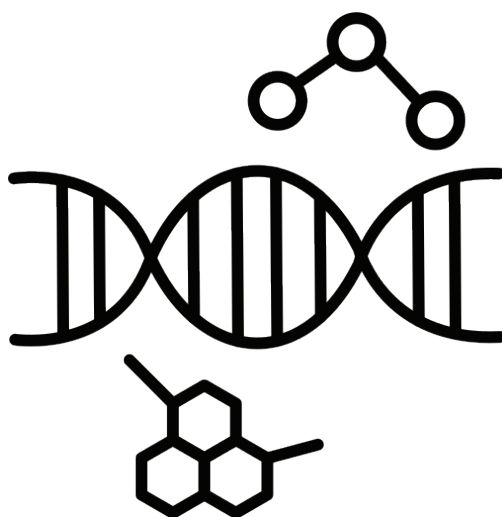


STRUCTURE AND FUNCTION OF BIOMOLECULES

BIOCHEMISTRY

7TH EDITION



Dr. Kailash Choudhary

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Design & Published by:
IFAS Publications

Address:
**Shivranjan Complex,
Someshwar Wadi, Near
Rajwada Hotel, Baner Road,
Pune 411 008.**

Contact No.: **+91-960 489 7388**

Website: **<https://ifasonline.com>**

7th Edition: **2026**

Price: **₹ 895.00**

ISBN: **978-81-69023-02-3**

PREFACE

A cell keeps no dictionary, yet it stores instructions, harvests energy, builds and dismantles, and answers the world around it — entirely in the grammar of molecules.

Biochemistry is the language in which life explains itself. To study it is to learn to read that language: to see why a single proton can decide the fate of a reaction, why a bond gives way exactly where it does, and how a handful of recurring chemical ideas can account for something as improbable as a living cell. This book was written to make that language learnable — and to make learning it a pleasure rather than an ordeal.

Biochemistry offers an excellent balance of basic and advanced topics, presented in a straightforward style that informs without burying the undergraduate under excessive detail. It provides a thorough, easy-to-understand introduction to the principles of the subject, and assumes only that the reader has had introductory biology as a prerequisite. Everything else is built up from first principles, in the order in which understanding actually develops.

The approach throughout is concept-based rather than encyclopaedic. Ideas are introduced where they are needed, illustrated by a wide spectrum of worked examples, and consolidated by questions that ask the reader to use a concept rather than merely recall it. The coverage runs from the basics of biochemistry through the biomolecules, the enzymes and metabolism, so that the chemistry of life is met as one continuous argument rather than a series of disconnected chapters.

Particular care has been taken with the topics that students most often struggle with. Solutions, pH and buffers, and thermodynamics are set out in a logical, structured format that keeps the core concepts in view instead of letting them disappear behind the arithmetic. Simple language is used deliberately, to clarify the facts that matter; the presentation is uncluttered; and the text is accompanied throughout by plenty of clear outline diagrams that carry the explanation rather than decorate it.

Above all, this book aims to build confidence. It is written for students preparing for graduate and postgraduate level examinations — readers who need not only to know biochemistry, but to trust what they know when it is tested. If a reader closes a chapter feeling that a difficult idea has become an obvious one, this book will have done its work.

Dr. Kailash Choudhary

ACKNOWLEDGEMENT

A book carries one name on its spine and the fingerprints of many hands on every page.

Writing Biochemistry has been an incredible journey. Every chapter in this volume reached its final shape only after endless inputs of time and effort — drafted, questioned, taken apart and rebuilt until the explanation was as clear as the science would allow. Many colleagues assisted me in compiling this book, and it is a genuine pleasure to record my debt to them here.

First of all, I would like to thank all the students at the Institute for Advanced Studies (IFAS). They have been this book's most demanding and most valuable teachers: it is in answering their questions that I learned to implement the concepts and ideas of biochemistry in their simplest form. Much of what is clear in these pages is clear because a student once told me that it was not.

I would like to make special mention of the effort made by Mr. Dadasaheb Sondge, COO, IFAS Publications, who extended immense support in myriad ways for bringing out the book in its present form.

My special thanks to Pranshu Dwivedi, Vishal Bhujbal and Digambar Jagtap for their suggestions and valuable contributions. I am grateful to the reviewers — Lalji Kannoujiya and Dr. Rakesh Kumawat — whose helpful suggestions improved the contents of this book at many points.

