an irreversible (exergonic) reaction that “commits” the intermediate it produces to continue down the pathway.

3. All metabolic pathways are regulated
In order to exert control on the flux of metabolites through a metabolic pathway, it is necessary to regulate its rate-limiting step. The first committed step, being irreversible, functions too slowly to permit its substrates and products to equilibrate. Since most of the other reactions in a pathway function close to equilibrium, the first committed step is often its rate-limiting step. Most metabolic pathways are therefore controlled by regulat-

ing the enzymes that catalyze their first committed steps. This is the most efficient way to exert control because it prevents the unnecessary synthesis of metabolites further along the pathway when they are not required. Specific aspects of such flux control are discussed in Section 16-4A.

4. Metabolic pathways in eukaryotic cells occur in specific cellular locations
The synthesis of metabolites in specific membrane-bounded subcellular compartments makes their transport between these compartments a vital component of eukaryotic metabolism. Biological membranes are selectively permeable to metabolites because of the presence in membranes of specific transport proteins. For example, ATP is generated in the mitochondria but much of it is utilized in the cytosol. The transport protein that facilitates the passage of ATP through the mitochondrial membrane is discussed in Section 18-4C, along with the characteristics of membrane transport processes in general. The synthesis and utilization of acetyl-CoA is also compartmentalized. This metabolic intermediate is utilized in the cytosolic synthesis of fatty acids, but is synthesized in mitochondria. Yet there is no transport protein for acetyl-CoA in the mitochondrial membrane. How cells solve this fundamental problem is discussed in Section 23-4D.

2. ORGANIC REACTION MECHANISMS

Almost all of the reactions that occur in metabolic pathways are enzymatically catalyzed organic reactions. Section 14-1 details the various mechanisms enzymes have at their disposal for catalyzing reactions: Acid–base catalysis, covalent catalysis, metal ion catalysis, electrostatic catalysis, proximity and orientation effects, and transition state binding. Yet, few enzymes alter the chemical mechanisms of these reactions, so much can be learned about enzymatic mechanisms from the study of nonenzymatic model reactions. We therefore begin our study of metabolic reactions by outlining the types of reactions we shall encounter and the mechanisms by which they have been observed to proceed in nonenzymatic systems.

Christopher Walsh has classified biochemical reactions into four categories: (1) group-transfer reactions; (2) oxidations and reductions; (3) eliminations, isomerizations, and rearrangements; and (4) reactions that make or break carbon–carbon bonds. Much is known about the mechanisms of these reactions and about the enzymes that catalyze them. The discussions in the next several chapters focus on these mechanisms as they apply to specific metabolic interconversions. In this section we outline the four reaction categories and discuss how our knowledge of their reaction mechanisms derives from the study of model or
ganic reactions. We begin by briefly reviewing the chemical logic used in analyzing these reactions.

### A. Chemical Logic

A covalent bond consists of an electron pair shared between two atoms. In breaking such a bond, the electron pair can either remain with one of the atoms (heterolytic bond cleavage) or separate such that one electron accompanies each of the atoms (homolytic bond cleavage) (Fig. 15-4). Homolytic bond cleavage, which usually produces unstable radicals, occurs mostly in oxidation-reduction reactions. Heterolytic C—H bond cleavage involves either carbanion and proton (H\(^+\)) formation or carbocation (carbonium ion) and hydride ion (H\(^-\)) formation. Since hydride ions are highly reactive species and carbon atoms are slightly more electronegative than hydrogen atoms, bond cleavage in which the electron pair remains with the carbon atom is the predominant mode of C—H bond breaking in biochemical systems. Hydride ion abstraction occurs only if the hydride is transferred directly to an acceptor such as NAD\(^+\) or NADP\(^+\).

Compounds participating in reactions involving heterolytic bond cleavage and bond formation are categorized into two broad classes: electron rich and electron deficient. Electron-rich compounds, which are called nucleophiles (nucleus lovers), are negatively charged or contain unshared electron pairs that easily form covalent bonds with electron-deficient centers. Biologically important nucleophilic groups include amino, hydroxyl, imidazole, and sulfhydryl functions (Fig. 15-5). The nucleophilic forms of these groups are also their basic forms. Indeed, nucleophilicity and basicity are closely related properties (Section 14-1B): A compound acts as a base when it forms a covalent bond with H\(^+\), whereas it acts as a nucleophile when it forms a covalent bond with an electron-deficient center other than H\(^+\), usually an electron-deficient carbon atom.

