complex formation:-

a) Lock and key model or Fischer's template theory:

This theory was proposed by a German biochemist, Emil Fischer. This is in fact the very first model proposed to explain an enzyme catalysed reaction. According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (now active site) just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind. This model does not give any scope for the flexible nature of enzymes, hence the model totally fails to explain many facts of enzymatic reactions the most important being the effect of allosteric modulators.

b) **Induced fit theory or Koshland's model:**

Koshland, in 1958, proposed a more acceptable and realistic model for enzyme substrate complex formation. As per this model, the active site is not rigid and pre-shaped. The essential features of the substrate binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis. Induced fit model has sufficient experimental evidence from the X-ray diffraction studies. Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

c) Substrate strain theory:

In this model, the substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product. ln fact, a combination of the induced fit model with the substrates train is considered to be operative in the enzymatic action.

MECHANISIII OF ENZYME GATATYSIS:

The formation of an enzyme-substrate complex (ES) is very crucial for the catalysis to occur, and for the product formation. lt is estimated that an enzyme catalysed reaction proceeds 10^6 to 10^{12} times faster than a non catalysed reaction. The enhancement in the rate of the reaction is mainly due to four processes:

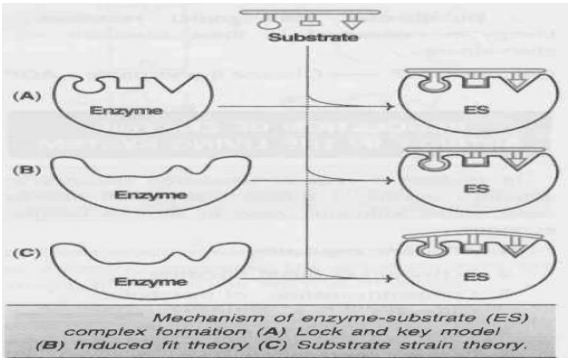
1. Acid-base catalysis;
2. Substrates train;
3. Covalent catalysis;
4. Entropy effects.

1. **Acid-base catalysis**: Role of acids and bases is quite important in enzymology. At the physiological pH, histidine is the most important amino acid, the protonated form of which functions as an acid and its corresponding conjugate as a base. The other acids are –OH group of tyrosine, -SH group of cysteine, and e-amino group of lysine. The conjugates of these acids and carboxyl ions (COO-) function as bases. Ribonuclease which cleaves phosphodiester bonds in a pyrimidine loci in RNA is a classical example of the role of acid and base in the catalysis.

2. **Substrate strain** : During the course of strain induction, the energy level of the substrate is raised, leading to a transition state. The mechanism of lysozyme (an enzyme of tears, that cleaves p-1,4 glycosidic bonds) action is believed to be due to a combination of substrates train and acid-basec atalysis.

3. **Covalent catalysis** : In the covalent catalysis, the negatively charged (nucleophilic) or posit ively charged (electrophilic) group is present at the active site of the enzyme. This group attacks the substrate that results in the covalent binding of the substrate to the enzyme. ln the serine proteases (so named due to the presence of serine at active site), covalent catalysis along with acid-base catalysis occur, e.g. chymotrypsin, trypsin, thrombin etc.

4. **Entropy effect** : Entropy is a term used in thermodynamics. lt is defined as the extent of disorder in a system. The enzymes bring about a decrease in the entropy of the reactants. This enables the reactants to come closer to the enzyme and thus increase the rate of reaction.



Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model (B) Induced fit theory (C) Substrate strain theory.

8. Enzyme inhibition

Many types of molecule exist which are capable of interfering with the activity of an individual enzyme. Any molecule which acts directly on an enzyme to lower its catalytic rate is called an **inhibitor**. Some enzyme inhibitors are normal body metabolites that inhibit a particular enzyme as part of the normal metabolic control of a pathway. Other inhibitors may be foreign substances, such as drugs or toxins, where the effect of enzyme inhibition could be either therapeutic or, at the other extreme, lethal.

Enzyme inhibition may be of two main types: **irreversible** or **reversible**, with reversible inhibition itself being subdivided into **competitive** and **noncompetitive** inhibition. Reversible inhibition can be overcome by removing the inhibitor from the enzyme, for example by dialysis, but this is not possible for irreversible inhibition.

1. **Irreversible inhibition:**

Inhibitors which bind irreversibly to an enzyme often form a covalent bond to an amino acid residue at or near the active site, and permanently inactivate the enzyme. Susceptible amino acid residues include Ser and Cys residues which have reactive –OH and –SH groups, respectively. The compound diisopropylphosphofluoridate (DIPF), a component of nerve gases, reacts with a Ser residue in the active site of the enzyme acetylcholinesterase, irreversibly inhibiting the enzyme and preventing the transmission of nerve impulses Iodoacetamide modifies Cys residues and hence may be used as a diagnostic tool in determining whether one or more Cys residues are required for enzyme activity . The antibiotic penicillin irreversibly inhibits the glycopeptide transpeptidase enzyme that forms the cross-links in the bacterial cell wall by covalently attaching to a Ser residue in the active site of the enzyme.

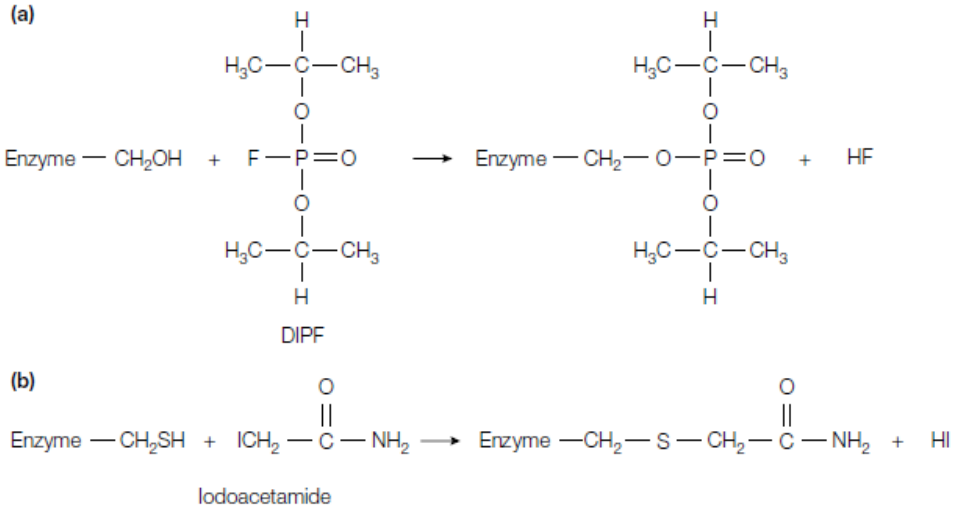


Fig. Structure and mechanism of action of (a) diisopropylphosphofluoridate (DIPF) and (b) iodoacetamide.

2. Reversible competitive inhibition:

A **competitive inhibitor** typically has close structural similarities to the normal substrate for the enzyme. Thus it competes with substrate molecules to bind to the active site. The enzyme may bind either a substrate molecule or an inhibitor molecule, but not both at the same time. The competitive inhibitor binds **reversibly to the active site**. At **high substrate concentrations** the action of a competitive inhibitor is overcome because a sufficiently high substrate concentration will successfully compete out the inhibitor molecule in binding to the active site. Thus there is no change in the Vmax of the enzyme but the apparent affinity of the enzyme for its substrate decreases in the presence of the competitive inhibitor, and hence Km increases. A good example of competitive inhibition is provided by succinate dehydrogenase. This enzyme uses succinate as its substrate and is competitively inhibited by malonate which differs from succinate in having one rather than two methylene groups. Many drugs work by mimicking the structure of the substrate of a target enzyme, and hence act as competitive inhibitors of the enzyme. Competitive inhibition can be recognized by using a Lineweaver–Burk plot. V0 is measured at different substrate concentrations in the presence of a fixed concentration of inhibitor. A competitive inhibitor increases the slope of the line on the Lineweaver–Burk plot, and alters the intercept on the x-axis (since Km is increased), but leaves the intercept on the y-axis unchanged (since Vmax remains constant).

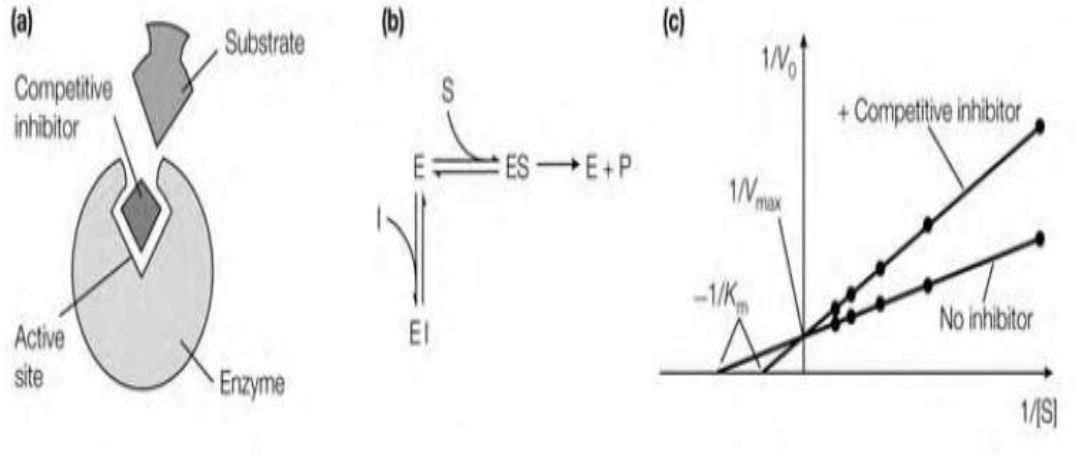


Fig. . The characteristics of competitive inhibition. (a) A competitive inhibitor competes with the substrate for binding at the active site of the enzyme; (b) the enzyme can bind either substrate or the competitive inhibitor but not both; (c) Lineweaver-Burk plot showing the effect of a competitive inhibitor on K_m and V_{max}.

3. Reversible noncompetitive inhibition:

A noncompetitive inhibitor binds reversibly at a site other than the active site and causes a change in the overall three-dimensional shape of the enzyme that leads to a decrease in catalytic activity. Since the inhibitor binds at a different site from the substrate, the enzyme may bind the inhibitor, the substrate or both the inhibitor and substrate together. The effects of a noncompetitive inhibitor cannot be overcome by increasing the substrate concentration, so there is a decrease in *V*max. In noncompetitive inhibition the affinity of the enzyme for the substrate is unchanged and so *K*m remains the same. An example of noncompetitive inhibition is the action of **pepstatin** on the enzyme **renin**. Noncompetitive inhibition can be recognized on a Lineweaver–Burk plot, since it increases the slope of the experimental line, and alters the intercept on the *y*-axis (since ***V*max is decreased**), but leaves the intercept on the *x*-axis unchanged (since ***K*m remains constant**).

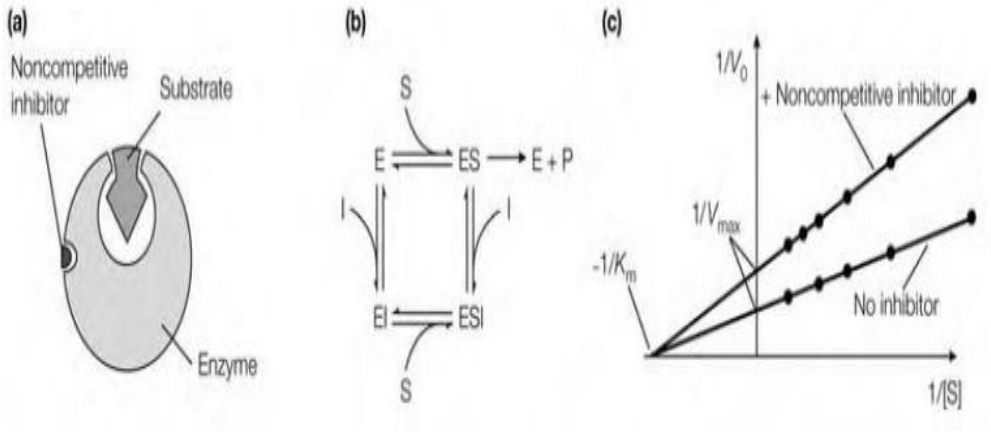
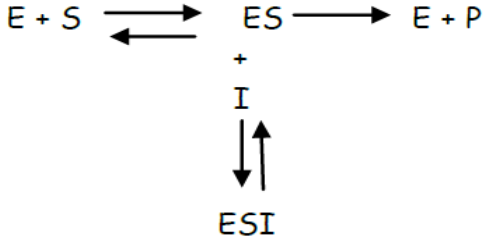


Fig. The characteristics of noncompetitive inhibition. (a) A noncompetitive inhibitor binds at a site distinct from the active site; (b) the enzyme can bind either substrate or the noncompetitive inhibitor or both; (c) Lineweaver-Burk plot showing the effect of a noncompetitive inhibitor on K_m and V_{max}.

4. Uncompetitive inhibition:

It is typically seen in multisubstrate reactions (here, there is a decrease in product formation because the second substrate cannot bind). The Inhibitor binds to ES, but not enzyme and both Km and Vmax are lowered, while as ratio Km/Vmax remains unchanged



Semester-II (Biotechnology)

Course Title: Enzymology and Metabolism

Unit–II

Carbohydrates Metabolism

Dear Students, we have covered Properties, Structures, Functions and Classification of Carbohydrates in Semester –I. In Semester-II we will be dealing with Carbohydrate Metabolism

Carbohydrate Metabolism: It denotes the various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates in living organisms.

Different topics which we will be covering here are:

1. Glycolysis.

2. Gluconeogenesis.

3. TCA Cycle.

4. Electron Transport Chain.

5. Oxidative Phosphorylation

6. Pentose Phosphate Pathway

Let us first start with Glycolysis: The pathway which occurs in nearly all organisms, both aerobic and anaerobic. Glycolsis is one of the most ancient known metabolic pathways. It occurs in the cytosol of the cell.

Glycolysis

Glycolysis is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, $CH_3COCOO^- + H^+$. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate), $FADH_2$ and NADH (reduced nicotinamide adenine dinucleotide).