This book is a team effort, and producing it would have been impossible without the outstanding people of IFAS Publications. I am thankful to the entire publication team, for helpful suggestions that improved the quality of the content and for the regular reminders that brought the project to timely completion. It has been a pleasure to work with Vikendra Metha and Kuldeep Singh, who took great care over the copy-editing and production of this book.

And finally, my humble greetings to all those who put in significant effort and remain unmentioned here. Their work is in this book even if their names are not.

Dr. Kailash Choudhary

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Atoms and Molecules

LEARNING OBJECTIVES

- | | |
|---|--|
| 1.1 Elemental composition of life & CHNOPS | 1.5 Electron arrangement, valence & electronegativity |
| 1.2 Atomic structure, isotopes & radioactivity | 1.6 Molecules & the four biomolecules |
| 1.3 Atomic number & atomic mass | 1.7 Life-supporting properties of water |
| 1.4 Atomic mass unit, mole & Avogadro's number | |

All living organisms, including you and me, are composed of matter, and matter is made of combinations of elements. An element is a substance that cannot be separated into simpler substances by chemical means, and an atom is the smallest particle of an element that retains the element's chemical properties. An atom of one element differs in size and mass from the atoms of every other element. Atoms can be broken down into still smaller particles, although they lose their chemical identity in the process. To date, 118 elements have been identified; 94 of them occur naturally on Earth and the others have been created by scientists through nuclear processes. The cells of all organisms — whether derived from plant, animal or microbe — contain essentially the same 25 elements in almost the same proportions. Thus, of the 118 known elements, only around 25 are essential for terrestrial life (Table 1.1).

Table 1.1. *The elemental composition of the living cell.*

Element by weight	Symbol	Percentage in body
Oxygen	O	65
Carbon	C	18.5
Hydrogen	H	9.5
Nitrogen	N	3.2
Calcium	Ca	1.5
Phosphorus	P	1.0
Potassium	K	0.4
Sulfur	S	0.3
Sodium	Na	0.2
Chlorine	Cl	0.2
Magnesium	Mg	0.1
Trace elements	B, Cr, Co, F, I, Fe, Mn, Mo, Se, Si, Sn, V, Zn	less than 0.1

1.1 Elemental Composition of Life Forms

Although around 25 types of element can be found in biomolecules, six are the most common. These are called the CHNOPS elements; the letters stand for the chemical abbreviations of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur. These six elements contribute almost 98% of the total mass of cells and provide the structural elements of protoplasm, from which the functional components of cells are formed. Carbohydrates and lipids contain C, H and O, while proteins and nucleic acids contain in addition N and S, and N and P respectively. Na, K, Mg, Ca and Cl are usually found in ionic forms, whose concentrations must remain within narrow limits.

The presence of B, Cr, V, Mn, Fe, Co, Cu, Zn, Si, Sn and Mo, though in ultra-trace amounts, is essential for the functioning of enzymes; these elements are used as inorganic cofactors in enzyme catalysis. Simple relative abundance in the seas, crust and atmosphere of the Earth does not make an element suitable for life. For example, aluminium is more abundant than carbon, yet it performs no essential function in life. Remarkably, the CHNOPS elements all occur within the first three periods of the periodic table (Table 1.2). Their unique intrinsic chemical properties make them suitable as building blocks for life. The important features required to function as structural elements are:

- Small atomic size.
- The flexibility to form 1-, 2-, 3- and 4-electron bonds.
- The capability to form multiple bonds.

Table 1.2. *The structural elements of protoplasm; the common elements in the cell lie in the first three periods.*

Period	I	II	III	IV	V	VI	VII	VIII
1	H							He
2	Li	Be	B	C	N	O	F	Ne
3	Na	Mg	Al	Si	P	S	Cl	Ar

Small atoms form the tightest and most stable bonds — a distinct advantage for structural elements. H, O, N and C are the smallest atoms capable of forming 1-, 2-, 3- and 4-electron bonds respectively. Using every possible type of electron bond permits maximum versatility in molecular design, as does the ability to form multiple bonds — a property confined almost entirely to C, N, O, P and S.

Why carbon and not silicon?