**Basic reaction of an amine**

\[
\text{R—N}^+\text{H}_2 + \text{H}^+ \rightarrow \text{R—N}^+\text{H}
\]

**Nucleophilic reaction of an amine**

\[
\text{R—N}^+\text{H}_2 + \text{C}=\text{O} \rightarrow \text{R—N}^+\text{C}=\text{OH}
\]

Electron-deficient compounds are called electrophiles (electron lovers). They may be positively charged, contain an unfilled valence electron shell, or contain an electronegative atom. The most common electrophiles in biochemical systems are H\(^+\), metal ions, the carbon atoms of carbonyl groups, and cationic imines (Fig. 15-6).

Reactions are best understood if the electron pair rearrangements involved in going from reactants to products can be traced. In illustrating these rearrangements we shall use the curved arrow convention in which the movement of an electron pair is symbolized by a curved arrow emanating from the electron pair and pointing to the electron-deficient center attracting the electron pair. For example, imine formation, a biochemically important reaction between an amine and an aldehyde or ketone, is represented:

**FIGURE 15-4.** Modes of C—H bond breaking. Homolytic cleavage yields radicals, whereas heterolytic cleavage yields either (i) a carbanion and a proton or (ii) a carboxication and a hydride ion.

**FIGURE 15-5.** Biologically important nucleophilic groups. Nucleophiles are the conjugate bases of weak acids.

In the first reaction step, the amine’s unshared electron pair adds to the electron-deficient carbonyl carbon atom while...
one electron pair from its C=O double bond transfers to
the oxygen atom. In the second step, the unshared electron
pair on the nitrogen atom adds to the electron-deficient
carbon atom with the elimination of water. At all times, the
rules of chemical reason apply to the system. For example,
there are never five bonds to a carbon atom or two bonds to
a hydrogen atom.

\[ \text{RCHO} + \text{Y}^- \rightarrow \text{RCOY} + \text{H}_2\text{O} \]

**Tetrahedral intermediate**

\[ \text{O}^- \quad \text{O}^- \quad \text{O}^- \]

**Trigonal bipyramid intermediate**

\[ \text{O}^- \quad \text{O}^- \quad \text{O}^- \]

**Resonance stabilized carbocation (oxonium ion)**

\[ \text{Y}^- \quad \text{Y}^- \quad \text{Y}^- \]

\[ \text{+ X}^- \]

**FIGURE 15-7.** Types of metabolic group-transfer reactions: (a) Acyl group transfer involves addition of a nucleophile (Y) to the electrophilic carbon atom of an acyl compound to form a tetrahedral intermediate. The original acyl carrier (X) is then expelled to form a new acyl compound. (b) Phosphoryl group transfer involves addition of a nucleophile (Y) to the electrophilic phosphorus atom of a tetrahedral phosphoryl group. This yields a trigonal bipyramidal intermediate whose apical positions are occupied by the leaving group (X) and the attacking group (Y). Elimination of the leaving group (X) to complete the transfer reaction results in the phosphoryl group's inversion of configuration. (c) Glycosyl group transfer involves the substitution of one nucleophilic group for another at C1 of a sugar ring. This reaction usually occurs via a double displacement mechanism in which the elimination of the original glycosyl carrier (X) is accompanied by the intermediate formation of a resonance-stabilized carbocation (oxonium ion) followed by the addition of the adding nucleophile (Y). The reaction also may occur via a single displacement mechanism in which Y directly displaces X with inversion of configuration.
trigonal bipyramidal intermediate whose apexes are occupied by the adding and leaving groups (Fig. 15-7b). The overall reaction results in the tetrahedral phosphoryl group's inversion of configuration. Indeed, chiral phosphoryl compounds have been shown to undergo just such an inversion. For example, Jeremy Knowles has synthesized ATP made chiral at its γ-phosphoryl group by isotopic substitution and demonstrated that this group is inverted upon its transfer to glucose in the reaction catalyzed by hexokinase (Fig. 15-8).

3. Glycosyl group transfer involves the substitution of one nucleophilic group for another at C1 of a sugar ring (Fig. 15-7c). This is the central carbon atom of an acetal. Chemical models of acetal reactions generally proceed via acid-catalyzed cleavage of the first bond to form a resonance-stabilized carbocation at C1 (an oxonium ion). The lysozyme-catalyzed hydrolysis of bacterial cell wall polysaccharides (Section 14-2B) is such a reaction.