The entire glycolsis pathway can be separated into two phases:

1. The Preparatory Phase - in which ATP is consumed and is hence also known as the investment phase
2. The Pay Off Phase - in which ATP is produced.

Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates.

Step I

The enzyme hexokinase phosphorylates (adds a phosphate group to) glucose in the cell's cytoplasm. In the process, a phosphate group from ATP is transferred to glucose producing glucose 6-phosphate.

Glucose ($C_6H_{12}O_6$) + hexokinase + ATP → ADP + Glucose 6-phosphate ($C_6H_{11}O_6P_1$)

Step II

The enzyme phosphoglucoisomerase converts glucose 6-phosphate into its isomer fructose 6-phosphate. Isomers have the same molecular formula, but the atoms of each molecule are arranged differently.

Glucose 6-phosphate ($C_6H_{11}O_6P_1$) + Phosphoglucoisomerase → Fructose 6-phosphate ($C_6H_{11}O_6P_1$)

Step III

The enzyme phosphofructokinase uses another ATP molecule to transfer a phosphate group to fructose 6-phosphate to form fructose 1, 6-bisphosphate.

Fructose 6-phosphate ($C_6H_{11}O_6P_1$) + phosphofructokinase + ATP

ADP + Fructose 1, 6-bisphosphate ($C_6H_{10}O_6P_2$)

Step IV

The enzyme aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate and glyceraldehyde phosphate.

Fructose 1, 6-bisphosphate ($C_6H_{10}O_6P_2$) + aldolase

Dihydroxyacetone phosphate ($C_3H_5O_3P_1$) + Glyceraldehyde phosphate ($C_3H_5O_3P_1$)

Step V

The enzyme triose phosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate and glyceraldehyde phosphate. Glyceraldehyde phosphate is removed as soon as it is formed to be used in the next step of glycolysis.

Dihydroxyacetone phosphate ($C_3H_5O_3P_1$) → Glyceraldehyde phosphate ($C_3H_5O_3P_1$)

Pay-off phase

The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH.

Step 6

The enzyme which catalyzes step 6 in Glycolysis is triose phosphate dehydrogenase. This enzyme serves two functions in this step. First the enzyme transfers hydrogen (H^-) from glyceraldehyde phosphate to the oxidizing agent nicotinamide adenine dinucleotide (NAD^+) to form NADH. Next triose phosphate dehydrogenase adds a phosphate (P) from the cytosol to the oxidized glyceraldehyde phosphate to form 1, 3-bisphosphoglycerate. This occurs for both molecules of glyceraldehydes phosphate produced in step-5.

A. Triose phosphate dehydrogenase + 2 H^- + 2 NAD^+ → 2 NADH + 2 H^+

B. Triose phosphate dehydrogenase + 2 P + 2 glyceraldehyde phosphate ($C_3H_5O_3P_1$)

↓

2molecules of 1,3-bisphosphoglycerate($C_3H_4O_4P_2$)

Step 7

In step 7, The enzyme phosphoglycerokinase transfers a P from 1,3-bisphosphoglycerate to a molecule of ADP to form ATP. This happens for each molecule of 1,3-bisphosphoglycerate. The process yields two 3-phosphoglycerate molecules and two ATP molecules.

2 molecules of 1,3-bisphoshoglycerate ($C_3H_4O_4P_2$) + phosphoglycerokinase + 2 ADP

↓

2molecules of 3-phosphoglycerate($C_3H_5O_4P_1$)+2ATP

Step 8

In step 8 of Glycolysis, the enzyme phosphoglyceromutase relocates the P from 3-phosphoglycerate from the third carbon to the second carbon to form 2-phosphoglycerate.

2 molecules of 3-Phosphoglycerate ($C_3H_5O_4P_1$) + phosphoglyceromutase

↓

2 molecules of 2-Phosphoglycerate($C_3H_5O_4P_1$)

Step 9

In this step, enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP). This happens for each molecule of 2-phosphoglycerate.

2 molecules of 2-Phosphoglycerate ($C_3H_5O_4P_1$) + enolase

↓

2 molecules of phosphoenolpyruvic acid (PEP) ($C_3H_3O_3P_1$)

Step 10

The enzyme pyruvate kinase transfers a P from PEP to ADP to form pyruvic acid and ATP. This happens for each molecule of PEP. This reaction yields 2 molecules of pyruvic acid and 2 ATP molecules.

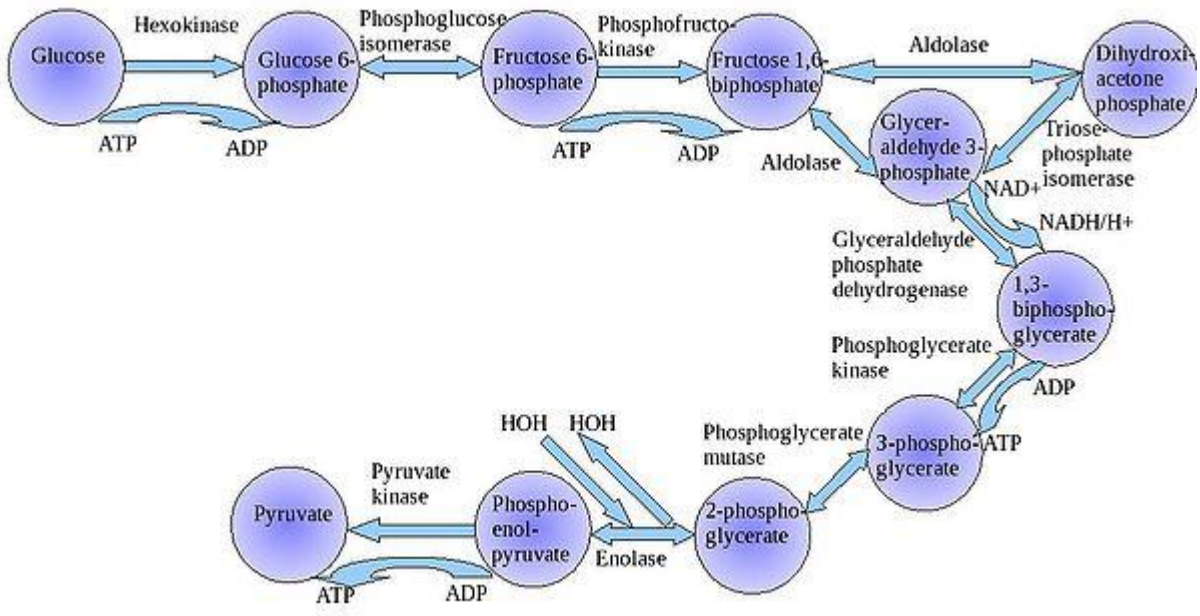
2 molecules of PEP ($C_3H_3O_3P_1$) + pyruvate kinase + 2 ADP →

↓

2 molecules of pyruvic acid ($C_3H_4O_3$) + 2 ATP

Summary

In summary, a single glucose molecule in glycolysis produces a total of 2 molecules of pyruvic acid, 2 molecules of ATP, 2 molecules of NADH and 2 molecules of water.



Metabolic pathway of Glycolysis

GLUCONEOGENESIS (GNG)

Next topic which we will be covering is Gluconeogenesis.

Let us first see what it is in simple terms

The synthesis of glucose from non-carbohydrate precursors is called gluconeogenesis. There are two major sites for gluconeogenesis, the liver and the kidneys. The liver and kidneys maintain the glucose level in the blood so that the brain, muscle and red blood cells have sufficient glucose to meet their requirement.

WHEN GLUCONEOGENESIS OCCURS

Gluconeogenesis occurs when glycogen is depleted in the body, during longer fasts and between meals, or after vigorous exercise. For these times, organisms need a method for synthesizing glucose from noncarbohydrate precursors. This is accomplished by a pathway called **gluconeogenesis,** which converts pyruvate and related three- and four-carbon compounds to glucose. Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms. The important precursors of glucose in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well.

PATHWAY

GNG pathway consists of a series of 10 enzymatic reactions. Seven are reverse of glycolytic reactions. Glycolysis has three irreversible steps catalyzed by hexokinase, phosphofructokinase and pyruvate kinase. In gluconeogenesis these irreversible steps have to be bypassed.

Conversion of Pyruvate to Phosphoenolpyruvate

The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate

(PEP). Pyruvate is first transported from the cytosol into Mitochondria. Then **pyruvate carboxylase,** a mitochondrial enzyme that requires the coenzyme **biotin,** converts the pyruvate to oxaloacetate.

Pyruvate + HCO_3^- + ATP $\longrightarrow$ oxaloacetate + ADP + Pi

As the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial **malate dehydrogenase,** at the expense of NADH:

Oxaloacetate + NADH + H $\rightleftharpoons$ **L-malate + NAD$^+$**

Malate leaves the mitochondrion through a specific transporter in the inner mitochondrial membrane and in the cytosol it is reoxidized to oxaloacetate, with the production of cytosolic NADH:

Malate + NAD$^+$ $\longrightarrow$ **oxaloacetate + NADH + H$^+$**

The oxaloacetate is then converted to PEP by phosphoenolpyruvate carboxykinase**.**

Conversion of Fructose 1,6-Bisphosphate to Fructose 6-Phosphate Is the Second Bypass

The second glycolytic reaction that cannot participate in gluconeogenesis is the phosphorylation of fructose 6-
phosphate by Phosphofructo kinase-1 (PFK-1) .

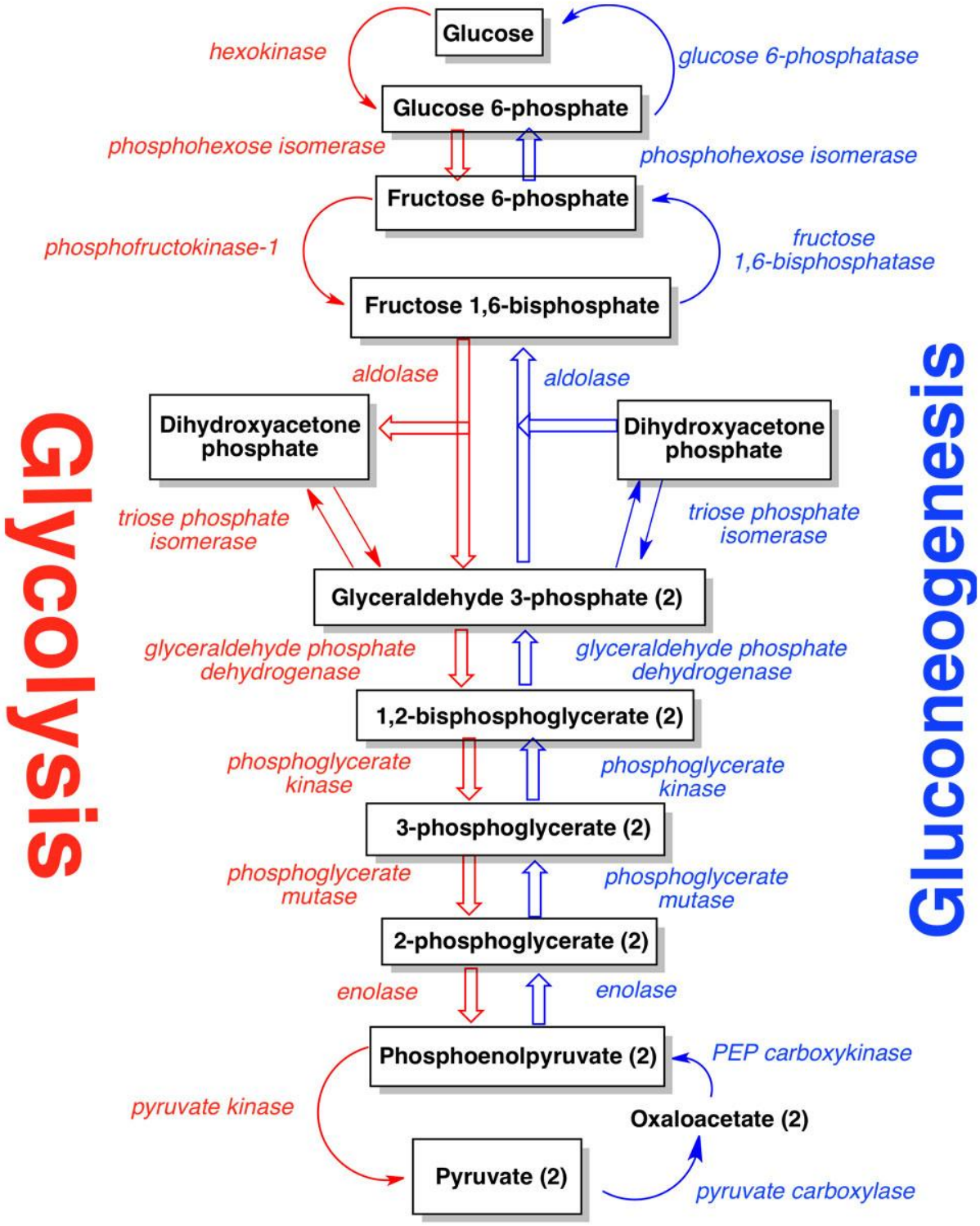
Fructose 1,6-bisphosphate + H_2O $\longrightarrow$ **fructose 6 -phosphate + Pi**

Conversion of Glucose 6-Phosphate to Glucose Is the Third Bypass

The third bypass is the final reaction of gluconeogenesis, the dephosphorylation of glucose 6-phosphate to
yield glucose. The reaction is catalyzed by **glucose 6-phosphatase which does n**ot require synthesis of ATP; it is a simple hydrolysis of a phosphate ester:

Glucose 6-phosphate + H_2O $\longrightarrow$ **Glucose + Pi**

Muscle and brain tissue do not contain this enzyme and so cannot carry out gluconeogenesis. Glucose produced by gluconeogenesis in the liver or kidney or ingested in the diet is delivered to brain and muscle through the bloodstream.


