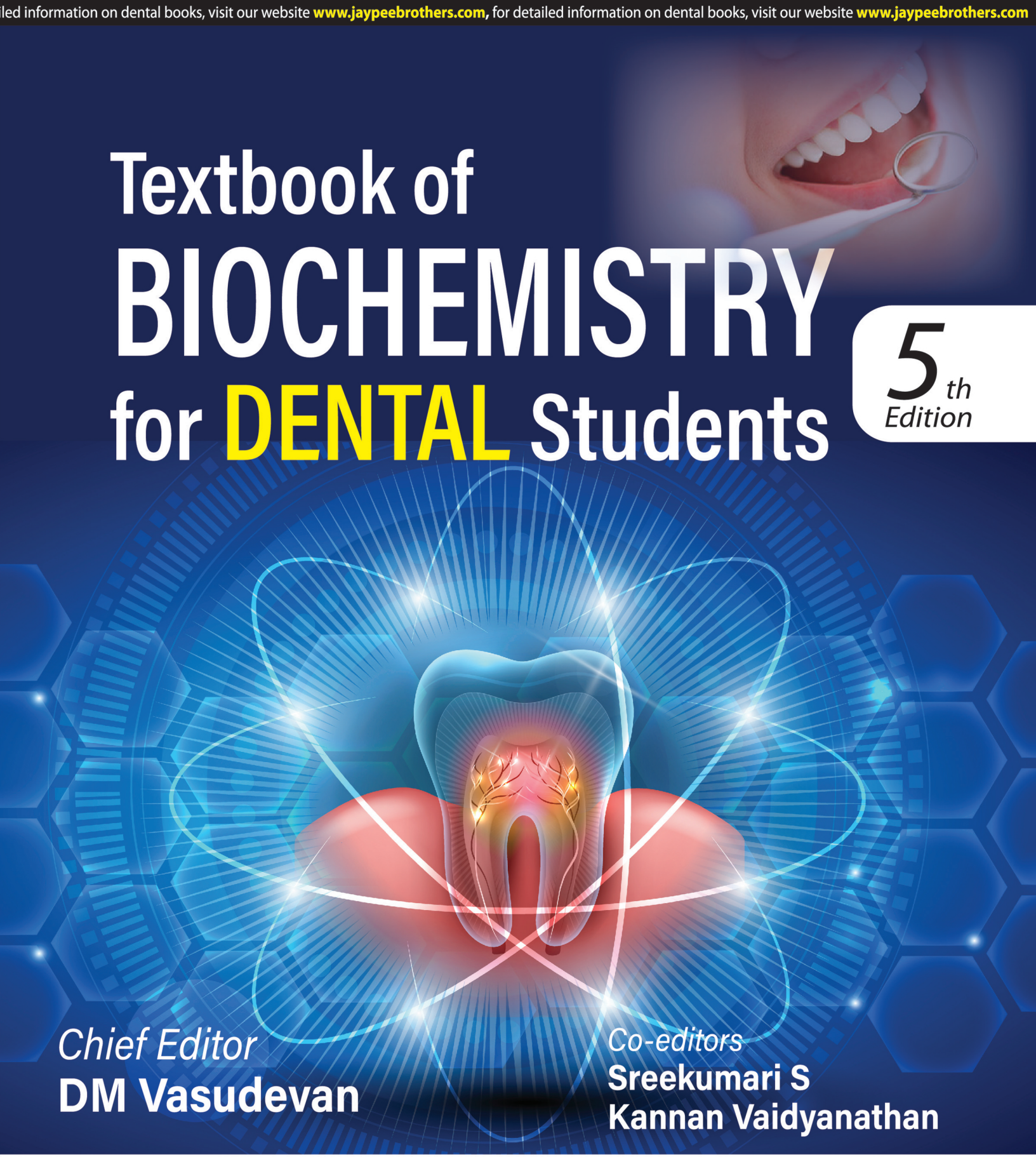
iled information on dental books, visit our website www.jaypeebrothers.com, for detailed information on dental books, visit our website www.jaypeebrothers.com

# Textbook of BIOCHEMISTRY for DENTAL Students

5th Edition



*Chief Editor*
**DM Vasudevan**

*Co-editors*
**Sreekumari S**
**Kannan Vaidyanathan**

*Assistant Editor*
**Sukhes Mukherjee**

**Genuine?**
Scratch, Scan & Validate

JAYPEE

# Contents

## Section 1: General Metabolism

CHAPTER

3

# Enzymology

## LEARNING OBJECTIVES

**The learner will be able to:**

- Enumerate the major classes of enzymes
- List the important coenzymes
- Indicate the theories of enzyme action
- Explain factors influencing enzyme activity
- Briefly describe different types of enzyme inhibition with examples
- Mention the regulation of enzyme activity in biological systems with examples
- Define isoenzymes and cite examples of different types
- Enumerate the clinically important enzymes

Once upon a time there was a rich merchant. In his last will and testament, he put aside his 17 white horses to his 3 sons to be shared thus; 1/2 for the 1st son, 1/3 for the 2nd son and 1/9 for the 3rd son. After his death, the sons started to quarrel, as the division could not produce a whole number. Then their brother-in-law told them that they should include his black horse also for the sharing purpose. Thus now they had 17 + 1 = 18 horses, and so the division was possible; 1st son got one-half or 9 horses; 2nd son got 6 and 3rd son had his 2 horses. Now all the 17 white horses were correctly divided among the sons. The remaining black horse was taken back by the brother-in-law. **Catalysts** are similar to this black horse. The reaction, although theoretically probable, becomes practically possible only with the help of catalysts. They enter into the reaction, but come out of the reaction without any change. Catalysts are substances that accelerate the rate of chemical reactions, but do not change the equilibrium.

**Enzymes are biocatalysts.** Life is possible due to the coordination of numerous metabolic reactions inside the cells. Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long time; but inside the body, with the help of enzymes, proteolysis takes place within a short time at body temperature. Lack of enzymes will lead to block in metabolic pathways causing inborn errors of metabolism.

The substance upon which an enzyme acts, is called the **substrate**. The enzyme will convert the substrate into the **product** or products. **Almost all enzymes are proteins** (recently a few RNA enzymes have been discovered). Enzymes follow the physical and chemical reactions of proteins. They are heat labile, soluble in water, precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid) and contain 16% weight as nitrogen.

## CLASSIFICATION OF ENZYMES

Early workers gave whimsical names such as Pepsin, Trypsin, Chymotrypsin, etc., some of which are still used. Later, enzymes are named by adding the suffix "-ase" to the substrate. Thus, the enzyme lactase acts on the substrate lactose. These are known as the **trivial names** of enzymes. But there may be more than one enzyme acting on the same substrate.

### IUBMB System of Nomenclature of Enzymes

Six enzyme classes have been recognized since the first enzyme classification and nomenclature list was first approved by the International Union of Biochemistry (IUBMB) in 1961 (modified in 1972 and 1978). In the 2018 revision, one more class has been added. Thus the enzymes are now grouped into 7 major classes **(Table 3.1)**. For example, Class 1 is called oxidoreductases. As per this classification system, the *name starts with EC (enzyme class) followed by 4 digits.* The first digit represents the class; the second digit stands for the subclass; the third digit is the sub-subclass; and the 4th digit gives the number of the particular enzyme in the list.

## COENZYMES

Enzymes may be simple proteins, or complex enzymes, containing a nonprotein part, called the **prosthetic group.**

JAYPEE BROTHERS

**TABLE 3.1:** Classification of enzymes.

| Class | Name | Function | Example |
|---|---|---|---|
| Class 1 | **Oxidoreductases** | Transfer of hydrogen | Alcohol dehydrogenase |
| Class 2 | **Transferases** | Transfer of groups other than hydrogen | Subclass: Kinase, transfer of phosphoryl group from ATP, e.g., hexokinase |
| Class 3 | **Hydrolases** | Cleave bond; add water | Acetylcholine esterase |
| Class 4 | **Lyases** | Cleave without adding water | Aldolase (Subclass: Hydratase; add water to double bond) |
| Class 5 | **Isomerases** | Intramolecular transfers | This class includes racemases and epimerases. Example, triose phosphate isomerase |
| Class 6 | **Ligases** | ATP-dependent condensation of two molecules | Acetyl-CoA carboxylase |
| Class 7* | **Translocases** | Catalyze movement of molecules across cell membranes | Carnitine-acylcarnitine translocase |

(* Class 7 was added in 2018).

The protein part of the enzyme is named as the **apoenzyme**, the prosthetic group the **coenzyme**; and these two portions combined together are called the **holoenzyme**. The coenzyme is essential for the biological activity of the enzyme. A coenzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction. One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of an enzyme. Coenzymes may be divided into: (a) Those taking part in reactions catalyzed by oxidoreductases by donating or accepting hydrogen atoms or electrons. (b) Those coenzymes taking part in reactions transferring groups other than hydrogen.