Silicon (Si) lies in the same group as carbon in the periodic table. Like carbon it can bond to four other atoms at once, form long chains (polymers), and bind to oxygen; it is the second-most abundant element in the Earth's crust after oxygen. It is these chemical similarities between silicon and carbon that planted the idea of silicon-based life. However, all known life on Earth is based on carbon. The advantages of carbon-based over silicon-based life are:

- Greater chemical stability of C–C bonds than of Si–Si bonds.
- The ability of carbon (but not silicon) to form multiple bonds; for example, the oxides of carbon are diffusible monomeric gases, whereas the oxide of silicon is a viscous polymer.
- The stability of C–C bonds — but not of Si–Si bonds — to rupture by nucleophilic reagents (electron-rich elements or compounds) such as O₂, H₂O or NH₃.

Similar factors uniquely qualify phosphorus and sulfur for use in energy-transfer reactions. Energy transfer is facilitated by bonds that are susceptible to nucleophilic attack. P and S resemble Si in that P–O–P and S–O–S bonds, like Si–Si bonds, are susceptible to nucleophilic rupture by virtue of their unoccupied third orbitals. However, unlike Si, P and S can form multiple bonds — a consequence of their smaller atomic diameters — which makes them far more versatile.

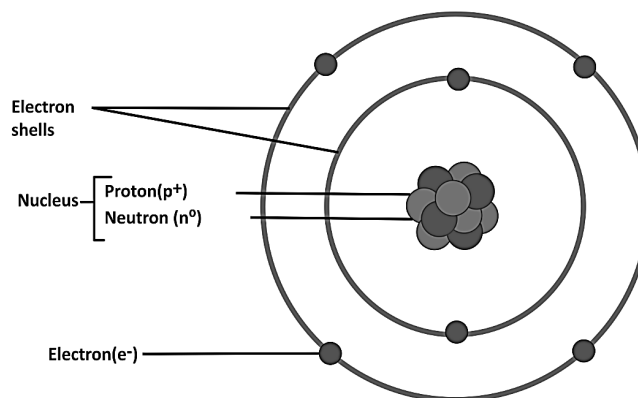
1.2 Structure of an Atom

Each kind of matter is composed of very small particles called atoms. In 1803, Dalton proposed the following statements, which together comprise the atomic theory of matter:

1. Matter consists of indivisible atoms.
2. All atoms of a given chemical element are identical in mass and in all other properties.
3. Different chemical elements have different kinds of atom, differing in particular in mass.
4. Atoms are indestructible and retain their identity in chemical reactions.
5. A compound forms from its elements through the combination of atoms of unlike elements in a small whole-number ratio.

Dalton's fourth postulate is clearly related to the law of conservation of mass: every atom of an element has a definite mass, and in a chemical reaction the atoms are merely rearranged, so the mass of the product remains the same. The fifth postulate is an attempt to explain the law of definite proportions: because the atoms have definite mass, a compound must contain its elements in definite proportions by mass. Although atoms were once thought to be the smallest particles, subatomic particles were later recognised. The three fundamental subatomic particles are the proton, the neutron and the electron. Besides these, about 35 other atomic particles are known to exist; many of these are extremely unstable and merely represent a bundle of energy.

Figure 1.1. Structure of an atom — a central positively charged nucleus of protons and neutrons, surrounded by electrons held in shells.



Regarding the arrangement of the fundamental particles inside the atom, Ernest Rutherford (1911) proposed the most satisfactory model, which is still accepted today with some modifications (Figure 1.1). Each atom has a positively charged nucleus at its centre, surrounded by a cloud of negatively charged electrons held in a series of orbitals or shells by electrostatic attraction. The nucleus contains two kinds of subatomic particle: protons, which are positively charged, and neutrons, which carry no charge.

A proton is a subatomic particle with a positive electric charge of +1 elementary charge and a mass slightly less than that of a neutron. Protons and neutrons, each with a mass of approximately one atomic mass unit, are together referred to as nucleons. A neutron is a subatomic particle of about the same mass as a proton but without electric charge; neutrons contribute to the structural stability of the nucleus. If there are too many or too few neutrons, the nucleus becomes unstable and may disintegrate by radioactive decay. Neutrons do not alter the chemical properties of the atom. Thus an atom can exist in several physically distinguishable but chemically identical forms, called isotopes.

Table 1.3. Mass and charge of the three fundamental subatomic particles.

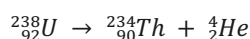
Particle	Mass	Charge
Proton	1.6726×10^{-27} kg	1.6022×10^{-19} C (positive)
Neutron	1.6749×10^{-27} kg	No charge
Electron	9.1×10^{-31} kg	1.6022×10^{-19} C (negative)

Isotopes are atoms of the same element having the same number of protons (atomic number) but different numbers of neutrons. They have the same chemical properties, owing to their identical electronic configuration, but different physical properties. In nature, almost all elements have multiple isotopes, some of which may be unstable (radioactive).