Glycolysis
Gluconeogenesis
Glucose
hexokinase
glucose 6-phosphatase
Glucose 6-phosphate
phosphohexose isomerase
phosphohexose isomerase
Fructose 6-phosphate
phosphofructokinase-1
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
aldolase
aldolase
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
triose phosphate isomerase
triose phosphate isomerase
Glyceraldehyde 3-phosphate (2)
glyceraldehyde phosphate dehydrogenase
glyceraldehyde phosphate dehydrogenase
1,2-bisphosphoglycerate (2)
phosphoglycerate kinase
phosphoglycerate kinase
3-phosphoglycerate (2)
phosphoglycerate mutase
phosphoglycerate mutase
2-phosphoglycerate (2)
enolase
enolase
Phosphoenolpyruvate (2)
PEP carboxykinase
pyruvate kinase
Oxaloacetate (2)
Pyruvate (2)
pyruvate carboxylase

Overview of TCA Cycle / Citric acid Cycle/ Krebs Cycle)

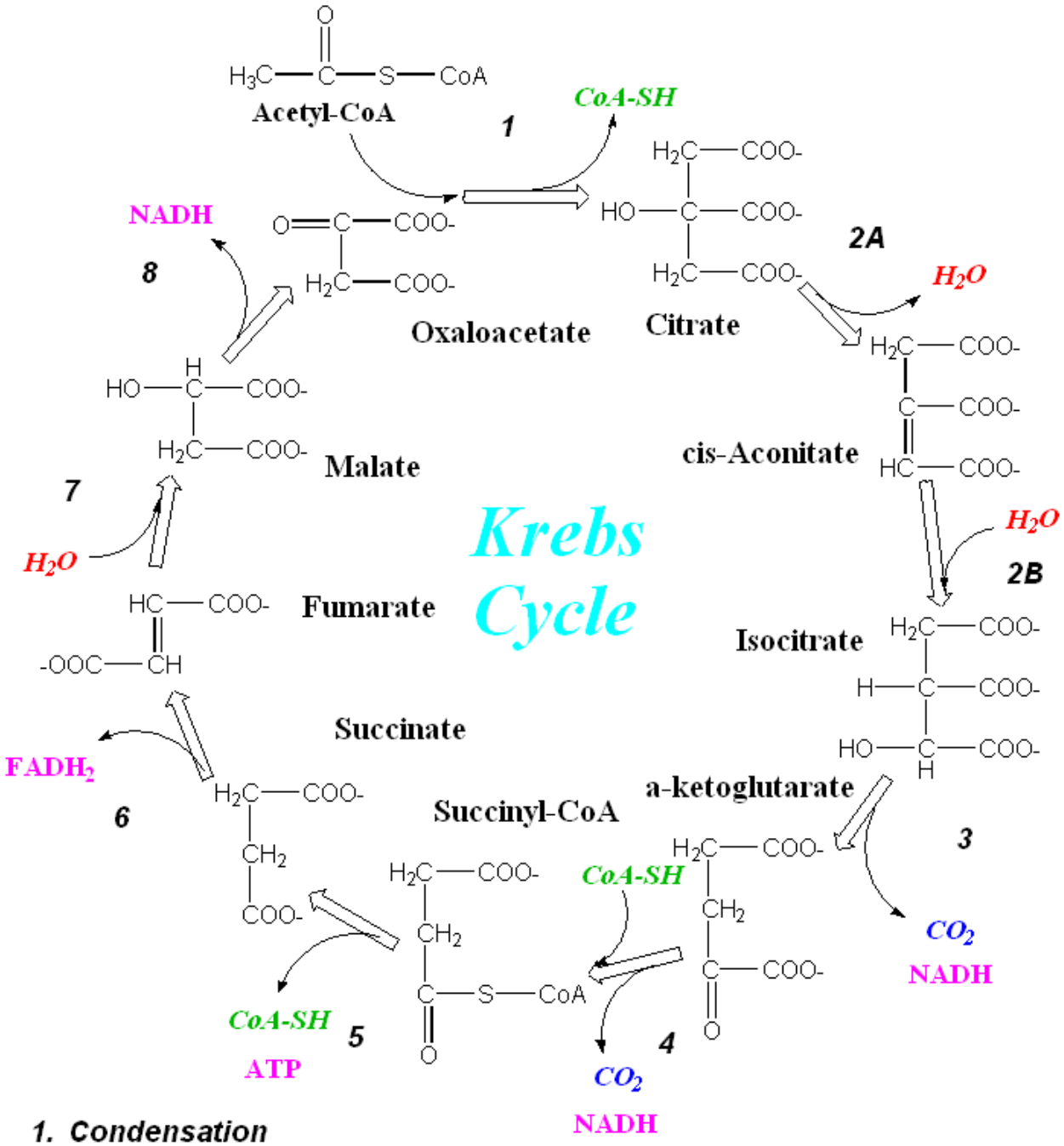
Important concepts:

- All fuel molecules are oxidized to citric acid cycle intermediates.
- The citric acid cycle is important for the biosynthesis of glucose, lipids, and some amino acids.
- 3.T he cycle was first elucidated by scientist "Sir Hans Adolf Krebs" (1900 to 1981).

The citric acid cycle breaks down a 6 carbon compound (citrate) to a 4 carbon compound (oxaloacetate). The high energy electrons are transferred to co-enzyme carriers (NAD+ and FAD) and destined for oxidative phosphorylation. The carbons are removed as CO2, and the H+ will follow with the electrons to O_2 to form H_2O. The citric acid cycle enzymes are located in the mitochondria, but they are mostly soluble.

The Net Equation

acetyl CoA + 3 NAD + FAD + ADP + HPO_4^{-2} ——————> 2 CO_2 + CoA + 3 $NADH^+$ + $FADH^+$ + ATP



1. *Condensation*
2a. *Dehydration*
2b. *Hydration*
3. *Oxidative decarboxylation*
4. *Oxidative decarboxylation*
5. *Substrate level phosphorylation*
6. *Dehydrogenation*
7. *Hydration*
8. *Dehydrogenation*

Reactions of TCA Cycle

➢ Reaction:1 Formation of Citrate

The first reaction of the cycle is the condensation of **acetyl-CoA** with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**.

Once Oxaloacetate is joined with acetyl-CoA, a water molecule attacks the acetyl leading to the release of coenzyme A from the complex.

➢ Reaction 2: Formation of Isocitrate

The **citrate** is rearranged to form an isomeric form, **isocitrate** by an enzyme **acontinase.**

In this reaction, **a water molecule is removed** from the citric acid and then put back on in another location. The overall effect of this conversion is that the –OH group is moved from the 3′ to the 4′ position on the molecule. This transformation yields the molecule **isocitrate.**

➢ Reaction 3: Oxidation of Isocitrate to α-Ketoglutarate

In this step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of **isocitrate** to form **α-ketoglutarate.**

In the reaction, generation of NADH from NAD is seen. The enzyme **isocitrate dehydrogenase** catalyzes the oxidation of the –OH group at the 4′ position of isocitrate to yield an intermediate which then has a carbon dioxide molecule removed from it to yield **alpha-ketoglutarate**.

➢ Reaction 4: Oxidation of α-Ketoglutarate to Succinyl-CoA

Alpha-ketoglutarate is oxidized, carbon dioxide is removed, and coenzyme A is added to form the 4-carbon compound **succinyl-CoA**.

During this oxidation, NAD^+ is reduced to NADH + H^+. The enzyme that catalyzes this reaction is **alpha-ketoglutarate dehydrogenase.**

➢ Reaction 5: Conversion of Succinyl-CoA to Succinate

CoA is removed from **succinyl-CoA** to produce **succinate.**

The energy released is used to make guanosine triphosphate (GTP) from guanosine diphosphate (GDP) and Pi by substrate-level phosphorylation. GTP can then be used to make ATP. The enzyme **succinyl-CoA synthase** catalyzes this reaction of the citric acid cycle.

➢ Reaction 6: Oxidation of Succinate to Fumarate

Succinate is oxidized to **fumarate**.

During this oxidation, FAD is reduced to FADH2. The enzyme **succinate dehydrogenase** catalyzes the removal of two hydrogens from succinate.

➢ Reaction 7: Hydration of Fumarate to Malate

The reversible hydration of **fumarate** to **L-malate** is catalyzed by **fumarase (fumarate hydratase).**

Fumarase continues the rearrangement process by adding **Hydrogen** and **Oxygen** back into the substrate that had been previously removed.

➢ Reaction 8: Oxidation of Malate to Oxaloacetate

Malate is oxidized to produce **oxaloacetate**, the starting compound of the citric acid cycle by **malate dehydrogenase**. During this oxidation, NAD^+ is reduced to NADH + $H^{+.}$

Importance/Significance of Krebs Cycle

1. Intermediate compounds formed during Krebs cycle are used for the synthesis of biomolecules like amino acids, nucleotides, chlorophyll, cytochromes and fats etc.
2. Intermediate like succinyl CoA takes part in the formation of chlorophyll.
3. Amino Acids are formed from α- Ketoglutaric acid, pyruvic acids and oxaloacetic acid.
4. Krebs cycle (citric Acid cycle) releases plenty of energy (ATP) required for various metabolic activities of cell.
5. By this cycle, carbon skeleton are got, which are used in process of growth and for maintaining the cells.

Regulation of the Citric Acid Cycle

The citric acid cycle is regulated at multiple points. However, in general it is safe to say that it is inhibited by ATP and NADH. The inhibition by NADH keeps it tightly regulated by oxygen supply, since NADH is converted to NAD+ by oxidative phosphorylation. The inhibition by ATP keeps the citric acid cycle in balance with energy supply. When ATP (energy supply) is high, the citric acid cycle is inhibited and precursors to the citric acid cycle (pyruvate, acetyl CoA and amino acids) are diverted into other pathways. Acetyl CoA, citrate, and succinylCoA are the end products of individual steps in the citric acid cycle and their accumulation inhibits the step involved in their production. That, of course, results in inhibition of the cycle as a whole. Finally, Ca^{++} stimulates the citric acid cycle at several points. This is important because electrical stimulation of the muscle causes an increase in intracellular calcium levels. Thus, during exercise the citric acid cycle will be maximally stimulated in muscle.

Our Next topic is Oxidative Phosphorylation.

Oxidative Phosphorylation •

- Energy is released when electrons are transported from higher energy NADH/FADH2 to lower energy O_2.
- This energy is used to phsophorylate ADP.
- This coupling of ATP synthesis to NADH/FADH2 oxidation is called oxidative phosphorylation.
- Oxidative phosphorylation is responsible for 90 % of total ATP synthesis in the cell.

Topics which we will be Covering in Oxidative Phosphorylation are:

1 .Electron-Transfer Reactions in Mitochondria

2. Regulation of Oxidative Phosphorylation

1. Electron Transfer-Reactions

The citric acid cycle oxidizes acetate into two molecules of CO2 while capturing the electrons in the form of 3 NADH molecules and one molecule of FADH2. These reduced molecules contain a pair of electrons with a high transfer potential. These electrons are ultimately going to be transferred by a system of electron carriers to O_2 to form H_2O. This process occurs in the mitochondria and is the major energy source used to produce ATP by oxidative phosphorylation.

The Components of the Electron Transport Chain

1. NADH.

2. Flavoprotein.

3. Co-enzyme Q (CoQ).

4. Cytochromes.

5. Iron-Sulfur Protein.

6. Copper proteins.

(These Components have been studied in detail in Semester-I. Here, we will see how they participate in **Electron Transport Chain (ETC**)

Overview of the Electron Transport Chain.

Electrons move along the electron transport chain going from donor to acceptor until they reach oxygen the ultimate electron acceptor.

The components of the electron transport chain are organized into 4 complexes. Each complex contains several different electron carriers.

1. Complex I also known as the NADH-coenzyme Q reductase or NADH dehydrogenase.

2. Complex II also known as succinate-coenzyme Q reductase or succinate dehydrogenase.

3. Complex III also known as coenzyme Q reductase.

4. Complex IV also known as cytochrome c reductase

.

Complex I: NADH to Ubiquinone

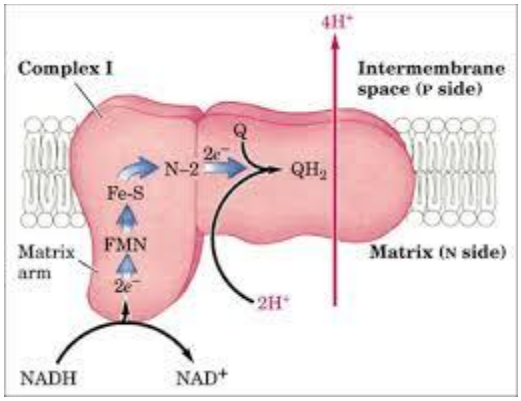
.

Complex I, also called **NADH:ubiquinone oxidoreductase** or **NADH dehydrogenase,** is a large enzyme composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six iron sulfur centers. Complex I catalyze two simultaneous and obligately coupled processes:

(1) The exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by

$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$ ands

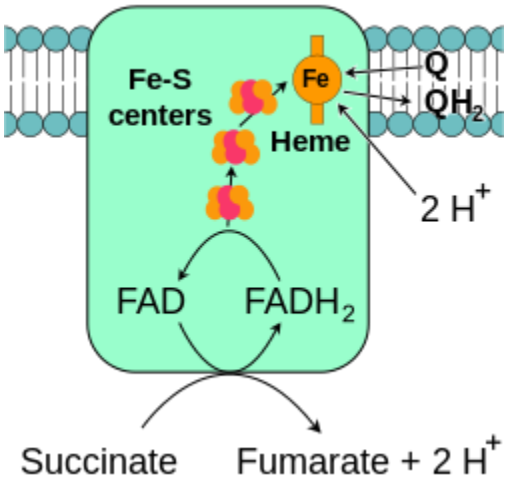
(2) The endergonic transfer of four protons from the matrix to the intermembrane space. Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is **vectorial.**



Complex I or NADH-Q oxidoreductas

<u>Complex II: Succinate to Ubiquinone</u> .It contains five prosthetic groups of two types and four different protein subunits (A, B, C, D)

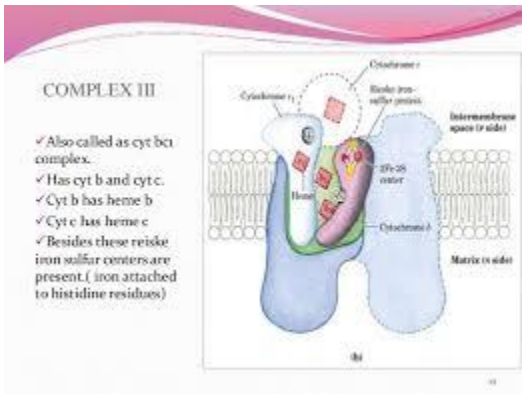
Subunits C and D are integral membrane proteins, each with three transmembrane helices. They contain a heme group, heme *b*, and a binding site for ubiquinone, the final electron acceptor in the reaction catalyzed by Complex II. Subunits A and B extend into the matrix, they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate. The heme *b* of Complex II is apparently not in the direct path of electron transfer; it may serve instead to reduce the frequency with which electrons "leak" out of the system, moving from succinate to molecular oxygen to produce the **reactive oxygen species (ROS)** hydrogen peroxide (H_2O_2) and the **superoxide radical.** Humans with point mutations in Complex II subunits near heme *b* or the quinone-binding site suffer from hereditary paraganglioma.