## First Group of Coenzymes

In the first group, the change occurring in the substrate is counter-balanced by the coenzymes. Therefore, such coenzymes may be considered as cosubstrates or secondary substrates. In the example shown in **Figure 3.1**, the substrate lactate is oxidized, and simultaneously the coenzyme (cosubstrate) is reduced. If the reaction is reversed, the opposite effect will take place. Other coenzymes engaged in oxidoreductase reactions are NADP, FAD and FMN.

## Nicotinamide Adenine Dinucleotide ($NAD^+$)

This is a coenzyme synthesized from nicotinamide, a member of the vitamin B complex. The structure of $NAD^+$ could be written as Nicotinamide-Ribose-P-P-Ribose-Adenine **(Fig. 16.5)**. The reversible reaction of lactate to pyruvate is catalyzed by the enzyme lactate dehydrogenase, but the actual transfer of hydrogen is taking place on the coenzyme, $NAD^+$ **(Fig. 3.1)**. Two hydrogen atoms are removed from lactate, out of which one hydrogen and two electrons are accepted by the $NAD^+$ to form NADH, and the remaining $H^+$ is released into the surrounding medium. The hydrogen is accepted by the **nicotinamide** group.

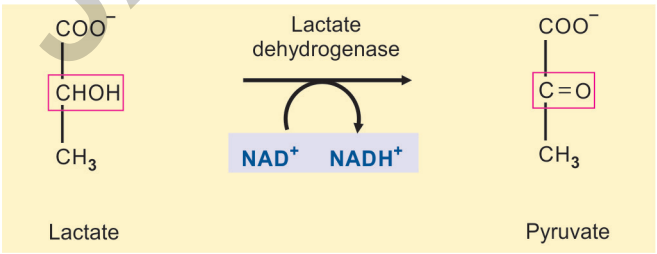



**Fig. 3.1:** Reaction of lactate dehydrogenase.

## Second Group of Coenzymes

Those coenzymes taking part in *reactions transferring groups other than hydrogen*, may be considered as the second category. A particular group or radical is transferred from the substrate to another substrate or from the coenzyme to the substrate. Here also coenzymes may be considered as cosubstrates. Most of them belong to the vitamin B complex group. A few such examples are given in **Table 3.2**.

## Adenosine Triphosphate (ATP)

Fiske and Subba Rao first isolated ATP from the muscle in 1929 and showed the importance of ATP in muscle contraction. ATP is considered to be the **energy currency** in the body. During the oxidation of food stuffs, energy is released, a part of which is stored as **chemical energy** in the form of ATP. The structure of ATP is shown in **Figure 3.2**. In the ATP molecule, the second and third phosphate bonds are '**high energy**' bonds. The endergonic

**TABLE 3.2:** Examples of coenzymes.

| Coenzyme | Group transferred |
|---|---|
| Thiamine pyrophosphate (TPP) | Hydroxy ethyl |
| Pyridoxal phosphate (PLP) | Amino group |
| Biotin | Carbon dioxide |
| Coenzyme-A (Co-A) | Acyl groups |
| Tetra hydrofolate ($FH_4$) | One carbon group |
| Adenosine triphosphate (ATP) | Phosphate |

JAYPEE BROTHERS

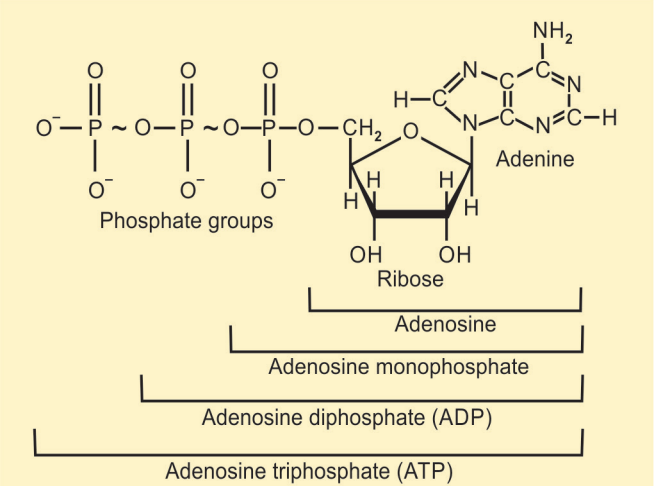



**Fig. 3.2:** Structure of adenosine triphosphate (ATP). Note that the second and third phosphate bonds are "high energy" bonds, as shown in the "squiggle" bonds.

reactions are carried out with the help of energy released from the hydrolysis of ATP. For example, one phosphate group is added to glucose to make it glucose-6-phosphate; this phosphate group is donated by the ATP; and this reaction is catalyzed by hexokinase **(Fig. 3.3)**.

### Salient Features of Coenzymes

- The protein part of the enzyme gives the necessary three-dimensional infrastructure for chemical reaction.
- But the group is transferred from or accepted by the coenzyme.
- The coenzymes are heat stable.
- They are low-molecular weight substances.
- The coenzymes combine loosely with the enzyme molecules and so, the coenzyme can be separated easily by dialysis.

Inside the cell, when the reaction is completed, the coenzyme is released from the apoenzyme, and can bind to another enzyme molecule. In the example shown in **Figure 3.4**, the reduced coenzyme, generated in the first reaction can take part in the second reaction. The coupling of these two reactions becomes essential in anaerobic glycolysis to regenerate $NAD^+$.

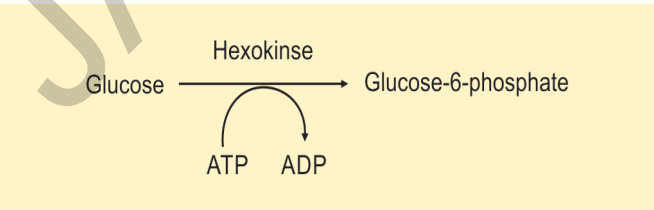



**Fig. 3.3:** Hexokinase reaction. Glucose is phosphorylated to glucose-6-phosphate; this phosphate group is donated by the ATP. Here the energy released from ATP hydrolysis is used for the synthesis of glucose-6-phosphate.

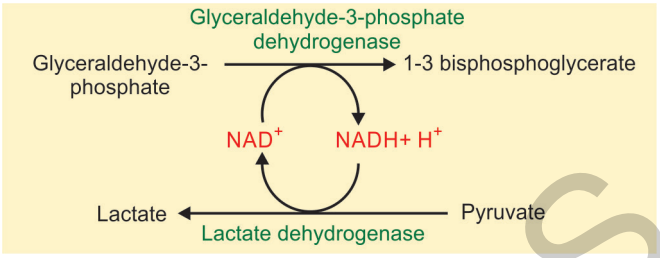



**Fig. 3.4:** One coenzyme molecule can work with different enzymes.

### Metalloenzymes

These are enzymes that require certain metal ions for their activity. Some examples are given in **Table 3.3**. In certain cases, e.g., copper in tyrosinase, the metal is tightly bound with the enzyme. In other cases, when the metal ion is removed from the enzyme, the activity of the enzyme will be minimal; but when the metal ion is added, the activity is enhanced. They are called **ion-activated enzymes,** e.g., magnesium ions will activate kinases.

## MODE OF ACTION OF ENZYMES

There are a few theories explaining the *mechanism of action of enzymes*.

### Lowering of Activation Energy

Substrates are remaining in an energy trough, and are to be placed at a higher energy level, whereupon spontaneous degradation can occur. Suppose, we want to make a fire; even if we keep a flame, the wood will not burn initially; we have to add kerosene or paper for initial burning. Similarly, the activation energy is to be initially supplied. **Activation energy** is defined as the energy required to convert all molecules in one mole of a reacting substance from the ground state to the transition state. Enzymes reduce the magnitude of this activation energy. This can be compared to making a tunnel in a mountain, so that the barrier could be lowered **(Fig. 3.5)**. For example, the activation energy for hydrolysis of sucrose by $H^+$ is 26,000 cal/mol, while the activation energy is only 9,000 cal/mol when hydrolyzed by sucrase.