C. Oxidations and Reductions

Oxidation–reduction (redox) reactions involve the loss or gain of electrons. The thermodynamics of these reactions is discussed in Section 15-5. Many of the redox reactions that occur in metabolic pathways involve C—H bond cleavage with the ultimate loss of two bonding electrons by the carbon atom. These electrons are transferred to an electron acceptor such as NAD⁺ (Fig. 12-2). Whether these reactions involve homolytic or heterolytic bond cleavage has not always been rigorously established. In most instances heterolytic cleavage is assumed when radical species are not observed. It is useful, however, to visualize redox C—H bond cleavage reactions as hydride transfers as diagrammed below for the oxidation of an alcohol by NAD⁺:

![Diagram of oxidation process]

The terminal acceptor for the electron pairs removed from metabolites by their oxidation is, for aerobic organisms, molecular oxygen (O₂). Recall that this molecule is a ground state diradical species whose unpaired electrons have parallel spins. The rules of electron pairing (the Pauli exclusion principle) therefore dictate that O₂ can only accept unpaired electrons; that is, electrons must be transferred to O₂ one at a time (in contrast to redox processes in which electrons are transferred in pairs). Electrons that are removed from metabolites as pairs must therefore be passed to O₂ via the electron-transport chain one at a time. This is accomplished through the use of conjugated coenzymes that have stable radical oxidation states and can therefore undergo both 1e⁻ and 2e⁻ redox reactions. One such coenzyme is FAD (whose structure and oxidation states are indicated in Fig. 14-28).

D. Eliminations, Isomerizations, and Rearrangements

Elimination Reactions Form Carbon–Carbon Double Bonds

Elimination reactions result in the formation of a double bond between two previously single-bonded saturated centers. The substances eliminated may be H₂O, NH₃, and...
alcohol (ROH), or a primary amine (RNH₂). The dehydration of an alcohol, for example, is an elimination reaction:

\[ \text{R} - \text{C} - \text{O} - \text{H} \rightarrow \text{R} - \text{C} = \text{C} \quad \text{+ H}_2\text{O} \]

Bond breaking and bond making in this reaction may proceed via one of three mechanisms (Fig. 15-9a): (1) concerted; (2) stepwise with the C—O bond breaking first to form a carbocation; or (3) stepwise with the C—H bond breaking first to form a carbanion.

Enzymes catalyze dehydration reactions by either of two simple mechanisms: (1) protonation of the OH group by an acidic group (acid catalysis), or (2) abstraction of the proton by a basic group (base catalysis). Moreover, in a stepwise reaction, the charged intermediate may be stabilized by an oppositely charged active site group (electrostatic catalysis). The glycolytic enzyme enolase (Section 16-2I) and the citric acid cycle enzyme fumarase (Section 19-3G) catalyze such dehydration reactions.

Elimination reactions may take one of two possible stereochemical courses (Fig. 15-9b): (1) trans (anti) eliminations, the most prevalent biochemical mechanism, and

(a) **Concerted**

\[ \text{R} - \text{C} - \text{C} - \text{R} ' \rightarrow \text{R} - \text{C} = \text{C} \quad \text{+ H}^+ \quad \text{+ OH}^- \]

(b) **Stepwise via a carbocation**

\[ \text{R} - \text{C} - \text{C} - \text{R} ' \rightarrow \text{R} - \text{C} - \text{C} \quad \text{+ R} ' \quad \text{+ H}^+ \quad \text{+ OH}^- \]

(c) **Stepwise via a carbanion**

\[ \text{R} - \text{C} - \text{C} - \text{R} ' \rightarrow \text{R} - \text{C} - \text{C} \quad \text{+ R} ' \quad \text{+ H}^+ \quad \text{+ OH}^- \]

FIGURE 15-9. Possible elimination reaction mechanisms using dehydration as an example. Reactions may be (a) either concerted, stepwise via a carbocation intermediate, or stepwise via a carbanion intermediate; and may occur (b) with either trans (anti) or cis (syn) stereochemistry.

(2) cis (syn) eliminations, which are biochemically less common.

**Biochemical Isomerizations Involve Intramolecular Hydrogen Atom Shifts**

Biochemical isomerization reactions involve the intramolecular shift of a hydrogen atom so as to change the location of a double bond. In such a process, a proton is removed from one carbon atom and added to another. The metabolically most prevalent isomerization reaction is the aldose—ketose interconversion, a base-catalyzed reaction that occurs via enediolate anion intermediates (Fig. 15-10). The glycolytic enzyme phosphoglucose isomerase catalyzes such a reaction (Section 16-2B).

Racemization is an isomerization reaction in which a hydrogen atom shifts its stereochemical position at a molecule's only chiral center so as to invert that chiral center. Such an isomerization is called an epimerization in a molecule with more than one chiral center.