Complex II (Succinate dehydrogenase)

Complex III: Ubiquinone to Cytochrome c

Complex III, also called **cytochrome bc_1 complex** or **ubiquinone:cytochrome *c* oxidoreductase.** It couples the transfer of electrons from ubiquinol (QH_2) to cytochrome *c* with the vectorial transport of protons from the matrix to the intermembrane space. Cytochrome *c* is a soluble protein of the intermembrane space. After its single heme accepts an electron from Complex III, cytochrome *c* moves to Complex IV to donate the electron to a binuclear copper center.



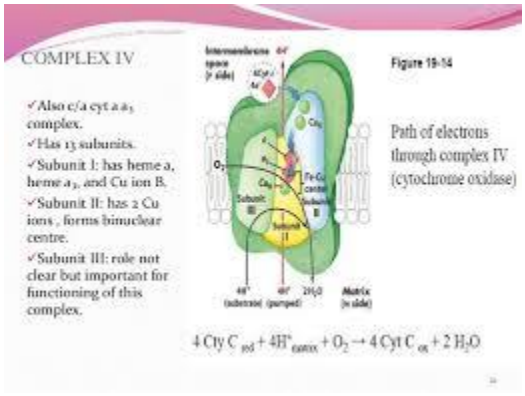
Complex IV: Cytochrome c to O_2

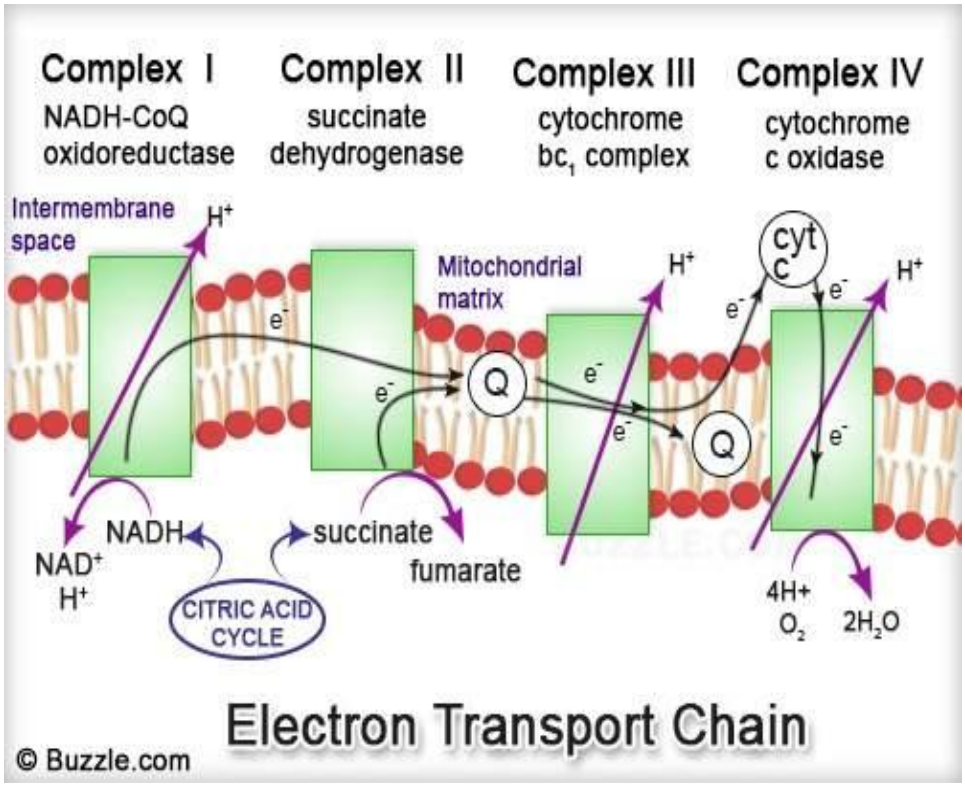
In the final step of the respiratory chain, **Complex IV,** also called **cytochrome oxidase,** carries electrons from cytochrome *c* to molecular oxygen, reducing it to H_2O. Complex IV is a large enzyme (13 subunits; *M*r 204,000) of the inner mitochondrial membrane.

Electron transfer through Complex IV is from cytochrome *c* to the Cu_A center, to heme *a*, to the heme a_3–Cu_B center, and finally to O_2.

The overall reaction catalyzed by Complex IV is:

$$4\ \text{Cyt}\ c\ (\text{reduced}) + 8H^+_N + O_2 \longrightarrow 4\text{cyt}\ c\ (\text{oxidized}) + 4H^+_P + 2H_2O$$




Complex I
NADH-CoQ
oxidoreductase
Complex II
succinate
dehydrogenase
Complex III
cytochrome
bc1 complex
Complex IV
cytochrome
c oxidase
Intermembrane
space
H+
Mitochondrial
matrix
cyt
c
e-
Q
NADH
NAD+
H+
succinate
fumarate
CITRIC ACID
CYCLE
4H+
O2
2H2O
Electron Transport Chain
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2. Regulation of Oxidative Phosphorylation

■ Oxidative phosphorylation is regulated by cellular energy demands. The intracellular [ADP] and the mass-action ratio [ATP]/([ADP][Pi]) are measures of a cell's energy status.

■ In ischemic (oxygen-deprived) cells, a protein inhibitor blocks ATP hydrolysis by the ATP synthase operating in reverse, preventing a drastic drop in [ATP].

■ In brown fat, which is specialized for the production of metabolic heat, electron transfer is uncoupled from ATP synthesis and the energy of fatty acid oxidation is dissipated as heat.

■ ATP and ADP concentrations set the rate of electron transfer through the respiratory chain via a series of nterlocking controls on

Pentose Phosphate Pathway

Also called (phosphogluconate pathway/
hexose monophosphate pathway)

In most animal tissues, glucose is catabolized via the glycolytic pathway into two molecules of pyruvate. Pyruvate is then oxidized via the citric acid cycle to generate ATP. There is another metabolic fate for glucose used to generate NADPH and specialized products needed by the cell. This pathway is called the pentose phosphate pathway. The pentose phosphate pathway produces NADPH which is the universal reductant in anabolic pathways. The second function of the pentose phosphate pathway is to generate pentoses,particularly ribose which is necessary for the synthesis of nucleic acids.

- It is convenient to think of the pentose phosphate pathway (Fig. 1) as operating in two phases.

The first phase is the oxidative phase.

The second phase is the nonoxidative phase.

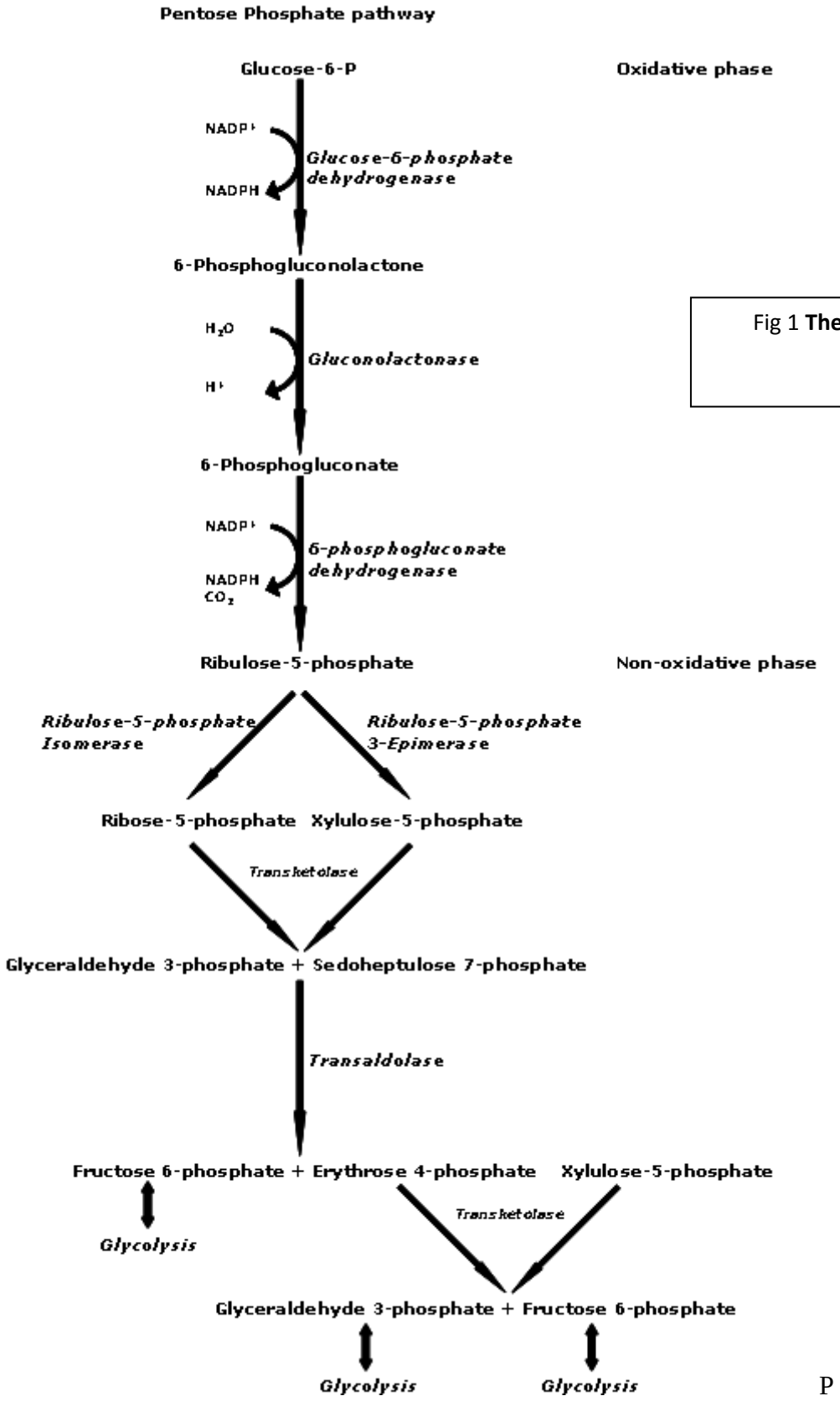


Fig 1 **The Pentose Phosphate pathway**

P

1. The Oxidative Phase Produces Pentose Phosphates and NADPH

In first reaction of the pentose phosphate pathway **(Fig. 1)** is the oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase (G6PD)** to form 6-phosphoglucono-d-lactone, an intramolecular ester. The lactone is hydrolyzed to the free acid 6-phosphogluconate by a specific **lactonase**, then 6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase** to form the ketopentose ribulose 5-phosphate; the reaction generates a second molecule of NADPH. **Phosphopentose isomerase** converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate.

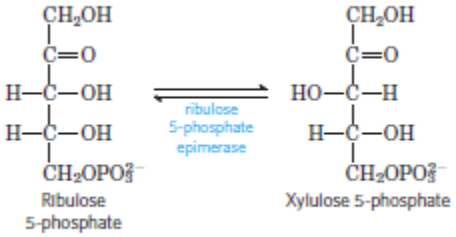
The overall reaction for this process is:

$$\text{Glucose 6-phosphate} + 2\ NADP^+ + H_2O \rightarrow \text{ribulose 5-phosphate} + 2\ NADPH + 2\ H^+ + CO_2$$

The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.

2. The Nonoxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate

In tissues that require primarily NADPH, the pentose phosphates produced in the oxidative phase of the pathway are recycled into glucose 6-phosphate. In this nonoxidative phase, ribulose 5-phosphate is first epimerized to xylulose 5-phosphate:



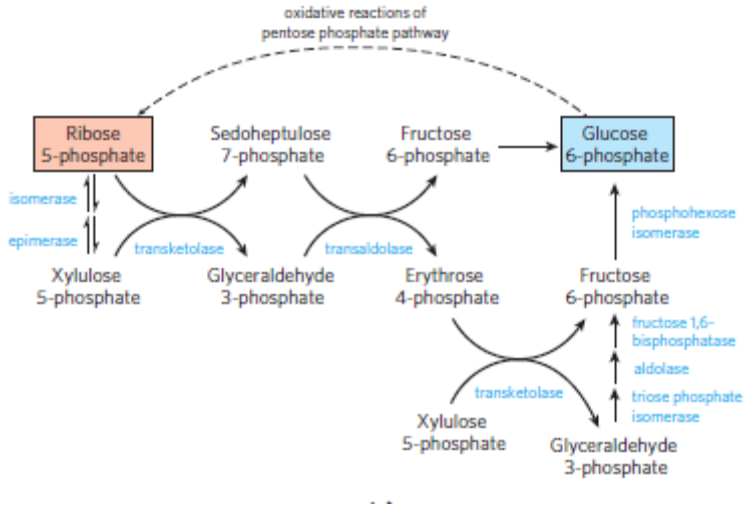


FIGURE 2 Non-oxidative reactions of the pentose phosphate pathway.

Then, in a series of rearrangements of the carbon skeletons **(Fig. 2)**, six five-carbon sugar phosphates are converted to five six-carbon sugar phosphates, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH. Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO2. Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars: transketolase and transaldolase.