**TABLE 3.3:** Metalloenzymes.

| *Metal* | *Enzymes containing the metal* |
|---|---|
| Zinc | Carbonic anhydrase, carboxy peptidase, alkaline phosphatase |
| Magnesium | Hexokinase, phosphofructokinase, enolase |
| Manganese | Hexokinase, enolase |
| Copper | Tyrosinase, cytochrome oxidase, superoxide dismutase |
| Iron | Cytochrome oxidase, xanthine oxidase |
| Calcium | Lecithinase, lipase |
| Molybdenum | Xanthine oxidase |

JAYPEE BROTHERS

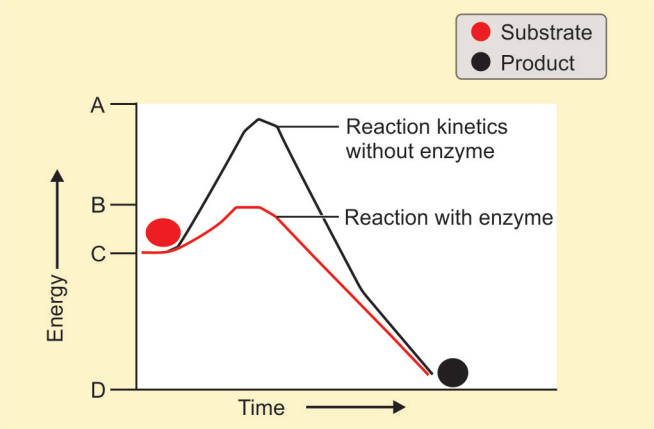



**Fig. 3.5:** Lowering of activation energy by enzymes. D = energy level of product. C to A = activation energy in the absence of enzyme. C to B is the activation energy in the presence of the enzyme. B to A = lowering of activation energy by the enzyme.

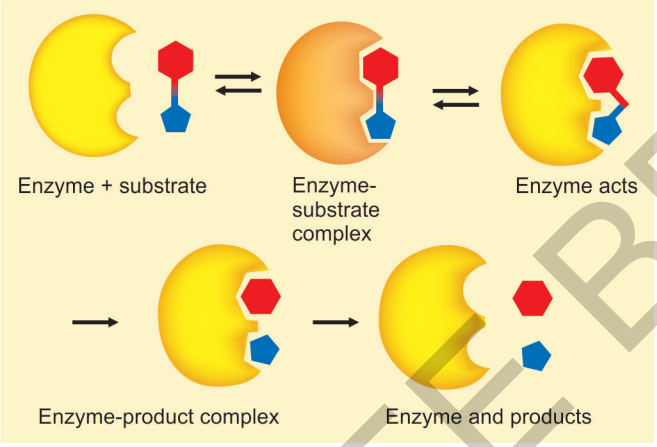



**Fig. 3.6:** Enzyme substrate complex.

## Michaelis-Menten Theory

Lenor Michaelis and Maud Menten (1913) put forward the **Enzyme–Substrate complex theory**. The enzyme (E) combines with the substrate (S), to form an enzyme-substrate (ES) complex, which immediately breaks down to the enzyme and the product (P) **(Fig. 3.6)**.

$$E + S \rightarrow E\text{–}S\ Complex \rightarrow E + P$$

## Fischer's Template Theory

It states that the three-dimensional structure of the active site of the enzyme is complementary to the substrate. Thus *enzyme and substrate fit each other* **(Fig. 3.7)**. The explanation is that the substrate fits on the enzyme, similar to a **lock and key**. The key will fit only to its own lock. Koshland proposed induced fit theory which explained among other things covalent inhibition.

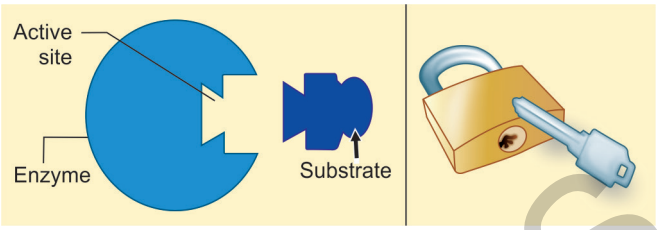



**Fig. 3.7:** Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory).

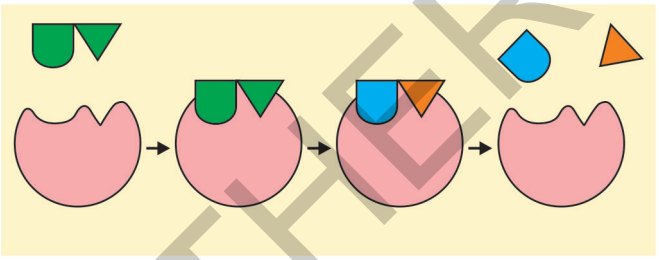


**Fig. 3.8:** Koshland's induced fit theory. 1 = Enzyme has shallow grooves; substrate alignment is not correct. 2 = Fixing of substrate induces structural changes in enzyme. 3 = Now the substrate correctly fits into the active site of the enzyme. 4 = Substrate is cleaved into two products.

## Koshland's Induced Fit Theory

The *substrate induces conformational changes in the enzyme*, such that the precise orientation of catalytic groups is effected **(Fig. 3.8)**.

## Active Site or Active Center

*That area of the enzyme where catalysis occurs is referred to as the active site or active centre.* For example, serine is the important amino acid at the catalytic site of trypsin. Proteolytic enzymes having a serine residue at the active center are called **serine proteases**, e.g., trypsin, chymotrypsin and coagulation factors. Salient features of the active sites of the enzymes are:

- Although all parts are required for keeping the exact three-dimensional structure of the enzyme, the reaction is taking place at the active site.
- The active site occupies only a small portion of the whole enzyme.
- The active site is situated in a crevice or cleft of the enzyme molecule.
- The amino acids or groups that directly participate in making or breaking the bonds (present at the active site) are called catalytic residues or catalytic groups.
- To the active site, the specific substrate is bound.
- The binding of substrate to the active site depends on the alignment of specific groups or atoms at the active site.
- During the binding process, these groups may realign themselves to provide a unique conformational orientation so as to promote the exact fitting of the substrate to the active site **(Fig. 3.8)**.

JAYPEE BROTHERS

## Specificity of Enzymes

- **Substrate specificity:** Each enzyme is specific for its substrate, e.g., glucokinase for glucose; fructokinase for fructose.
- **Bond specificity**: One enzyme will catalyze the hydrolysis or formation of a particular bond, e.g., trypsin will hydrolyze peptide bonds formed by basic amino acids only.
- **Group specificity:** Some enzymes will act on a group of substrates catalyzing the same reaction, e.g., hexokinase can add a phosphate group to all monosaccharides. The same proteolytic enzyme will hydrolyze the same type of peptide bond on all proteins.
- **Stereo specificity:** Enzymes will act only on one stereo isomer. Only D sugars are metabolized by the body. Glycerol kinase will add a phosphate group to only the 3rd hydroxyl group of the glycerol.

# THERMODYNAMICS

From the standpoint of energy, the enzymatic reactions are divided into three types:

### 1. Exergonic or Exothermic Reaction

Energy is released from the reaction, and therefore reaction essentially goes to completion, e.g., **urease** enzyme:

$$\text{Urea} \rightarrow \text{ammonia} + CO_2 + \text{energy}$$

At the equilibrium of this reaction, the substrate will be only 0.5% and the product will be 99.5%. Such reactions are generally irreversible.

### 2. Isothermic Reaction

When energy exchange is negligible, and the reaction is easily reversible, e.g.,

$$\text{Glycogen} + \text{Pi} \rightarrow \text{Glucose-1-Phosphate}$$

At the equilibrium of this reaction, 77% glycogen will be unutilized and 23% glucose-1-phosphate will be formed.

### 3. Endergonic or Endothermic Reaction

Energy is consumed and external energy is to be supplied for these reactions. In the body this is usually accomplished by coupling the endergonic reaction with an exergonic reaction, as shown in **Figure 3.9**. A good example is the **hexokinase reaction (Fig. 3.3)**.

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-Phosphate} + \text{ADP}$$

#### *Enzyme Kinetics*

The reaction rate or velocity is proportional to the concentration of reacting molecules.

$$A + B \rightarrow C + D$$

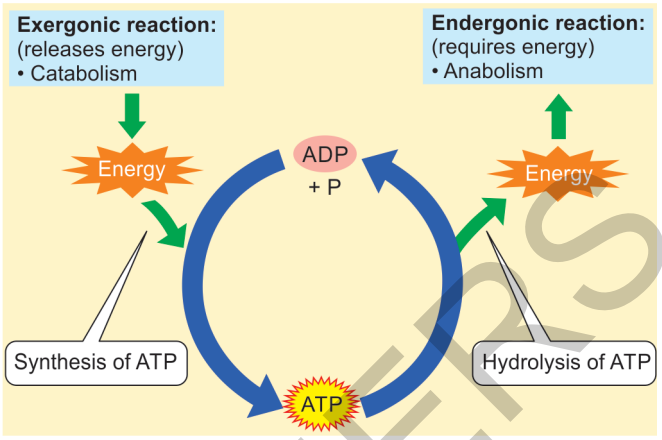



**Fig. 3.9:** Body couples exergonic and endergonic reactions for synthetic purposes. (ATP: adenosine triposphate).

At equilibrium, forward reaction and backward reaction are equal, so that

$$A + B \underset{K2}{\overset{K1}{\rightleftharpoons}} C + D$$

At equilibrium, forward and backward reactions are equal. Equilibrium is a dynamic state. Even though no net change in concentrations of substrate and product occurs, molecules are always interconverted. Enzyme makes it quicker to reach the equilibrium. Catalysts increase the rate of reaction, but do not alter the equilibrium.