**Rearrangements Produce Altered Carbon Skeletons**

Rearrangement reactions break and reform C—C bonds so as to rearrange a molecule's carbon skeleton. There are few such metabolic reactions. One is the conversion of L-methylmalonyl-CoA to succinyl-CoA by methylmalonyl-CoA mutase, an enzyme whose prosthetic group is a vitamin B₁₂ derivative:

\[ \text{H}^+ \quad \text{methylmalonyl-CoA} \quad \text{mutase} \quad \text{H}^+ \quad \text{CoO}^- \]

\[ \text{H} \quad \text{C} - \text{C} - \text{H} \quad \text{C} - \text{S} - \text{CoA} \quad \text{CoA} - \text{S} - \text{C} \quad \text{O} \]

\[ \text{L-Methylmalonyl-CoA} \quad \text{Succinyl-CoA} \]

**Carbon skeleton rearrangement**

\[ \text{C} - \text{C} - \text{C} \quad \text{C} - \text{C} - \text{C} \]

FIGURE 15-10. The mechanism of aldose—ketose isomerization. The reaction occurs with acid—base catalysis and proceeds via cis-enediolate intermediates.
This reaction is involved in the oxidation of fatty acids with an odd number of carbon atoms (Section 23-2E) and several amino acids (Section 24-3E).

**E. Reactions That Make and Break Carbon–Carbon Bonds**

Reactions that make and break carbon–carbon bonds form the basis of both degradative and biosynthetic metabolism. The breakdown of glucose to CO₂ involves five such cleavages, whereas its synthesis involves the reverse process. Such reactions, considered from the synthetic direction, involve addition of a nucleophilic carbanion to an electrophilic carbon atom. The most common electrophilic carbon atoms in such reactions are the sp²-hybridized carbonyl carbon atoms of aldehydes, ketones, esters, and CO₂.

\[
\begin{align*}
-C^+ & \rightarrow C=O \rightarrow C-C-\text{OH} \\
\text{(electrophilic center)} & \rightarrow B: + R-C-C-R' \\
& \text{(enolate)}
\end{align*}
\]

Stabilized carbanions must be generated to add to these.

---

**FIGURE 15-11.** Examples of C–C bond formation and cleavage reactions: (a) aldol condensation, (b) Claisen ester condensation, and (c) decarboxylation of a β-keto acid. All three types of reaction involve generation of a resonance-stabilized carbanion followed by addition of this carbanion to an electrophilic center.
3. EXPERIMENTAL APPROACHES TO THE STUDY OF METABOLISM

A metabolic pathway can be understood at several levels:

1. In terms of the sequence of reactions by which a specific nutrient is converted to end products, and the energetics of these conversions.

2. In terms of the mechanisms by which each intermediate is converted to its successor. Such an analysis requires the isolation and characterization of the specific enzymes that catalyze each reaction.

3. In terms of the control mechanisms that regulate the flow of metabolites through the pathway. An exquisitely complex network of regulatory processes renders metabolic pathways remarkably sensitive to the needs of the organism; the output of a pathway is generally only as great as required.

As you might well imagine, the elucidation of a metabolic pathway on all of these levels is a complex process, involving contributions from a variety of disciplines. Most of the techniques used to do so involve somehow perturbing the system and observing the perturbation’s effect on growth or on the production of metabolic intermediates. One such technique is the use of metabolic inhibitors that block metabolic pathways at specific enzymatic steps. Another is the study of genetic abnormalities that interrupt specific metabolic pathways. Techniques have also been developed for the dissection of organisms into their component organs, tissues, cells, and subcellular organelles, and for the purification and identification of metabolites as well as the enzymes that catalyze their interconversions. The use of isotopic tracers to follow the paths of specific atoms and molecules through the metabolic maze has become routine. New techniques utilizing NMR technology are able noninvasively to trace metabolites as they react in vivo. This section outlines the use of these various techniques.

A. Metabolic Inhibitors, Growth Studies, and Biochemical Genetics

Pathway Intermediates Accumulate in the Presence of Metabolic Inhibitors

The first metabolic pathway to be completely traced was the conversion of glucose to ethanol in yeast by a process known as glycolysis (Section 16-1A). In the course of these studies, certain substances, called metabolic inhibitors, were found to block the pathway at specific points, thereby causing preceding intermediates to build up. For instance, iodoacetate causes yeast extracts to accumulate fructose-1,6-bisphosphate, whereas fluoride causes the buildup of two phosphate esters, 3-phosphoglycerate and 2-phosphoglycerate. The isolation and characterization of these intermediates was vital to the elucidation of the glycolytic path-

---

**FIGURE 15-12.** The stabilization of carbanions:

(a) Carbanions adjacent to carbonyl groups are stabilized by the formation of enolates. (b) Carbanions adjacent to carbonyl groups hydrogen bonded to general acids are stabilized electrostatically or by charge neutralization. (c) Carbanions adjacent to protonated imines (Schiff bases) are stabilized by the formation of enamines. (d) Metal ions stabilize carbanions adjacent to carbonyl groups by the electrostatic stabilization of the enolate.