Reactants	Products	Enzymes
ribulose 5-phosphate	→ ribose 5-phosphate	Ribulose 5-Phosphate Isomerase
ribulose 5-phosphate	→ xylulose 5-phosphate	Ribulose 5-Phosphate 3-Epimerase
xylulose 5-phosphate + ribose 5-phosphate	→ glyceraldehyde 3-phosphate + sedoheptulose 7-phosphate	transketolase
sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate	→ erythrose 4-phosphate + fructose 6-phosphate	transaldolase
xylulose 5-phosphate + erythrose 4-phosphate	→ glyceraldehyde 3-phosphate + fructose 6-phosphate	transketolase

Regulation

The first step of the phosphopentose pathway is the irreversible committed step. This reaction is catalyzed by glucose-6-phosphate dehydrogenase. This step is of course allosterically regulated. The product of this reaction NADPH is a strong inhibitor. So when the cytosol concentration of NADPH is high, the enzyme's activity is low. It is also allosterically regulated by fatty acid acyl esters of coenzyme A. The transcription of the gene for this enzyme is under hormonal regulation.

B.SC-II SEMESTER

UNIT-IV: PROTEIN AND NUCLEIC ACID METABOLISM

1. Digestion and Absorption of protein in gastrointestinal tract.

Protein is a large, complex molecule that must undergo a series of processes during digestion. During digestion and absorption, protein passes through many organs. Once protein is digested, the body can utilize its nutrients to build and repair many of the cells in the body. The body also uses the calories from protein which are released during the digestion process for energy when carbohydrates and fats are not available.

a. **Protein Digestion in the Stomach:** Once protein is chewed and swallowed, hydrochloric acid and pepsin begin protein digestion in the stomach. HCl helps to kill bacteria in food that could cause infection. It also makes the stomach very acidic with a pH of 1.5. This acidic environment is necessary for HCl to react with pepsinogen to form pepsin so that it can break the central peptide bond in proteins. Rennin is an enzyme that is present in infants to help break down milk protein. Food is mixed with fluid secreted by the stomach lining to produce chime and circular, longitudinal and oblique muscles distort and distent the stomach in regular waves of contraction to mix the chyme. While as the cardiac sphincter prevents chyme going back up the esophagus (acid reflux). The mixture of enzymes, water and other exocrine secretions make up the acidic gastric juice (about 2 liters/day).

I-Digestion in the stomach

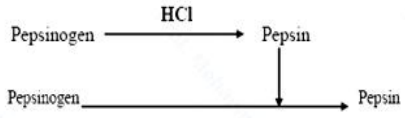
Protein digestion begins in the stomach by gastric juice.

1- Role of gastric HCl

- It causes denaturation of proteins.
- It converts proteins to metaproteins, which are easily digested.
- It activates pepsinogen to pepsin.
- It makes pH in the stomach suitable for the action of pepsin.

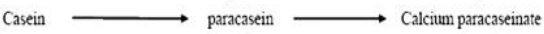
2- Pepsin

- It is an endopeptidase acting on central peptide bond in which amino group belongs to aromatic amino acids e.g. phenyl alanine, tyrosine and tryptophan.
- It is secreted in an inactive form called pepsinogen.
- Its optimum pH: 1.5-2.2
- It is activated by HCl then by autoactivation.



3- Rennin

- It is a milk-clotting enzyme.
- It is present in stomachs of infants and young animals.
- Its optimum pH: 4
- It acts on casein converting it to soluble paracasein, which in turn binds calcium ions forming insoluble calcium paracaseinate. Calcium paracaseinate is then digested by pepsin.



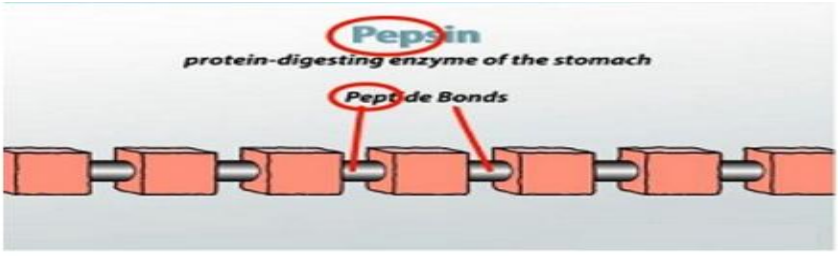
4- Gelatinase

It is an enzyme that liquefies gelatin.
The end products of protein digestion in the stomach are proteoses, peptones and large polypeptides.

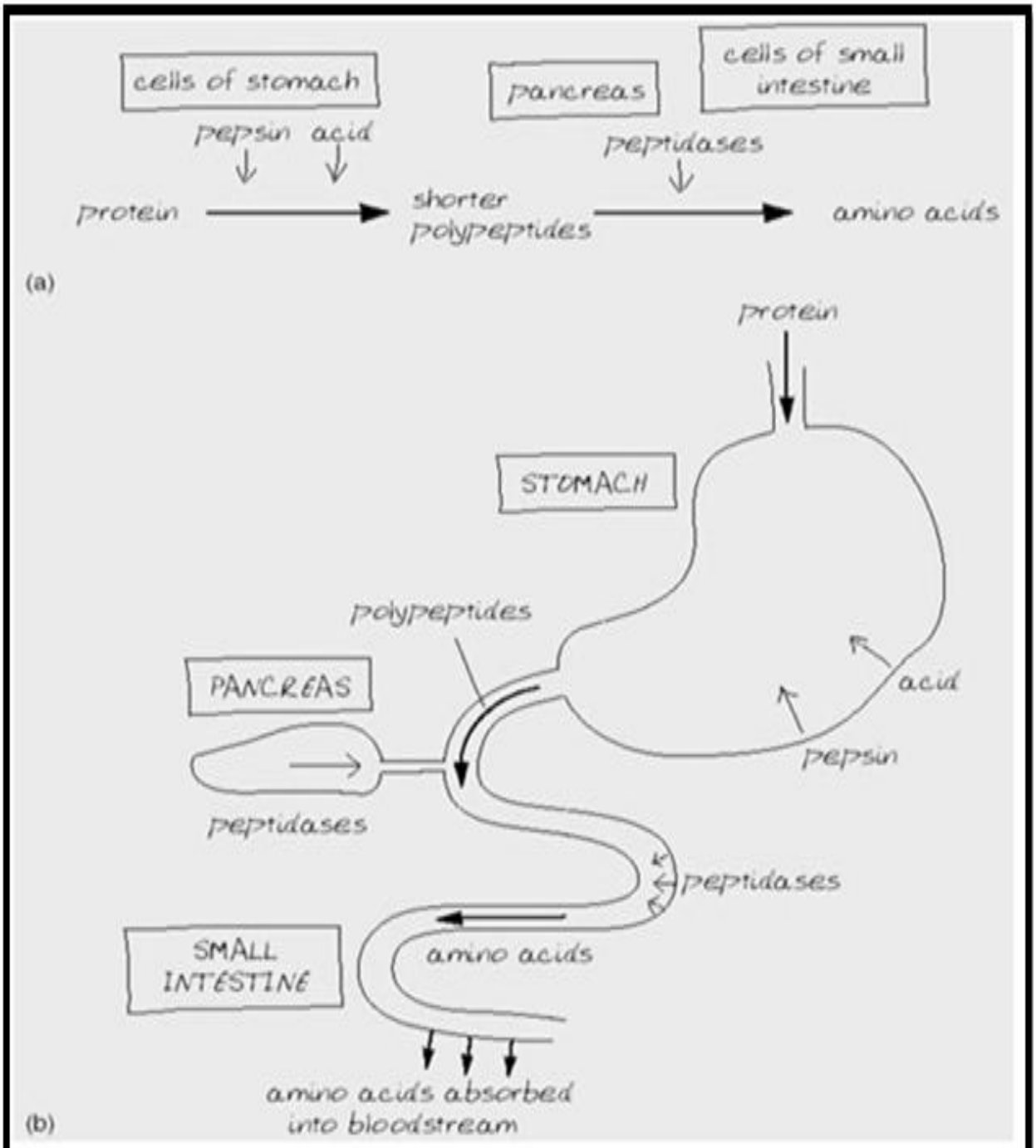
b. **Protein Digestion in the Small Intestine:** Chyme is released into the duodenum in small amounts through the pyloric sphincter.The pancreas releases digestive enzymes (Trypsinogen, chymotrypsinogen and procarboxypeptidase) into the small intestine which are secreted by acini. In the duodenum, the first section of the small intestine, trypsin breaks down proteins into single amino acids by a process called hydrolysis. During hydrolysis, a water molecule is placed between two amino acids, breaking the bond. Trypsin also activates the enzymes chymotrypsin, carboxypeptidase and elastase that are released into the small intestine for amino acid chain breakdown.

c. **Protein Absorption**: Protein absorption takes place in the jejunum and ileum portions of the small intestine. This process requires energy. Adenosine triphosphate is the energy source the body utilizes during protein absorption. The body uses the carrier protein transport system to absorb amino acids. Each amino acid group has a carrier protein that is responsible for transporting it from the intestines to the mucosa cells. Sodium and potassium are minerals needed for the amino acids to pass from the intestines through the villi and into the bloodstream.

d. **Protein Metabolism and Synthesis**: The single molecule amino acids, or free amino acids, that are absorbed through the wall of the small intestine are now used for the last part of protein metabolism, protein synthesis. The proteins ingested from animal and plant protein sources are made into new tissues or used for tissue repairs in the body (hair, skin, nails, muscle) or they are broken down and used for energy. When there is too much protein in the body, the excess gets
e.converted into fat for storage. Amino acids are not just the structural components of proteins. Each one of the 20 naturally occurring amino acids undergoes its own metabolism and performs specific functions. Some of the amino acids also serve as precursors for the synthesis of many biologically important compounds such as melanin,s erotoninc, reat inee tc.). Certain amino acids may directly act as neurotransmitters (e.g. glycine aspartate, glutamate). Protein metabolism is more appropriately learnt as metabolism of amino acids.



Pepsin is an enzyme in the stomach that breaks down the peptide bonds in protein.



Two possible ways of summarising information on protein digestion: (a) a flow chart, and (b) a diagram

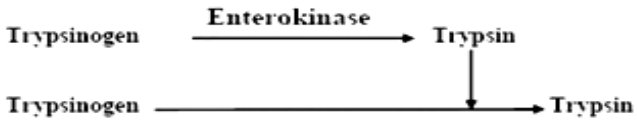
Digestion in the small intestine

Digestion of proteins is completed in the small intestine by proteolytic enzymes present in pancreatic and intestinal juices.

A. Pancreatic Juice

1- Trypsin

- It is an endopeptidase that hydrolyzes central peptide bond in which the carboxyl group belongs to basic amino acids e.g. arginine, lysine and histidine.
- It is secreted in an inactive form called trypsinogen.
- Its optimum pH: 8
- It is activated by enterokinase enzyme then by autoactivation.



2- Chymotrypsin

- It is an endopeptidase that hydrolyzes central peptide bond in which the carboxyl group belongs to aromatic amino acids.
- It is secreted in an inactive form called chymotrypsinogen.
- It is activated by trypsin.
- Its optimum pH: 8

3- Elastase

- It is an endopeptidase acting on peptide bonds formed by glycine, alanine and serine.
- It is secreted in an inactive form called proelatase.
- It is activated by trypsin.
- It digests elastin and collagen.
- Its optimum pH: 8

4- Carboxypeptidase

- It is an exopeptidase that hydrolyzes the terminal (peripheral) peptide bond at the carboxyl terminus (end) of the polypeptide chain.
- It is secreted in an inactive form called procarboxypeptidase.
- It is activated by trypsin.
- Its optimum pH: 7.4

B. Intestinal Juice

1- Aminopeptidase

- It is an exopeptidase that acts on the terminal peptide bond at the amino terminus of the polypeptide chain.
- It releases a single amino acid

2- Tripeptidase

- It acts on tripeptides
- It releases a single amino acid and dipeptide

3- Dipeptidase

- It acts on dipeptides
- It releases 2 amino acids

The end products of protein digestion in the small intestine are amino acids

Mechanisms of amino acids absorption

There are two mechanisms for amino acids absorption.
1- Carrier proteins transport system
2- Glutathione transport system (γ Glutamyl cycle)

1- Carrier proteins transport system

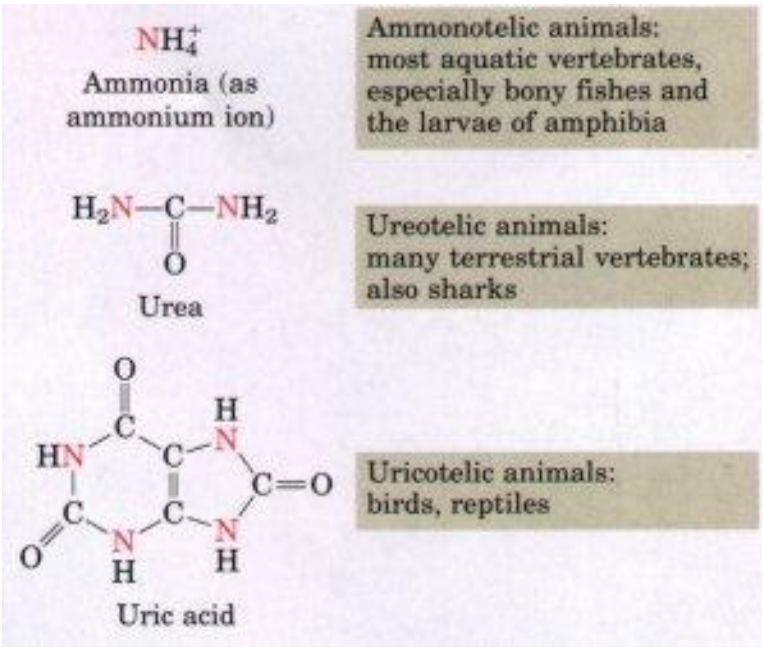
- It is the main system for amino acid absorption.
- It is an active process that needs energy.
- The energy needed id derived from ATP.
- Absorption of one amino acid molecule needs one ATP molecule.
- There are 7 carrier proteins, one for each group of amino acids.
- Each carrier protein has to sites one for amino acid and one for Na^+.
- It co-transports amino acid and Na^+ from intestinal lumen to cytosol of intestinal mucosa cells.
- The absorbed amino acid passes to the portal circulation, while Na^+ is extruded out of the cell in exchange with K^+ by sodium pump.