# FACTORS INFLUENCING ENZYME ACTIVITY

### 1. Enzyme Concentration

*The velocity of the reaction is increased proportionately with the concentration of enzyme*, when substrate concentration is unlimited **(Fig. 3.10)**. Hence this property is made use of in determining the level of a particular enzyme in plasma, serum or tissues.

### 2. Substrate Concentration

If the velocity is plotted against the substrate concentration, a typical curve **(Fig. 3.11)** will be obtained. As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the

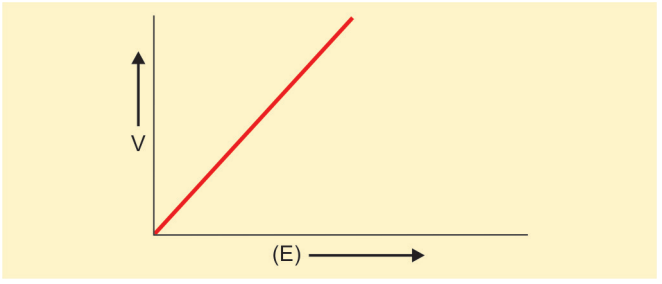



**Fig. 3.10:** Effect of enzyme concentration.

JAYPEE BROTHERS

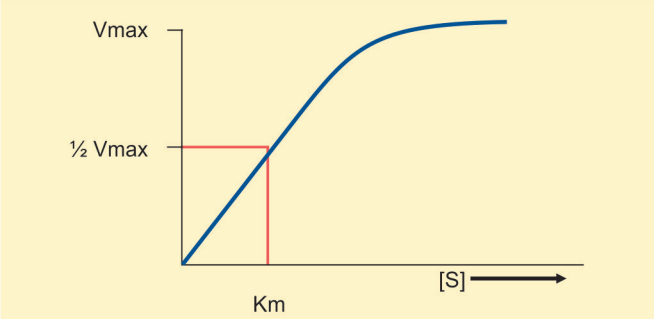



**Fig. 3.11:** Effect of substrate concentration (substrate saturation curve).

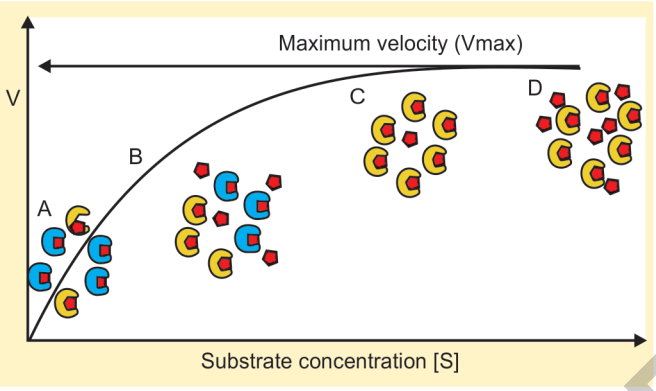



**Fig. 3.12:** The effect of substrate concentration on enzyme activity is explained. Enzyme molecules are shown as half-circles. Substrate molecules are shown as red dots. A = Substrate molecules are low; so only a few enzyme molecules are working and velocity is less. B = At half-maximal velocity (Km), 50% of enzyme molecules are bound with the substrate. C = As a lot of substrate molecules are available all the enzyme molecules are bound. D = Further increase in the substrate will not increase the velocity further.

curve flattens afterwards. **The maximum velocity thus obtained is called Vmax**. It represents the maximum reaction rate attainable in the presence of excess substrate (at **substrate saturation level**).

This behavior is explained in **Figure 3.12**. At lower concentrations of substrate (point A in the curve), some enzyme molecules remain idle. As the substrate is increased, more and more enzyme molecules are working. At half-maximal velocity, 50% of enzymes are attached to the substrate (point B in the curve). As more substrate is added, all enzyme molecules are saturated (point C). Further increase in the substrate cannot make any effect on the reaction velocity (point D).

### *Michaelis Constant*

According to Michaelis theory, the formation of enzyme-substrate complex is a reversible reaction, while the breakdown of the complex to enzyme + product is irreversible.

$$E + S \xrightarrow{K1} E\text{–}S \underset{K2}{\overset{K3}{\rightleftharpoons}} E + P$$

If the concentration of the substrate is increased, the forward reaction K1 is increased, and so K3 as well as total velocity is correspondingly enhanced. The three different constants may be made into one equation,

$$Km = \frac{K2 + K3}{K1}$$

This Km is called as **Michaelis Constant**. It is further shown that

$$v = ½\ Vmax$$

In **Figure 3.13**, 50% velocity in the Y-axis is extrapolated to the corresponding point in the X-axis, which gives the numerical value of Km.

### *Definition of Km*

**Substrate concentration** (expressed in moles/L) **at half-maximal velocity** is the Km value. It denotes that 50% of *enzyme molecules are bound with substrate* molecules at that particular substrate concentration.

**Km is independent of enzyme concentration**. If enzyme concentration is doubled, the Vmax also will be doubled. But the ½ Vmax (Km) will remain exactly the same **(Fig. 3.13)**.

**Km is the signature of the enzyme.** Km value is thus a constant for an enzyme. It is the *characteristic feature of a particular enzyme* for a specific substrate.

**Km denotes the affinity of the enzyme to the substrate.** The *lesser the numerical value of Km, the affinity of the enzyme for the substrate is more.* To cite an example, Km of **glucokinase** is 10 mmol/L and that of **hexokinase** is 0.05 mmol/L. Therefore, hexokinase has more affinity for glucose than glucokinase.

## 3. Effect of Concentration of Products

In a reversible reaction, S ⟷ P, when equilibrium is reached, as per the law of mass action, the reaction rate is slowed down. So when product concentration is increased, the reaction is slowed, stopped or even reversed. In inborn errors of metabolism, one enzyme of a metabolic pathway is blocked. For example,

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D$$

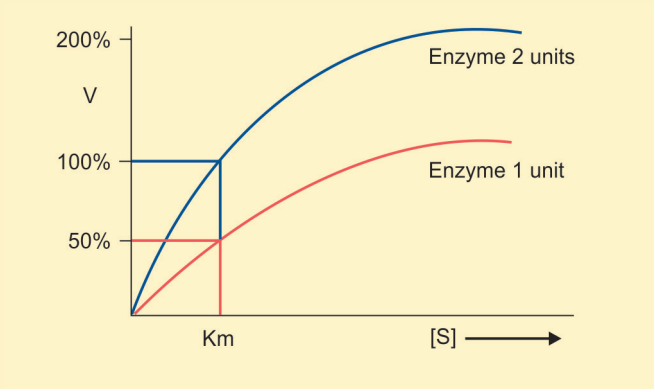



**Fig. 3.13:** Effect of enzyme concentration on Km.

JAYPEE BROTHERS

If E3 enzyme is absent, C will accumulate, which in turn, will inhibit E2. Consequently, in course of time, the whole pathway is blocked.

### 4. Effect of Temperature

The velocity of enzyme reaction increases when the temperature of the medium is increased; reaches a maximum and then falls (Bell shaped curve). The temperature at which the maximum amount of the substrate is converted to the product per unit time is called the optimum temperature **(Fig. 3.14)**. As the temperature is increased, more molecules get activation energy, or molecules are at an increased rate of motion. So, their collision probabilities are increased and so the reaction velocity is enhanced. But when the temperature is more than 50°C, heat denaturation and consequent loss of tertiary structure of protein occurs. So, the activity of the enzyme is decreased. Most human enzymes have an optimum temperature of around 37°C.

### 5. Effect of pH

Each enzyme has an optimum pH, on both sides of which the velocity will be drastically reduced. The graph will show a bell-shaped curve **(Fig. 3.15)**. The pH decides the charge on the amino acid residues at the active site. Usually, enzymes have an optimum pH between 6 and 8. Some important exceptions are Pepsin (with optimum pH 1–2); acid phosphatase (pH 4–5) and alkaline phosphatase (optimum pH 9–10).