2- Glutathione transport system (γ Glutamyl cycle)

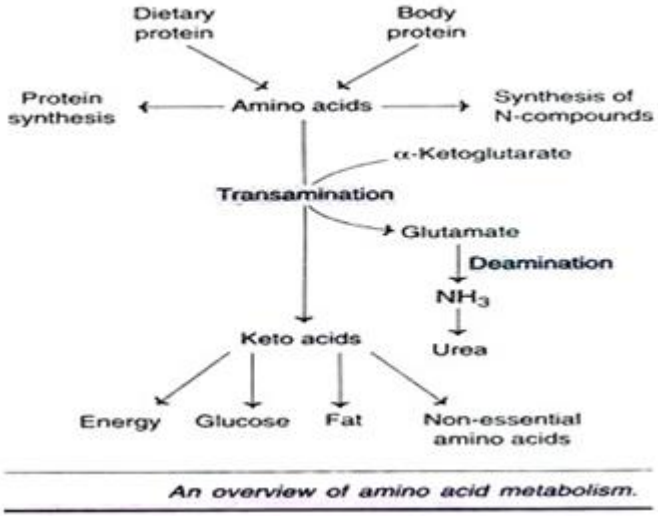
- Glutathione is used to transport amino acids from intestinal lumen to cytosol of intestinal mucosa cells.
- It is an active process that needs energy.
- The energy needed id derived from ATP.
- Absorption of one amino acid molecule needs 3 ATP molecules.
- Glutathione reacts with amino acid in the presence of glutamyl transpeptidase to form glutamyl amino acid.
- glutamyl amino acid releases amino acid in the cytosol of intestinal mucosa cells with formation of 5-oxoproline that is used for regeneration of glutathione to begin another turn of the cycle.

Metabolism of amino acids

The alpha-amino groups of the 20 L-amino acids commonly found in proteins are removed during the oxidative degradation of the amino acids. If not reused for synthesis of new amino acids or other nitrogenous products, these amino groups are channeled into a single excretory end product Many aquatic organisms simply release ammonia as NH_4^+ into the surrounding medium. Most terrestrial vertebrates first convert the ammonia into urea (humans, other mammals, and adult amphibians) or uric acid (birds, reptiles).



Excretory forms of amino group nitrogen in different forms of life.

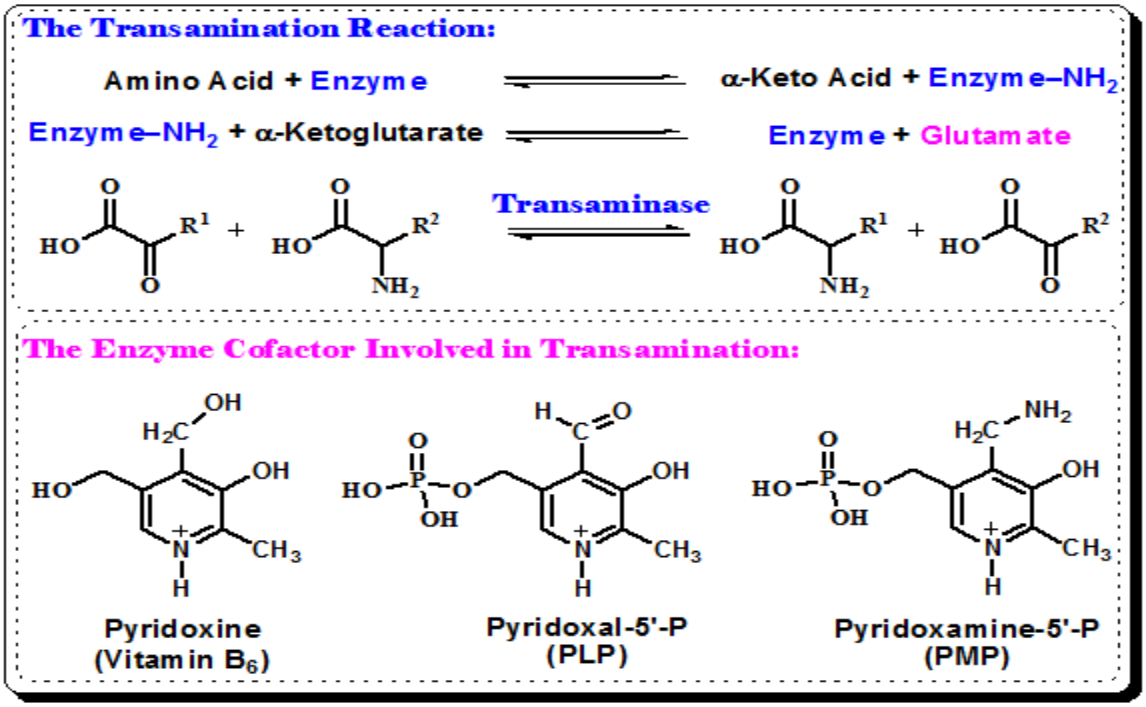


An overview of amino acid metabolism.

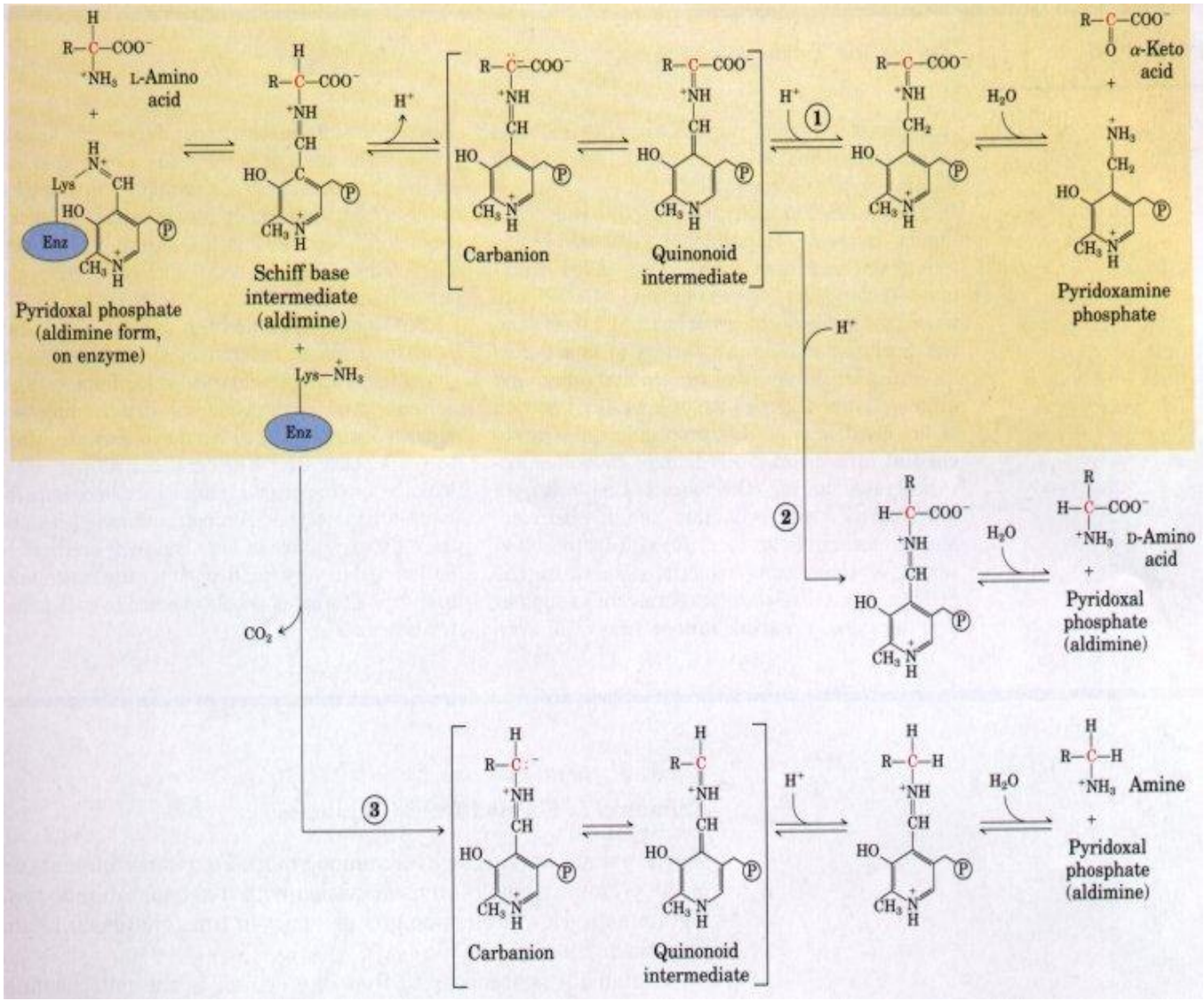
TRANSAMINATION REACTION:

- The transfer of an amino ($-NH_2$) group from an amino acid to a keto acid is known as transamination reaction.
- This process involves the interconversion of a pair of amino acids and a pair of keto acids catalysed by a group of enzymes called Transaminases or also called aminotransferases.
- All transaminases require pyridoxal phosphate (PLP) as conenzyme.

MECHANISM OF TRANSAMINATION REACTION:

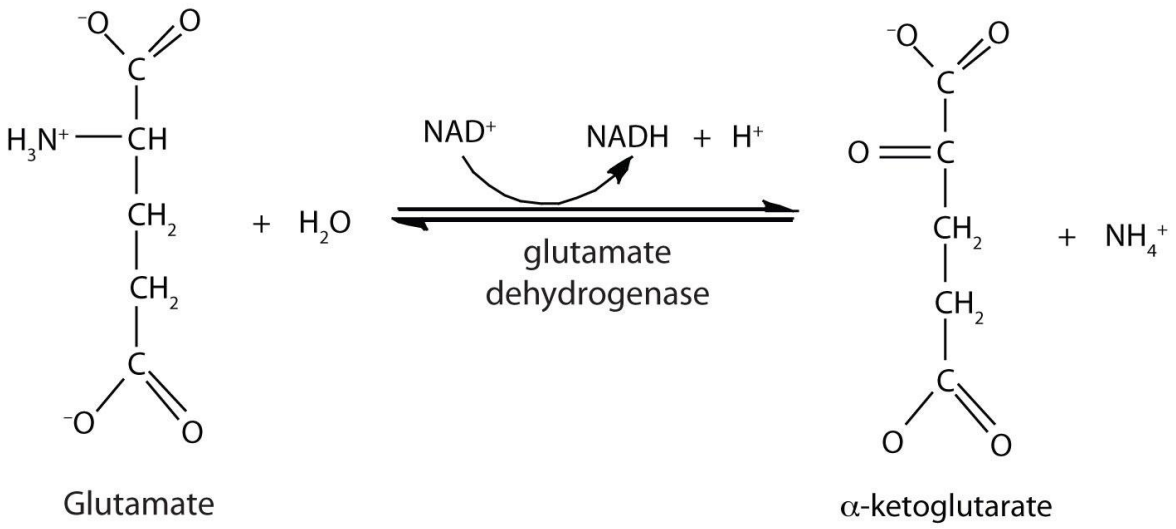


- All aminotransferases share a common prosthetic group and a common reaction mechanism.
- The prosthetic group is **pyridoxal phosphate** (PLP), the coenzyme form of pyridoxine or vitamin B_6.
- Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of arninotransferases.
- It undergoes reversible transformations between its aldehyde form, pyridoxal phosphate, which can accept an amino group, and its aminated form, pyridoxamine phosphate, which can donate its amino group to an α-keto acid
- Pyridoxal phosphate is generally bound covalently to the enzyme's active site through an imine (Schiff base) linkage to the ε-amino group of a Lys residue .



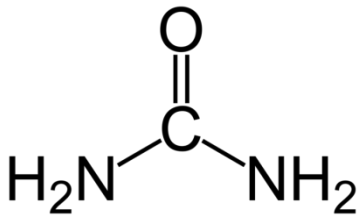
DEAMINATION REACTION

- **Deamination is the removal of** amine group from a molecule. Enzymes that catalyse this reaction are called **deaminases**.
- In the human body, deamination takes place primarily in the liver, however glutamate is also deaminated in the kidneys. In situations of excess protein intake, deamination is used to break down amino acids for energy.
- The amino group is removed from the amino acid and converted to ammonia. The rest of the amino acid is made up of mostlycarbon and hydrogen, and is recycled or oxidized for energy
- Ammonia is toxic to the human system, and enzymes convert it to urea or uric acid by addition of carbon dioxidemolecules (which is not considered a deamination process) in the urea cycle, which also takes place in the liver.
- Urea and uric acid can safely diffuse into the blood and then be excreted in urine.



UREA CYCLE

- The **urea cycle** (also known as the **ornithine cycle**) is a cycle of biochemical reactions occurring in many animals that produces urea ($(NH_2)_2CO$) from ammonia (NH_3).
- In mammals, the urea cycle takes place primarily in the liver, and to a lesser extent in the kidney.
- The urea cycle consists of five reactions: first two reactions taking place in mitochondriaand rest three in cytosol.
- The cycle converts two amino groups, one from NH_4^+ and one from Asp, and a carbon atom from HCO_3^-, to the relatively nontoxic excretion product urea utilising four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP).



STRUCTURE OF UREA

Enzymes involved in urea cycle:

L-Asp: L-aspartate

CPS-1: Carbamoyl phosphate synthetase I

OTC: Ornithine transcarbamoylase

ASS: vargininosuccinatesynthetase

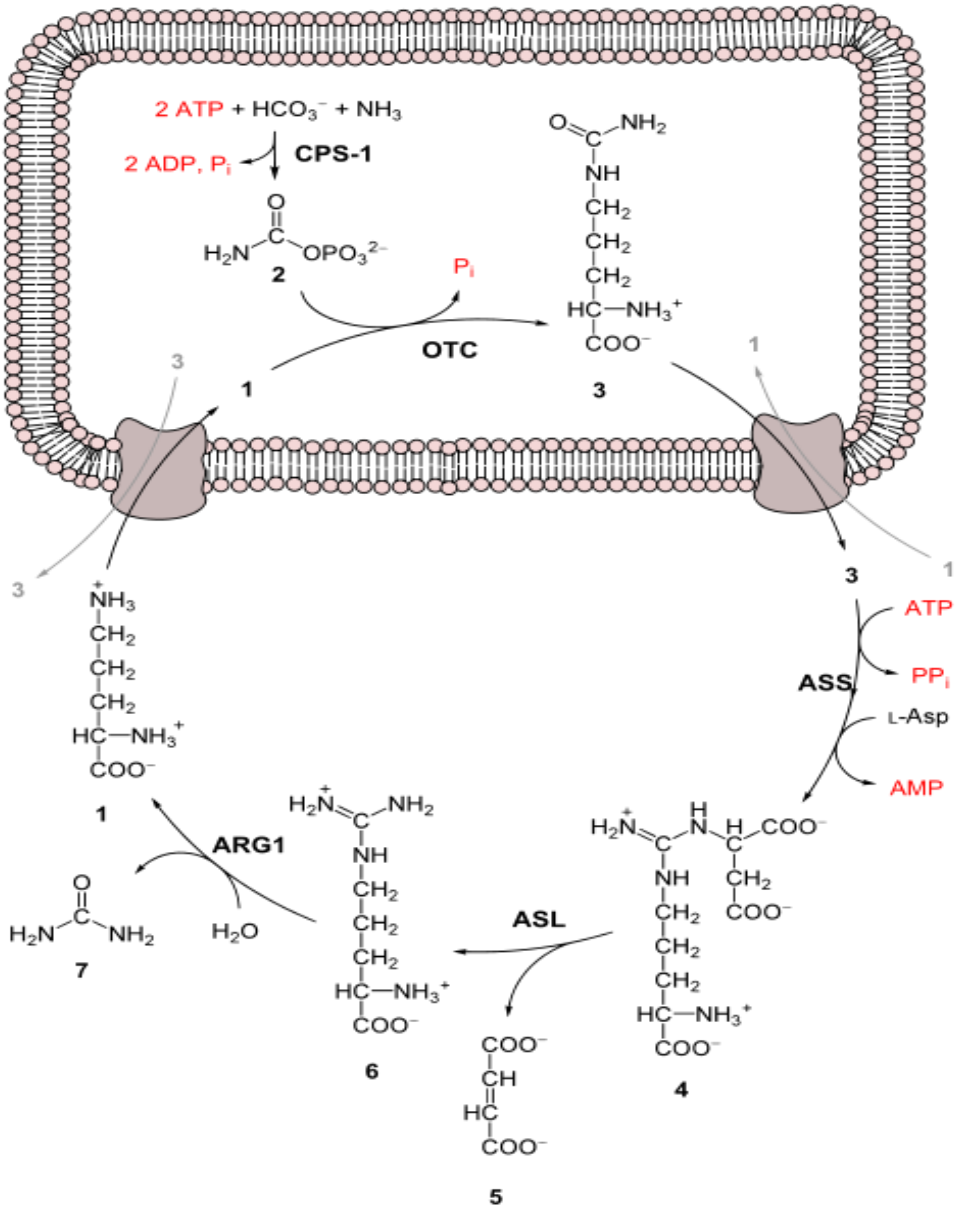
ASL: argininosuccinatelyase

ARG1: Arginase 1

UREA CYCLE

1. L-ornithine2. carbamoyl phosphate 3. L-citrulline

4. argininosuccinate 5. fumarate 6. L-arginine 7. urea



Reactions of the urea cycle				
Step	**Reactants**	**Products**	**Catalyzed by**	**Location**
1	$NH_3 + HCO_3^- + 2ATP$	carbamoyl phosphate + 2ADP + P_i	CPS1	mitochondria
2	carbamoyl phosphate + ornithine	citrulline + P_i	OTC	mitochondria
3	citrulline + aspartate + ATP	argininosuccinate + AMP + PP_i	ASS	cytosol
4	argininosuccinate	Arg + fumarate	ASL	cytosol
5	Arg + H_2O	ornithine + urea	ARG1	cytosol

Overall equation of the urea cycle is:

NH3 + CO2 + aspartate + 3 ATP + 2 H2O → urea + fumarate + 2 ADP + 2 Pi + AMP + PPi

REGULATION OF UREA CYCLE

1. **Carbamoyl phosphate synthase I** is rate limiting reaction or committed step in urea synthesis. N-Acetylglutamic acid. The synthesis of carbamoyl phosphate and the urea cycle are dependent on the presence of NAcGlu, which allosterically activates CPS1. NAcGlu is an obligate activator of Carbamoyl phosphate synthase. Synthesis of NAcGlu by NAGS (N-acetylglutamate synthase) is stimulated by both Arg, allosteric stimulator of NAGS, and Glu, a product in the transamination reactions and one of NAGS's substrates, both of which elevated when free amino acids are elevated. So Glu not only is a substrate for NAGS but also serves as an activator for the urea cycle.

2. **Substrate concentrations** -The remaining enzymes of the cycle are controlled by the concentrations of their substrates. Thus, inherited deficiencies in cycle enzymes other than ARG1 do not result in significant decreases in urea production (if any cycle enzyme is entirely missing, death occurs shortly after birth). Rather, the deficient enzyme's substrate builds up, increasing the rate of the deficient reaction to normal.

Difference between deamination and transamination

Transamination	Oxidative deamination
It is the transfer of amino group from an AA to a keto acid with simultaneous production of a corresponding keto acid & AA respectively.	An oxidation (dehydrogenation) process, where an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia .
Reactions are catalyzed by aminotransferases /transaminases. It needs pyridoxal PO4 as co-enzyme.	It is catalyzed by L-AA oxidase / D-AA oxidase /Glu DH.

AMINO ACID METABOLISM DISORDERS

Table 67.2 Inborn errors of amino acid metabolism

Disorder	*Metabolic defect (enzyme/other)*
I. Phenylalanine and tyrosine	
1. Phenylketonuria	Phenylalanine hydroxylase
2. Tyrosinemia type II	Tyrosine transaminase
3. Neonatal tyrosinemia	p-Hydroxy phenylpyruvate dioxygenase
4. Alkaptonuria	Homogentisate oxidase
5. Tyrosinosis (tyrosinemia type I)	Maleyl acetoacetate isomerase or fumaryl acetoacetate hydrolase
6. Albinism	Tyrosinase
II. Sulfur amino acids (methionine, cysteine and cystine)	
7. Cystinuria	Defect in renal reabsorption
8. Cystinosis	Impairment in cystine utilization (defect in lysosomal function)
9. Homocystinuria type I	Cystathionine synthetase
10. Homocystinuria type II	N^5, N^{10}-Methylene THF reductase
11. Homocystinuria type III	N^5-Methyl THF-homocysteine methyltransferase
12. Cystathionuria	Cystathioninase
III. Glycine	
13. Glycinuria	Defect in renal reabsorption
14. Primary hyperoxaluria	Glycine transaminase
IV. Tryptophan	
15. Hartnup's disease	Defective intestinal absorption
V. Branched chain amino acids (valine, leucine and isoleucine)	
16. Maple syrup urine disease	Branched chain α-keto acid dehydrogenase
17. Intermittent branched chain ketonuria	Variant of the above enzyme (less severe)
18. Hypervalinemia	Valine transaminase
19. Isovaleric acidemia	Isovaleryl CoA dehydrogenase
VI. Histidine	
20. Histidinemia	Histidase
VII. Proline	
21. Hyperprolinemia type I	Proline oxidase

Nucleotide Biosynthesis

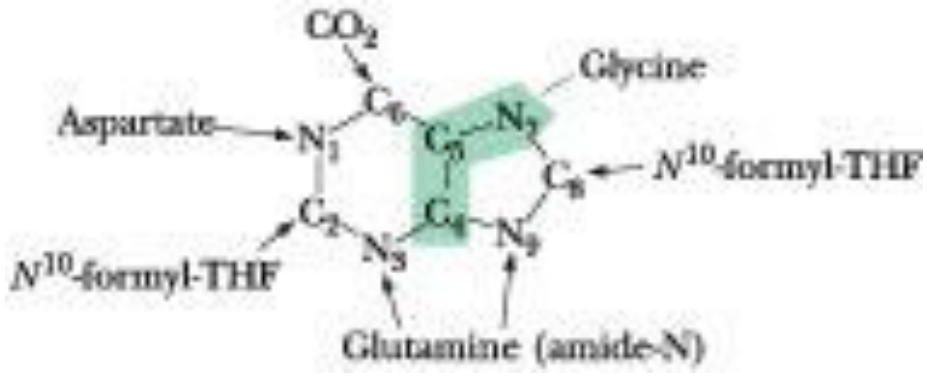
- Nucleotides are ubiquitous constituents of life, actively participating in the majority of biochemical reactions. Recall that ATP is the "energy currency" of the cell, that uridine nucleotide derivatives of carbohydrates are common intermediates in cellular transformations of carbohydrates and that biosynthesis of phospholipids proceeds via cytosine nucleotide derivatives.
- GTP serves as the immediate energy source driving the endergonic reactions of protein synthesis. Many of the coenzymes (such as coenzyme A, NAD, NADP, and FAD) are derivatives of nucleotides.
- Nucleotides also act in metabolic regulation, as in the response of key enzymes of intermediary metabolism to the relative concentrations of AMP, ADP, and ATP.
- Cyclic derivatives of purine nucleotides, cAMP and cGMP, have no other role in metabolism than regulation.
- Nucleotides are the monomeric units of nucleic acids. Deoxynucleoside triphosphates (dNTPs) and nucleoside triphosphates (NTPs) serve as the immediate substrates for the biosynthesis of DNA and RNA.
- Nearly all organisms can make the purine and pyrimidine nucleotides via so-called *de novo* biosynthetic pathways. (De novo means "anew"; a less literal but more apt translation might be "from scratch" because *de novo* pathways are metabolic sequences that form complex end products from rather simple precursors.)
- Many organisms also have salvage pathways to recover purine and pyrimidine compounds obtained in the diet or released during nucleic acid turnover and degradation. While the ribose of nucleotides can be catabolized to generate energy, the nitrogenous bases do not serve as energy sources; their catabolism does not lead to products used by pathways of energy conservation. Compared to slowly dividing cells, rapidly proliferating cells synthesize larger amounts of DNA and RNA per unit time. To meet the increased demand for nucleic acid synthesis, substantially greater quantities of nucleotides must be produced. The pathways of nucleotide biosynthesis thus become attractive targets for the clinical control of rapidly dividing cells such as cancers or

infectious bacteria. Many antibiotics and anticancer drugs are inhibitors of purine or pyrimidine nucleotide biosynthesis.

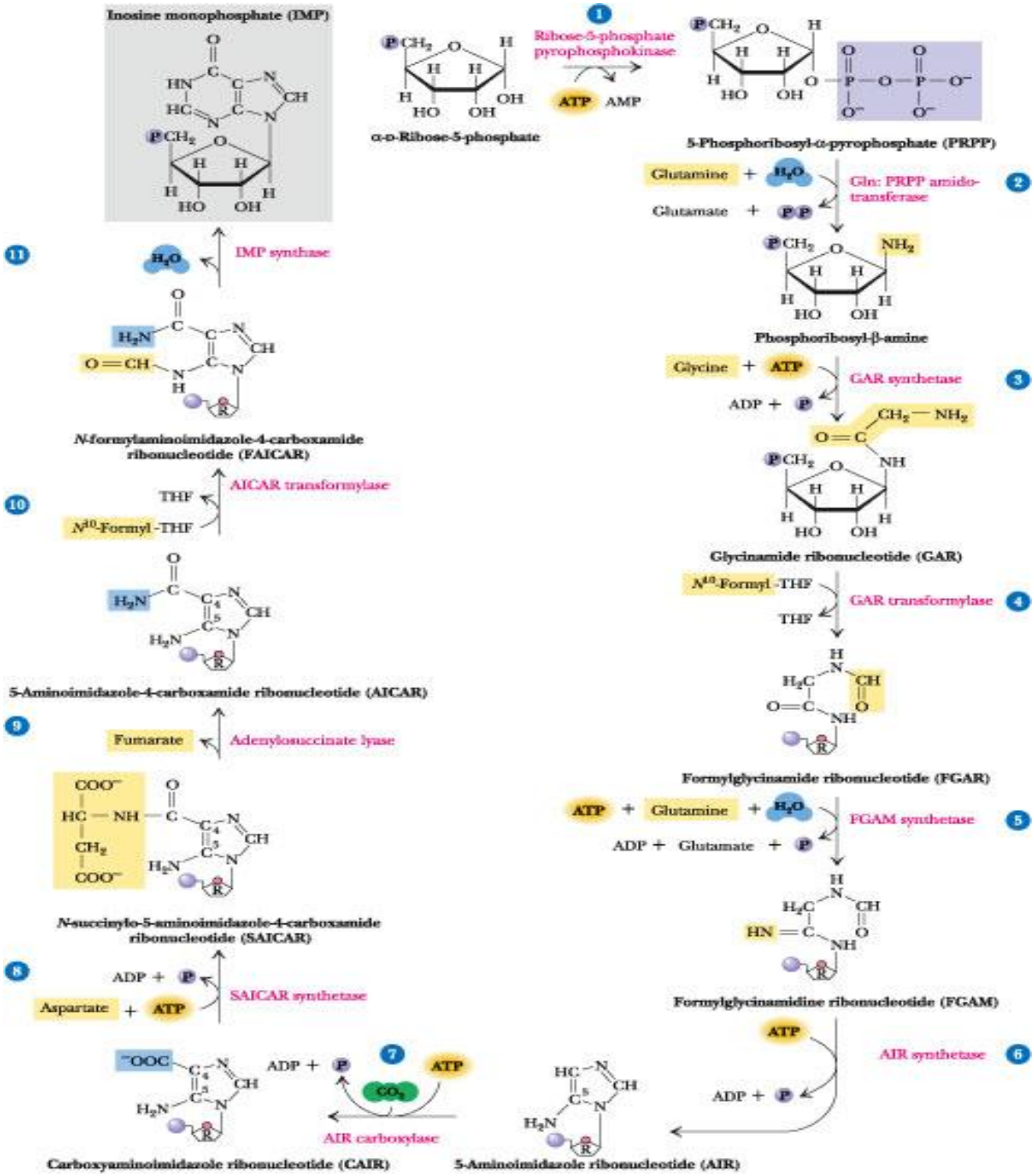
The Biosynthesis of Purines

Substantial insight into the *de novo* pathway for purine biosynthesis was provided in 1948 by John Buchanan, who cleverly exploited the fact that birds excrete excess nitrogen principally in the form of uric acid, a water-insoluble purine analog. Buchanan fed isotopically labeled compounds to pigeons and then examined the distribution of the labeled atoms in *uric acid*. By tracing the metabolic source of the various atoms in this end product, he showed that the nine atoms of the purine ring system are contributed by aspartic acid (N-1), glutamine (N-3 and N-9), glycine (C-4, C-5, and N-7), CO_2 (C-6), and THF one-carbon derivatives (C-2 and C-8).