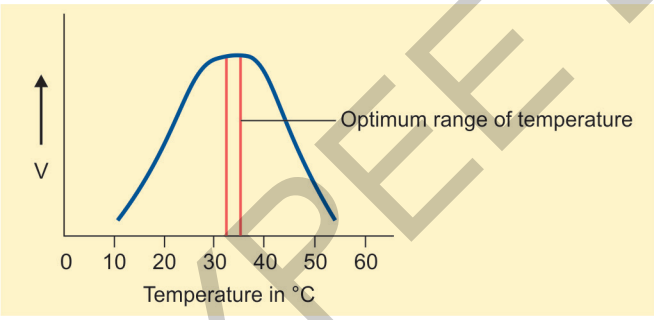



**Fig. 3.14:** Effect of temperature on velocity.

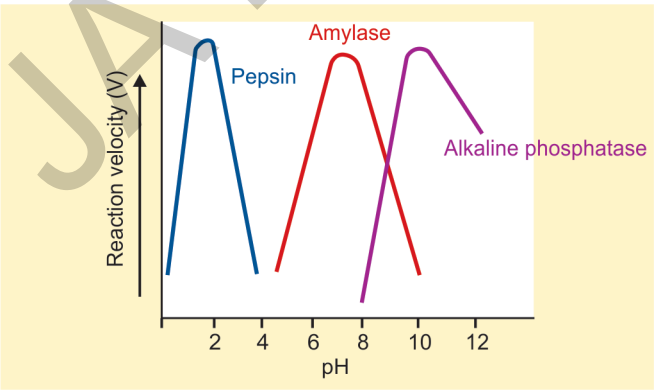



**Fig. 3.15:** Effect of pH on enzyme velocity.

### Enzyme Activation

In the presence of certain **metallic ions**, some enzymes show higher activity. Thus, chloride ions will activate salivary amylase and calcium will activate lipases. Another type of activation is the conversion of an inactive **pro-enzyme** or **zymogen** to the active enzyme. Thus, by splitting a single peptide bond, and removal of a small polypeptide from **trypsinogen**, the active trypsin is formed. This results in the unmasking of the active center. Similarly, trypsin activates **chymotrypsinogen**. All the gastrointestinal enzymes are synthesized in the form of proenzymes, and only after secretion into the alimentary canal, they are activated. This prevents the autolysis of cellular structural proteins.

### Covalent Modification

The process of altering the activity of enzymes by adding or removing groups (breaking or making covalent bonds) is called a covalent modification. Two typical examples are partial proteolysis and addition or removal of phosphate groups. Activation of zymogens to the functional enzyme is by partial proteolysis, which exposes the active site to which the substrate can bind. All digestive enzymes are secreted as zymogens that are further activated, e.g., trypsinogen to trypsin, pepsinogen to pepsin. Covalent modification by phosphorylation and dephosphorylation is brought about by the action of hormones. The binding of hormones to G protein-coupled receptors leads to the activation of a cyclic AMP mediated cascade activation pathway. (details given in Chapter 21). Protein kinases are activated that add phosphate groups to enzyme proteins. Some enzymes become active on phosphorylation, e.g., glycogen phosphorylase. The removal of the phosphate group by specific protein phosphatases activates some enzymes, e.g., glycogen synthase. Opposing metabolic pathways are thus reciprocally regulated so that futile reaction cycles will not take place.

## ENZYME INHIBITION

All the reactions in the body are appropriately controlled. Control of the whole pathway is achieved by inhibition of such **key enzymes** or regulatory enzymes.

### 1. Competitive Inhibition (Reversible)

The *inhibitor molecules compete with the normal substrate molecules to attach with the active site of the enzyme.*

$$E + S \longrightarrow E\text{–}S \longrightarrow E + P$$
$$E + I \longrightarrow E\text{–}I$$

Since E-I (enzyme–inhibitor complex) can react only to reform the enzyme and inhibitor, the number of enzyme molecules available for E-S formation is reduced. Suppose 100 molecules of the substrate and 100 molecules of the inhibitor are competing for 100 molecules of the enzyme. So,

JAYPEE BROTHERS

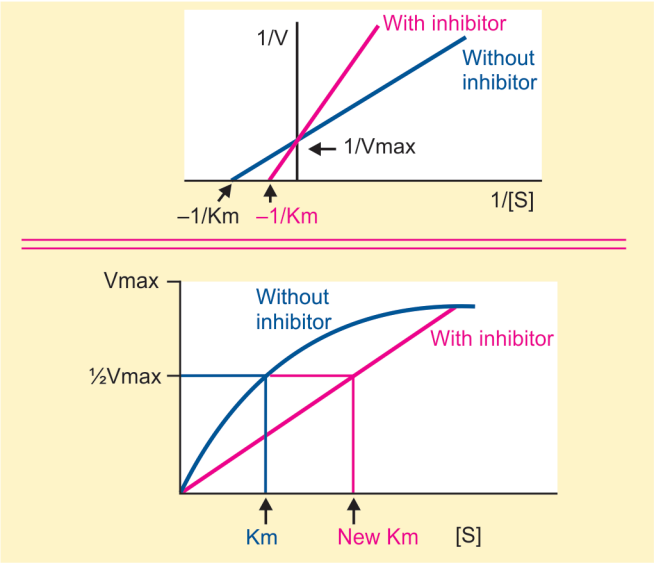



**Fig. 3.16:** Substrate saturation curve in the presence and absence of competitive inhibitor.

half of the enzyme molecules are trapped by the inhibitor, and only half the molecules are available for catalysis to form the product. Since the effective concentration of the enzyme is reduced, the reaction **velocity is decreased (Fig. 3.16)**.

In competitive inhibition, the inhibitor will be a **structural analogue** of the substrate. There will be a similarity in three-dimensional structure between the substrate (S) and inhibitor (I). For example, the succinate dehydrogenase reaction is inhibited by malonate, which are structural analogues of succinate **(Fig. 3.17)**. Competitive inhibition is usually **reversible.** *Excess substrate abolishes the inhibition.* If substrate concentration is enormously high when compared to inhibitor, then the inhibition is reversed **(Fig. 3.16)**. From the graphs, it is seen that in the case of competitive inhibition, the **Km is increased** in the presence of a competitive inhibitor *but Vmax is not changed.* Thus, competitive inhibitor apparently increases the Km.

## Clinical Significance

**The pharmacological action** of many drugs may be explained by the principle of competitive inhibition. A few important examples are given below: **Sulfonamides** are commonly employed antibacterial agents **(Fig. 3.18)**.

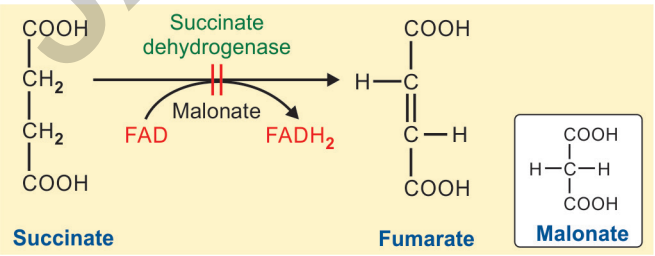



**Fig. 3.17:** Malonate inhibits succinate dehydrogenase.

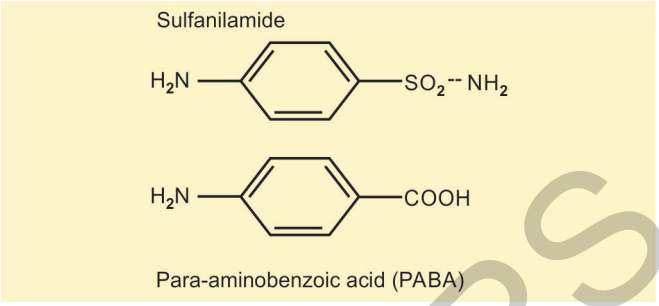



**Fig. 3.18:** Competitive inhibition.

Bacteria synthesize folic acid by combining PABA with pteroylglutamic acid. The bacterial wall is impermeable to folic acid. Sulfa drugs, being structural analogues of PABA, will inhibit the folic acid synthesis in bacteria, and they die. The drug is nontoxic to human cells, because human beings cannot synthesize folic acid. Preformed folic acid is essential for man. **Methotrexate** (4-amino-$N^{10}$-methyl folic acid) is a structural analogue of folic acid, and so can competitively inhibit folate reductase enzyme. This is essential for DNA synthesis and cell division. Therefore, methotrexate is used as an anticancer drug.

## 2. Noncompetitive Inhibition (Irreversible)

A variety of **poisons**, such as iodoacetate, heavy metal ions (silver, mercury) and oxidizing agents act as irreversible noncompetitive inhibitors. There is no competition between substrate and inhibitor **(Fig. 3.19)**. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site. Since these i*nhibitors have no structural resemblance to the substrate*, an increase in the substrate concentration generally does not relieve this inhibition **(Fig. 3.20)**. **Cyanide** inhibits cytochrome oxidase. **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis. The inhibitor combines with the enzymes and the reaction becomes **irreversible**. The velocity (Vmax) is reduced. But the **Km value is not changed**, because the remaining enzyme molecules have the same affinity for the

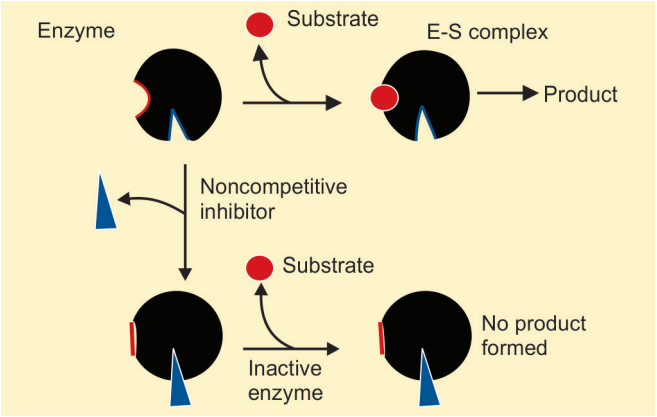



**Fig. 3.19:** Noncompetitive inhibition explained.

JAYPEE BROTHERS

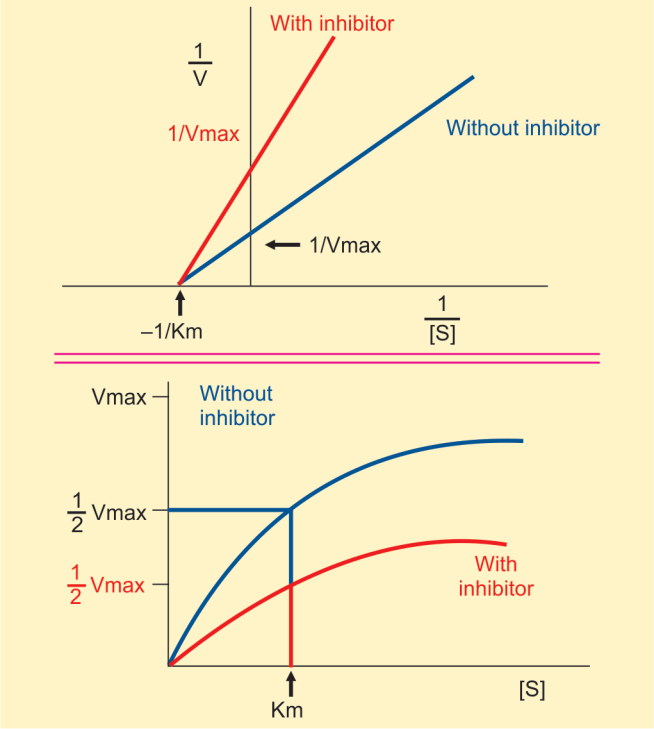



**Fig. 3.20:** Noncompetitive inhibition.

**TABLE 3.4:** Comparison of two types of inhibitions.

| | *Competitive inhibition* | *Noncompetitive inhibition* |
|---|---|---|
| Structure of inhibitor | Substrate analogue | Unrelated molecule |
| Inhibition is | Reversible | Generally irreversible |
| Excess substrate | Inhibition relieved | No effect |
| Km | Increased | No change |
| Vmax | No change | decreased |
| Significance | Drug action | Toxicological |

substrate. *Increasing the substrate concentration will abolish competitive inhibition, but will not abolish noncompetitive inhibition.* A comparison of the two types of inhibitions is shown in **Table 3.4**.

## 3. Allosteric Regulation

The allosteric enzyme has one catalytic site where the substrate binds and another **separate allosteric site** where the modifier binds (*allo* = other). In most cases, the substrate saturation curve is sigmoid in shape **(Figs. 3.21 and 3.22)**. Most allosteric enzymes are made up of subunits, e.g., aspartate transcarbamoylase has 6 subunits and pyruvate kinase has 4 subunits. A selected list of allosteric enzymes is shown in **Table 3.5**.

### *Key Enzymes*

Allosteric enzymes are utilized by the body for regulating metabolic pathways. Such a **regulatory enzyme** in a particular pathway is called the **key enzyme** or **rate limiting** enzyme. The flow of the whole pathway is constrained as if there is a bottle neck at the level of the key enzyme. For example, the glycolytic pathway is regulated by phosphofructokinase which catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate.

Fructose-6-phosphate + ATP ⟶ Fructose-1,6-bisphosphate + ADP

The reaction is allosterically inhibited by ATP and activated by AMP. The glycolytic pathway operates to produce ATP. So, when the ATP level in the cell is high (high energy charge), the pathway slows down and is activated when the energy charge is low as indicated by a high AMP level.

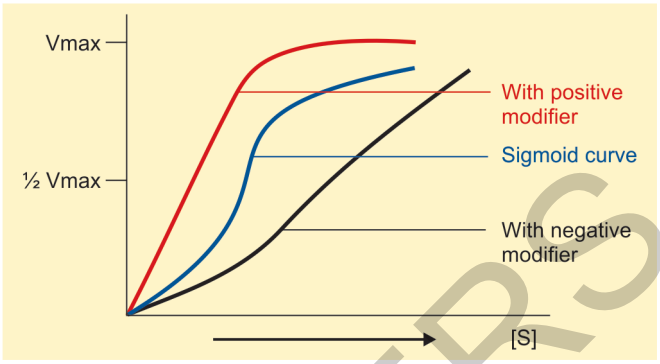



**Fig. 3.21:** Allosteric inhibition.

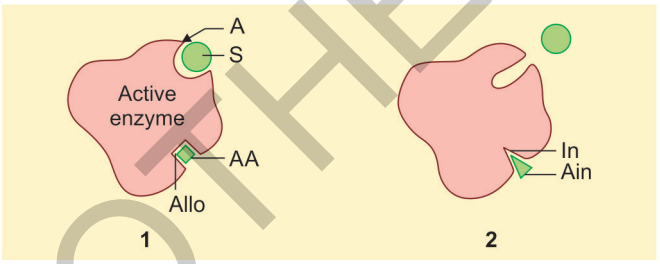



**Fig. 3.22:** Action of allosteric enzymes. A = Active site. Allo = Allosteric site. S = Substrate. AA = Allosteric activator. In = Inhibitor site. Ain = Allosteric inhibitor. The enzyme has a separate active site and an allosteric site. In figure 1, the activator is fixed at the allosteric site, when the active site has correct conformation, and the substrate is correctly fixed. Figure 2 shows that the inhibitor is fixed at the allosteric site when the active site is deformed and the substrate cannot be fixed.

**TABLE 3.5:** Examples of allosteric enzymes.

| *Enzyme* | *Allosteric inhibitor* | *Allosteric activator* | *Chapter* |
|---|---|---|---|
| ALA synthase | Heme | — | 13 |
| Aspartate transcarbamoylase | CTP | ATP | 26 |
| HMGCoA reductase | Cholesterol | — | 10 |
| Phosphofructokinase | ATP, citrate | AMP, F-2,6-P | 5 |

JAYPEE BROTHERS

### *Induction*

Induction is effected through the process of derepression. The *inducer will relieve the repression on the operator site* and will remove the block on the biosynthesis of the enzyme molecules. A classical example is the induction of lactose-utilizing enzymes in the bacteria when the media contains lactose in the absence of glucose. In human beings, ALA synthase is induced by barbiturates.

### *Repression*

Even though both inhibition and repression reduce the enzyme velocity, the mechanisms are different. In the case of **inhibition,** the inhibitor acts on the enzyme directly; and the number of enzyme molecules is not changed by the inhibitor. On the contrary, the **repression** acts at the gene level; and the number of enzyme molecules is reduced in the presence of repressor molecules. The key enzyme of heme synthesis, **ALA synthase** is autoregulated by the heme by means of repression **(Fig. 3.23)**. The structural gene is transcribed and later translated to produce the enzyme molecules. Details are shown in Chapter 26.

## ISOENZYMES

*They are physically distinct forms of the same enzyme activity. Multiple molecular forms of an enzyme are described as isoenzymes* or isozymes. If we take a few Rupee coins and examine them carefully, there will be minor variations of ridges on the rims and the number of dots below the year. In the market all these coins have the same face value; but to an experienced numismatist, these variations will explain from which mint it was produced. Similarly, different molecular forms of the same enzyme synthesized from various tissues are called isoenzymes. For example, lactate dehydrogenase has 5 forms. The study of isoenzymes is useful to understand diseases of different organs.