The metabolic origin of the nine atoms in the purine ring system below:



IMP Biosynthesis: Inosinic Acid Is the Immediate Precursor to GMP and AMP



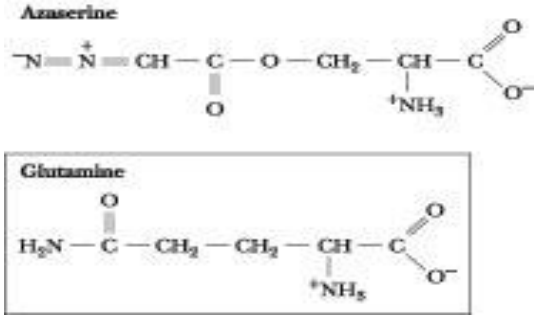
The *de novo* pathway for purine synthesis.

- The first purine product of this pathway, IMP (inosinic acid or inosine monophosphate), serves as a precursor to AMP and GMP.
- ***Step1****:* PRPP synthesis from ribose-5-phosphate and ATP by ribose-5-phosphate pyrophosphokinase.
- ***Step2****:* 5-Phosphoribosyl-b-1-amine synthesis from a-PRPP, glutamine, and H_2O by glutamine phosphoribosyl pyrophosphate amidotransferase.
- ***Step3****:* Glycinamide ribonucleotide (GAR) synthesis from glycine, ATP, and 5-phosphoribosyl-b-amine by glycinamide ribonucleotide synthetase.
- ***Step4****:* Formylglycinamide ribonucleotide synthesis from N^{10}-formyl-THF and GAR by GAR transformylase.
- ***Step5****:* Formylglycinamidine ribonucleotide (FGAM) synthesis from FGAR, ATP, glutamine, and H_2O by FGAM synthetase (FGAR amidotransferase). The other products are ADP, P_i, and glutamate.
- ***Step6****:* 5-Aminoimidazole ribonucleotide (AIR) synthesis is achieved via the ATP-dependent closure of the imidazole ring, as catalyzed by FGAM cyclase (AIR synthetase). (Note that the ring closure changes the numbering system.)
- ***Step7****:* Carboxyaminoimidazole ribonucleotide (CAIR) synthesis from CO_2, ATP, and AIR by AIR carboxylase.
- ***Step8****:* N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR) synthesis from aspartate, CAIR, and ATP by SAICAR synthetase.
- ***Step9****:* 5-Aminoimidazole carboxamide ribonucleotide (AICAR) formation by the nonhydrolytic removal of fumarate from SAICAR. The enzyme is adenylosuccinase.
- ***Step10****:* 5-Formylaminoimidazole carboxamide ribonucleotide (FAICAR) formation from AICAR and N^{10}-formyl-THF by AICAR transformylase.
- ***Step11****:* Dehydration/ring closure yields the authentic purine ribonucleotide IMP. The enzyme is IMP synthase.

The *de novo* synthesis of purines occurs in an interesting manner: The atoms forming the purine ring are successively added to *ribose-5-phosphate*; thus, purines are directly synthesized as nucleotide derivatives by assembling the atoms that comprise the purine ring system directly on the ribose.

Step 1, ribose-5-phosphate is activated via the direct transfer of a pyrophosphoryl group from ATP to C-1 of the ribose, yielding *5-phosphoribosyl-a-pyrophosphate (PRPP)*. The enzyme is ribose-5-phosphate pyrophosphokinase. PRPP is the limiting substance in purine biosynthesis. The two major purine nucleoside diphosphates, ADP and GDP, are negative effectors of ribose-5-phosphate pyrophosphokinase. However, because PRPP serves additional metabolic needs, the next reaction is actually the committed step in the pathway.

Step 2: is catalyzed by glutamine phosphoribosyl pyrophosphate amidotransferase. The anomeric carbon of the substrate PRPP is in the a-configuration; the product is a b-glycoside . The N atom of this *N*-glycoside becomes N-9 of the nine-membered purine ring; it is the first atom added in the construction of this ring. Glutamine phosphoribosyl pyrophosphate amidotransferase is subject to feedback inhibition by GMP and GDP.



The structure of azaserine. Azaserine acts as an irreversible inhibitor of glutamine-dependent enzymes by covalently attaching to nucleophilic groups in the glutamine-binding site.

The G series of nucleotides interacts at a guanine-specific allosteric site on the enzyme, whereas the adenine nucleotides act at an A-specific site. The pattern of inhibition by these nucleotides is competitive, thus ensuring that residual enzyme activity is expressed until sufficient amounts of both adenine and guanine nucleotides are synthesized. Glutamine phosphoribosyl pyrophosphate amidotransferase is also sensitive to inhibition by the glutamine analog *azaserine* . Azaserine has

been employed as an antitumor agent because it causes inactivation of glutamine-dependent enzymes in the purine biosynthetic pathway.

Step 3 is carried out by glycinamide ribonucleotide synthetase (GAR *synthetase*) via its ATP-dependent condensation of the glycine carboxyl group with the amine of *5-phosphoribosyl-b amine*. The reaction proceeds in two stages. First, the glycine carboxyl group is activated via ATP-dependent phosphorylation. Next, an amide bond is formed between the activated carboxyl of glycine and the b-amine. Glycine contributes C-4, C-5, and N-7 of the purine.

Step 4 is the first of two THF-dependent reactions in the purine pathway. GAR transformylase transfers the N^{10}-formyl group of N^{10}-formyl-THF to the free amino group of GAR to yield a-N-formylglycinamide ribonucleotide (FGAR). Thus, C-8 of the purine is "formyl-ly" introduced. Although all of the atoms of the imidazole portion of the purine ring are now present, the ring is not closed until Reaction 6.

Step 5 is catalyzed by FGAR amidotransferase (also known as *FGAM synthetase*). ATP-dependent transfer of the glutamine amido group to the C-4-carbonyl of FGAR yields*formylglycinamidine ribonucleotide (FGAM).* As a glutamine-dependent enzyme, FGAR amidotransferase is, like glutamine phosphoribosyl pyrophosphate amidotransferase (Reaction 2), irreversibly inactivated by azaserine. The imino-N becomes N-3 of the purine.

Step 6 is an ATP-dependent dehydration that leads to formation of the imidazole ring. ATP is used to phosphorylate the oxygen atom of the formyl group, activating it for the ring closure step that follows. Because the product is *5-aminoimidazole ribonucleotide,* or *AIR,* this enzyme is called AIR synthetase. In avian liver, the enzymatic activities for Steps 3, 4, and 6 (GAR synthetase, GAR transformylase, and AIR synthetase) reside on a single, 110-kD multifunctional polypeptide.

In Step 7, carbon dioxide is added at the C-4 position of the imidazole ring by AIR carboxylase in an ATP-dependent reaction; the carbon of CO_2 will become C-6 of the purine ring. The product is *carboxyaminoimidazole ribonucleotide (CAIR).*

In Step 8, the amino-N of aspartate provides N-1 through linkage to the C-6 carboxyl function of CAIR. ATP hydrolysis drives the condensation of Asp with CAIR. The product is *N-succinylo-5-aminoimidazole-4-carboxamide-ribonucleotide-(SAICAR).*

SAICAR synthetase catalyzes the reaction.

The enzymatic activities for Steps 7 and 8 reside on a single, bifunctional polypeptide in avian liver.

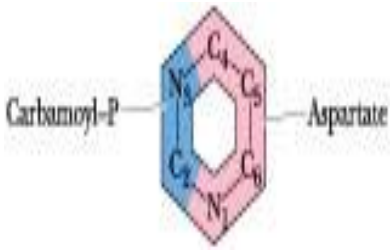
Step 9 removes the four carbons of Asp as fumaric acid in a nonhydrolytic cleavage. The product is *5-aminoimidazole-4-carboxamide ribonucleotide (AICAR);* the enzyme isadenylosuccinase *(adenylosuccinate lyase).* Adenylosuccinase acts again in that part of the purine pathway leading from IMP to AMP and derives its name from this latter activity (see following). AICAR is also an intermediate in the histidine biosynthetic pathway, but because the purine nucleotide ATP is the precursor to AICAR in that pathway, no net purine synthesis is achieved.

Step 10 adds the formyl carbon of N^{10}-formyl-THF as the ninth and last atom necessary for forming the purine nucleus. The enzyme is called AICAR transformylase; the products are THF and *N-formylaminoimidazole-4-carboxamide ribonucleotide,* or *FAICAR.*

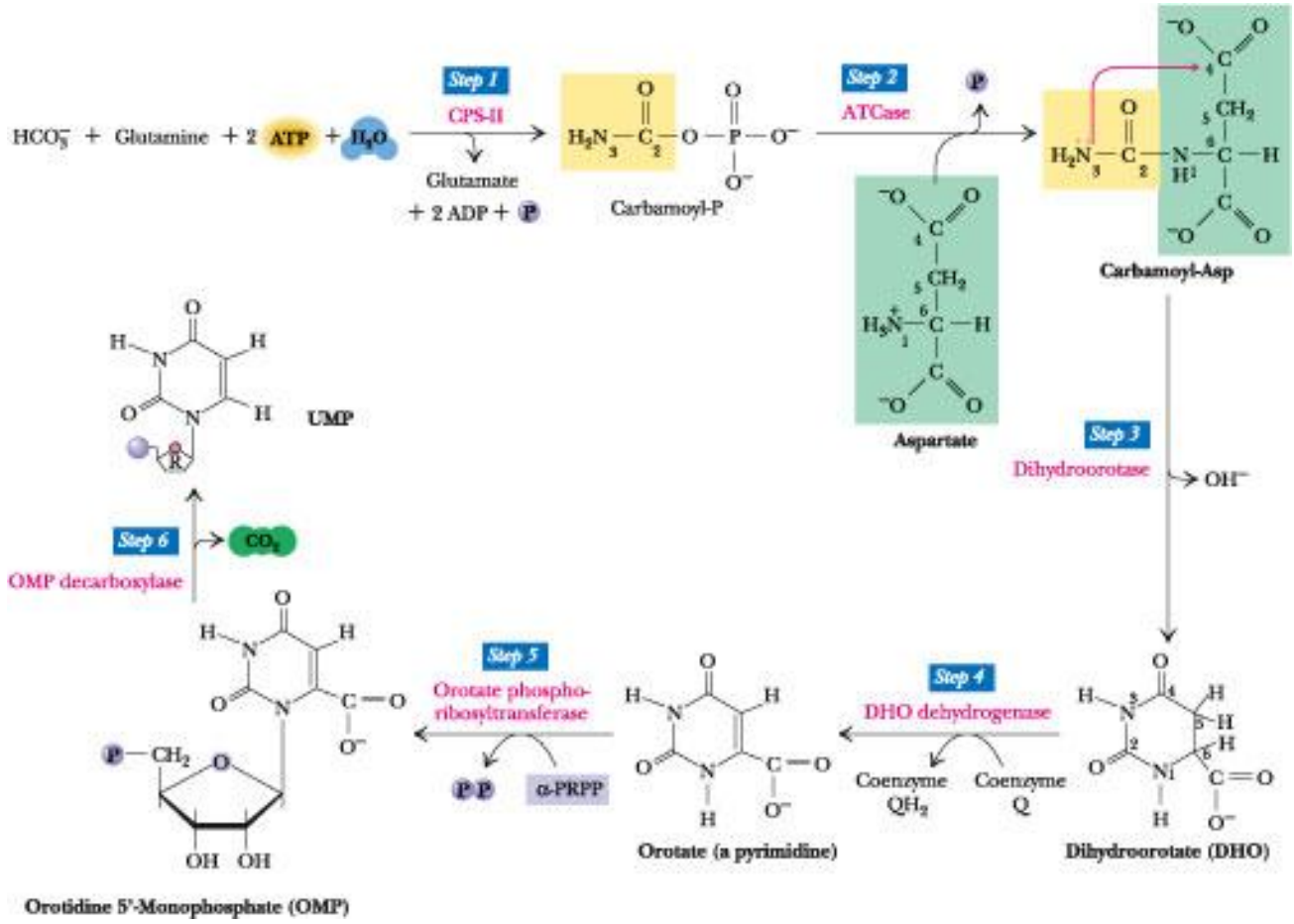
Step 11 involves dehydration and ring closure and completes the initial phase of purine biosynthesis. The enzyme is IMP cyclohydrolase (also known as *IMP synthase* and*inoisinicase*).

The Biosynthesis of Pyrimidines

In contrast to purines, pyrimidines are not synthesized as nucleotide derivatives. Instead, the pyrimidine ring system is completed before a ribose-5-P moiety is attached.



Also, only two precursors, carbamoyl-P and aspartic acid, contribute atoms to the six-membered pyrimidine ring compared to seven precursors for the 9 purine atoms



The *de novo* pyrimidine biosynthetic pathway

Step1*:* Carbamoyl-P synthesis.

Step2*:* Condensation of carbamoyl phosphate and aspartate to yield carbamoyl-aspartate is catalyzed by aspartate transcarbamoylase (ATCase).

Step3*:* An intramolecular condensation catalyzed by dihydroorotase gives the six-membered heterocyclic ring characteristic of pyrimidines. The product is dihydroorotate (DHO).

Step4*:* The oxidation of DHO by dihydroorotate dehydrogenase gives orotate. (In bacteria, NAD^+ is the electron acceptor from DHO.)

Step5*:* PRPP provides the ribose-5-P moiety that transforms orotate into orotidine-5'-monophosphate, a pyrimidine nucleotide. Note that orotate phosphoribosyltransferase joins N-1 of the pyrimidine to the ribosyl group in appropriate b-configuration. PP_i hydrolysis renders this reaction thermodynamically favorable.

Step 6*:* Decarboxylation of OMP by OMP decarboxylase yields UMP.