## CLINICAL ENZYMOLOGY

Plasma contains many **functional enzymes,** which are actively secreted into plasma. For example, enzymes of blood coagulation. On the other hand, there are a few **non-functional enzymes** in plasma, which are coming out from cells of various tissues due to normal wear and tear. Their normal levels in the blood are very low; but are drastically increased during cell death (necrosis) or disease. Therefore, assays of these enzymes are very useful in the diagnosis of diseases.

### Lactate Dehydrogenase

Lactate dehydrogenase (LDH) enzyme is a tetramer with 4 subunits. But the subunit may be either H (heart) or M (muscle) polypeptide chains. These two are the products of 2 different genes. So 5 combinations of H and M chains are possible; H4, H3M, H2M2, M3H and M4 varieties, forming **5 isoenzymes (Fig. 3.24)**. All these 5 forms are seen in all persons. The M4 form is seen in skeletal muscles; while the H4 form is seen in the heart. Normally LDH-2 (H3M1) concentration in blood is greater than LDH-1 (H4); but this pattern is reversed in myocardial infarction; this is called a **flipped pattern.** The isoenzymes are usually separated by cellulose acetate **electrophoresis**. In myocardial infarction, LDH activity is increased. Within a few hours after the heart attack, the enzyme level starts to increase, reaches a peak on the 5th day, and reaches normal levels by 10–12 days. LDH is seldom used in the diagnosis of myocardial infarction nowadays, and is of historical importance only.

### Creatine Kinase

Creatine kinase (CK) value in serum is increased in **myocardial infarction.** The CK level starts to rise **within 4 hours** of infarction. Therefore, CK estimation is very useful to detect early cases, where ECG changes may be ambiguous. The CK level is not increased in hemolysis or in congestive cardiac failure; and therefore, CK has an advantage over LDH.

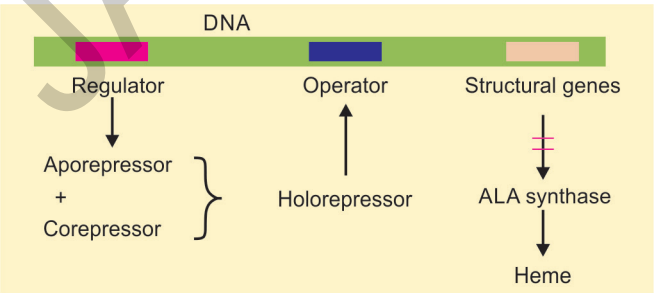



**Fig. 3.23:** Repression of ALA synthase.

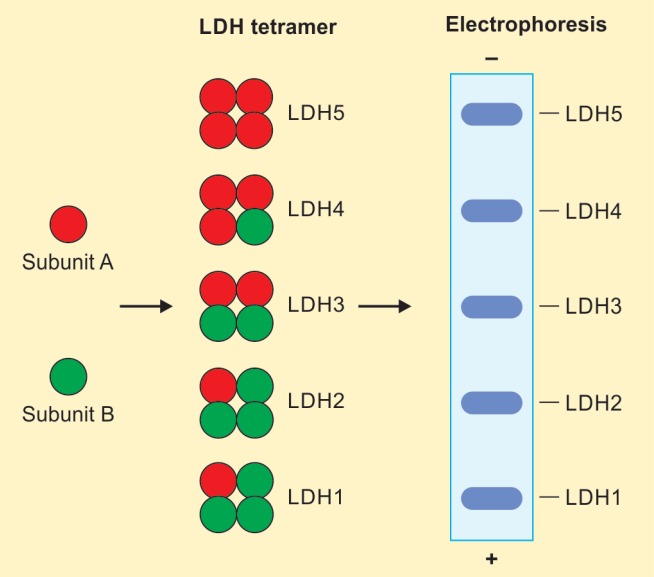



**Fig. 3.24:** Isoenzymes of lactate dehydrogenase (LDH).

JAYPEE BROTHERS

## Creatine Kinase and Muscle Diseases

The level of CK in serum is very much elevated in **muscular dystrophies**. The level is very high in the early phases of the disease. CK level is highly elevated in crush injury, fracture and acute cerebrovascular accidents. Estimation of total CK is employed in muscular dystrophies and MB isoenzyme is estimated in myocardial infarction. Cardiac biomarkers are described in Chapter 9.

## Isoenzymes of Creatine Kinase

CK is a dimer. The subunits are called B for the brain and M for muscle. Therefore, three isoenzymes are seen in circulation. MM (CK3) is originating from skeletal muscles. MB (CK2) is from the heart and BB (CK1) is from the brain. Hence the *detection of MB-isoenzyme is important in myocardial infarction.*

## Alanine Amino Transferase (ALT )

In old literature, it was called serum glutamate pyruvate transaminase (SGPT). The enzyme needs pyridoxal phosphate as the coenzyme. Details of the reaction are shown in **Figure 2.17**. Normal serum level of ALT for male is <45 U/L and for female is <35 U/L. Very high values (100–1000 U/L) are seen in **acute hepatitis,** either toxic or viral in origin. Both ALT and AST levels are increased in liver disease, but ALT >> AST. A rise in ALT levels may be noticed several days before clinical signs such as jaundice are manifested. Moderate increase (25–100 U/L) may be seen in chronic liver diseases such as cirrhosis, and malignancy in the liver.

## Aspartate Amino Transferase (AST)

In earlier days it was called glutamate oxaloacetate transaminase (GOT). Along with ALT, it is used as a marker of hepatic injury. Very high levels are seen in acute viral hepatitis and hepatocellular carcinoma. AST is also elevated muscle diseases and cardiac ischemia. Alcoholic hepatitis is characterized by elevated AST.

## Alkaline Phosphatase

Alkaline phosphatase (ALP) is a nonspecific enzyme that hydrolyses aliphatic, aromatic or heterocyclic compounds. The pH optimum for the enzyme reaction is between 9 and 10. It is produced by **osteoblasts** of bone, and is associated with the calcification process. The **normal serum** value of ALP is 40–125 U/L. In children, the upper level of normal value may be more, because of the increased osteoblastic activity in children. A moderate increase (2–3 times) in ALP level is seen in **hepatic diseases** such as infective hepatitis, alcoholic hepatitis or hepatocellular carcinoma. Very high levels of ALP (10-12 times of upper limit) may be noticed in **extrahepatic obstruction** (obstructive jaundice) caused by gallstones or by pressure on the bile duct by the carcinoma of the head of the pancreas. Drastically high levels of ALP (10–25 times of upper limit) are also seen in **bone diseases** where osteoblastic activity is enhanced such as Paget's disease (osteitis deformans), rickets, osteomalacia, osteoblastoma, metastatic carcinoma of bone and hyperparathyroidism.

## Acid Phosphatase

Acid phosphatase (ACP) hydrolyzes phosphoric acid ester at pH between 4 and 6. ACP is secreted by prostate cells, RBC, platelets and WBC. The prostate isoenzyme is inactivated by **tartaric acid**. ACP total value is increased in **prostate cancer** and highly elevated in metastatic bone disease, especially from a primary from the prostate. In these conditions, the tartrate labile isoenzyme is elevated. This assay is very helpful in follow up of treatment of prostate cancers. ACP is therefore an important **tumor marker** (Chapter 30). Alterations of enzymes in various diseases are shown in **Table 3.6**.

**TABLE 3.6:** Enzyme patterns (enzyme profiles) in diseases.

| | | |
|---|---|---|
| I | ***Hepatic diseases*** | |
| | 1. Alanine aminotransferase (ALT) | Marked increase in parenchymal liver diseases |
| | 2. Alkaline phosphatase (ALP) | Marked increase in obstructive liver disease |
| II | ***Myocardial infarction*** | |
| | 1. Creatine kinase (CK-MB) | The first enzyme to rise following infarction, CK-MB isoenzyme is specific |
| | 2. Aspartate aminotransferase (AST) | Rises after the rise in CK and returns to normal in 4–5 days |
| | 3. Lactate dehydrogenase (LDH) | Last enzyme to rise. LDH-1 becomes more than 2 (flipped pattern) |
| III | ***Muscle diseases*** | |
| | Creatine kinase (CK-MM) | Marked increase in muscle diseases. CK-MM fraction is elevated |
| IV | ***Bone diseases*** | |
| | Alkaline phosphatase (ALP) | Marked elevation in osteoblastic bone activity as in rickets. Heat labile bone isoenzyme is elevated. Also in Paget's disease |
| V | ***Prostate cancer*** | |
| | 1. Prostate specific antigen (PSA) | Marker for prostate cancer. Mild increase in benign prostate enlargement |
| | 2. Acid phosphatase (ACP) | Marker for prostate cancer. Metastatic bone disease, especially from a primary prostate. Inhibited by L tartrate |

JAYPEE BROTHERS

## Other Uses of Enzymes

Many enzymes are used in the treatment of diseases. Examples of therapeutic uses of enzymes are shown in **Table 3.7**. Recombinant enzymes are used in the cloning and treatment of genetic disorders, like Pompe's disease. Enzymes are also used for diagnosis; for example, glucose oxidase enzyme is used in the reagent for the detection of glucose. They may be rendered fixed before use, as "immobilized enzymes".

**TABLE 3.7:** Therapeutic use of enzymes.

| *Enzyme* | *Therapeutic application* |
|---|---|
| Asparaginase | Acute lymphoid leukemia |
| Streptokinase | To lyse intravascular clot |
| Urokinase | do |
| Hyaluronidase | Enhances local anesthetics |
| Pancreatin | Pancreatic insufficiency |
| Papain | Anti-inflammatory |

## A QUICK LOOK

### General characteristics of enzymes

- Enzymes, facilitate reactions without being consumed or altered in the process.
- Enzymes are biocatalysts that are essential for biochemical reactions to proceed in the human body.
- Enzymes accelerate reactions at body temperature, which would otherwise require extreme conditions.
- Enzymes are proteins and show heat sensitivity.
- Biological activity of enzymes is dependent on the structural conformation of the enzyme protein.

### Enzyme classification

- According to the IUBMB system for enzyme classification, there are seven major classes of enzymes based on the type of reaction they catalyze.
- Enzymes can be classified into: (i) Oxidoreductases (ii) Transferases (iii) Hydrolases (iv) Lyases (v) Isomerases (vi) Ligases and (vii) Translocases.
- Enzymes requiring the presence of a certain metal ion for their activity are called metalloenzymes.
- Examples are zinc in carbonic anhydrase, iron in catalase and peroxidase, calcium in lipase, etc.

### Coenzymes

- Apoenzyme (protein part) combines with coenzyme (prosthetic group) to form the functional holoenzyme.
- Coenzymes are essential nonprotein components for enzyme functionality.
- Coenzymes may take part in reactions as cosubstrates, but are regenerated.
- Some vitamin coenzymes are derivatives of nucleotide phosphates, e.g., $NAD^+$, FAD. The deficiency of coenzymes can affect the rate of enzymatic reactions.

### Modes of action of enzymes

- Area of an enzyme where the catalysis occurs is called the 'active site'.
- Enzymes catalyze reactions by lowering the activation energy, but does not change the equilibrium constant.
- Michaelis-Menten theory states that an enzyme (E) combines with a substrate (S) to form an enzyme-substrate (E-S) complex, which breaks down to give rise to the product (P).
- Fischer's lock and key hypothesis puts forward a rigid structure for the active site where the substrate binds.
- Koshland's induced fit theory proposes a conformational change in the active site to allow the binding of regulatory molecules.
- Thermodynamics of enzymatic reaction explains the types of reactions (exergonic, isothermic, endergonic).

### Enzyme kinetics

- Enzyme activity is influenced by the enzyme concentration, substrate concentration, pH, temperature and the presence of inhibitors. Velocity is directly proportional to the concentration of enzymes.
- Velocity at saturating concentration of substrate is called maximum velocity or Vmax.
- Km value (Michaelis-Menten constant), the substrate concentration at half maximum velocity is a constant for each enzyme–substrate pair.
- Km value is characteristic of a given enzyme. No two enzymes can have the same Km value. It denotes the affinity of the enzyme to its substrate.
- The lesser the Km, the greater the affinity and vice versa.
- Km value indicates the affinity of the enzyme for substrate; higher the affinity, lower the Km.
- Velocity is the maximum for each enzyme at an optimum pH and temperature.

### Types of enzyme inhibitions

- Inhibition may be reversible or irreversible.
- Enzyme inhibition can be competitive or noncompetitive or uncompetitive. Competitive inhibition is usually reversible.
- Competitive inhibitor is a structural analogue of substrate that binds to the catalytic site.
- The competitive inhibitor increases the Km and its effect can be reversed by increasing the substrate concentration.

JAYPEE BROTHERS

- Many drugs are competitive inhibitors of specific enzymes, e.g., folic acid synthesis is inhibited by sulfonamides since they are structurally similar to PABA.
- Actions of drugs such as sulfonamides, methotrexate, dicoumarol and isoniazid are based on the principle of competitive inhibition.
- Noncompetitive inhibitor binds to a site other than the catalytic site and reduces the Vmax.
- Noncompetitive inhibition is irreversible and can be caused by toxins or poisons.
- Allosteric enzymes can be regulated by the binding of positive or negative modifiers to the allosteric site, thus affecting substrate binding to the active catalytic site.
- Suicide inhibition is irreversible. The inhibitor makes use of the natural reaction of the enzyme for inhibition, e.g., ornithine decarboxylase.
- Covalent modification by reversible protein phosphorylation or zymogen activation is a common mechanism of short-term regulation.
- Induction and repression are other mechanisms by which enzyme activities are regulated. These are taking place at the gene level.

**Isoenzymes**

- Isoenzymes are physically distinct forms of the same enzyme activity. They may be products of the same gene or different genes.
- Examples are lactate dehydrogenase (LDH), and creatine kinase (CK).

JAYPEE BROTHERS

for detailed information on dental books, visit our website www.jaypeebrothers.com, for detailed information on dental books, visit our website www.jaypeebroth

# Textbook of BIOCHEMISTRY for DENTAL Students

In this fifth edition, the contents have been updated, while deleting some irrelevant points from the previous edition. Moreover, important points are shown in bold letters to aid in memorization. A few figures have been added, and some figures and tables have been improved. There are about 400 figures and 150 tables in total, making the book more student-friendly.

**DM Vasudevan** MBBS MD FAMS FRCPath has 55 years of teaching experience. He was also the Principal of two medical colleges (Amrita School of Medicine, Kochi, Kerala and Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim) for 9 years and Vice Principal (Medical College, Thrissur, Kerala) for 7 years. He obtained his MBBS from the University of Kerala; MD (Biochemistry) from All India Institute of Medical Sciences (AIIMS), New Delhi, India and conducted post-doctoral research at the Swiss Institute for Cancer Research, Lausanne, Switzerland. He has been a visiting scientist at the University of Health Sciences, Bethesda, USA. He is a Fellow of the Royal College of Pathologists, London. He is the recipient of the prestigious "BC Roy National Award for eminent medical teacher" adjudged by the Medical Council of India and received from the then President of India in 1994. He is a member of various academic bodies of different universities and serves on the editorial boards of various scientific journals. He has published more than 260 research papers in international and national peer-reviewed journals. He has presented more than 200 papers and delivered keynote addresses at international conferences in the United States, the United Kingdom, Europe and the Far East. Already, 35 students have completed their PhD under his guidance. He was the President of the Indian Association of Clinical Biochemists of India (ACBI) in 2010.

**Sreekumari S** MBBS MD has more than 40 years of teaching experience to her credit. She has served as a Professor of Biochemistry at Govt Medical College, Kottayam, Kerala, India; Govt Medical College, Thrissur; Jubilee Mission Medical College, Thrissur; and Sree Gokulam Medical College and Research Foundation, Thiruvananthapuram.

**Kannan Vaidyanathan** MBBS MD FRCP has 25 years of teaching experience. He obtained his MD (Biochemistry) from Kerala and conducted extensive research at the Indian Institute of Science, Bengaluru, Karnataka, India. He has visited many advanced laboratories worldwide and presented papers at various international conferences. He has more than 50 publications to his credit. He has served as a Professor of Biochemistry at Amrita Institute of Medical Sciences, Kochi and Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala. He is now a Professor and Head, Department of Biochemistry, Believers Church Medical College and Hospital, Thiruvalla. He is the President of the Indian Association of Clinical Biochemists of India for the year 2024.

**Sukhes Mukherjee** PhD MAMS FRSPH FAScT FLS has more than 20 years of teaching experience. He is PhD from Amrita Vishwa Vidyapeetham. He has published more than 80 papers in peer reviewed journals and has presented more than 80 papers at international conferences. He is a Member of the Editorial Board of 15 reputed journals.

*Printed in India*

**Available at all medical bookstores or buy online at www.ejaypee.com**

**JAYPEE BROTHERS**
**Medical Publishers (P) Ltd.**
**EMCA House, 23/23-B, Ansari Road,**
**Daryaganj, New Delhi - 110 002, INDIA**
**www.jaypeebrothers.com**

Join us on facebook.com/JaypeeMedicalPublishers
Follow us on instagram.com/JaypeeMedicalPublishers

Shelving Recommendation
**DENTISTRY**

ISBN 978-93-5696-842-4
9 789356 968424