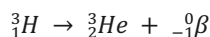
Radioactivity

Radioactivity is the phenomenon of the spontaneous emission of highly energetic radiation by unstable nuclei. Three types of radioactive particle are emitted — alpha (α) rays, beta (β) rays and gamma (γ) rays. The alpha rays have the least penetrating power, while the gamma rays have the greatest.

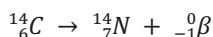
Alpha rays, when passed through an electric field, deflect towards the negative plate, indicating that alpha particles carry a positive charge. Each alpha particle has a mass of 4 units and a charge of +2, so it is identical to a helium nucleus. An example of a nucleus that undergoes alpha decay is uranium-238; its alpha decay is:



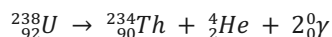
Beta (negatron) rays deflect towards the positive plate in an electric field, and the constituent particles of beta rays are called β particles. Each β particle has the mass and charge of an electron. During beta decay, a neutron splits into a proton and an electron; the proton stays in the nucleus, increasing the atomic number by one while the mass number remains unchanged, and the electron is ejected as beta radiation. For example, while most hydrogen exists as the stable isotope hydrogen-1 (one proton, no neutrons), the heavy isotope hydrogen-2 (deuterium) has one proton and one neutron, and small amounts of the unstable, radioactive isotope hydrogen-3 (tritium) have one proton and two neutrons. Tritium undergoes beta decay at a steady rate into stable helium:



Similarly, most carbon on Earth exists as the stable isotope carbon-12 (six protons, six neutrons), but small amounts of the unstable, radioactive isotope carbon-14 (six protons, eight neutrons) also occur. Carbon-14 undergoes beta decay at a slow but steady rate into nitrogen-14. This forms the basis of the technique known as carbon-14 dating, used in archaeology to determine the age of organic materials:



Gamma rays show no deflection in an electric field; they carry no charge and have negligible mass. In the alpha decay of U-238, two gamma rays of different energies are emitted in addition to the alpha particle:



Two further relationships are worth noting. Isobars are atoms of different elements having the same mass number but different atomic numbers — for example carbon ($Z = 6$, $A = 14$) and nitrogen ($Z = 7$, $A = 14$), or potassium ($Z = 19$, $A = 40$) and calcium ($Z = 20$, $A = 40$). Isotones are atoms of different elements that contain the same number of neutrons, although both their atomic and mass numbers differ — for example ${}^{14}_6\text{C}$, ${}^{15}_7\text{N}$ and ${}^{16}_8\text{O}$, each of which has 8 neutrons.

1.3 Atomic Number and Atomic Mass

The atomic number of any atom equals the number of protons in its nucleus. It is characteristic of a chemical element and determines the element's place in the periodic table. An atom of hydrogen has a nucleus of a single proton, so hydrogen, with atomic number 1, is the lightest element; an atom of carbon has six protons and an atomic number of 6.

The electric charge carried by each proton is exactly equal and opposite to the charge carried by a single electron. Since an atom as a whole is electrically neutral, the number of negatively charged electrons surrounding the nucleus equals the number of positively charged protons in it; thus the number of electrons in a neutral atom also equals the atomic number. It is these electrons that determine the chemical behaviour of an atom.

The relative mass of an atom or molecule compared with that of a hydrogen atom is termed its atomic mass or molecular mass respectively. Atomic mass equals the number of protons plus neutrons in the atom, while the atomic weight is the average, allowing for the relative abundances of the different isotopes. Electrons are ignored because they are much lighter and contribute almost nothing to the total (an electron is almost 1840 times lighter than a proton or neutron). Thus the major isotope of carbon (${}^{12}\text{C}$) has an atomic mass of 12, as it has 6 protons and 6 neutrons, whereas the unstable radioactive isotope ${}^{14}\text{C}$ has an atomic mass of 14.

1.4 Atomic Mass Unit

The dalton (Da), or atomic mass unit (amu), is the standard unit for measuring atomic mass. One dalton (1 amu) is approximately equal to the mass of a hydrogen atom — that is, approximately the mass of one nucleon (a single proton or neutron) — and is numerically equivalent to 1 g/mol. One proton or neutron weighs approximately $1/(6.022 \times 10^{23})$ gram, so one gram of hydrogen contains 6.022×10^{23} atoms.

Atoms are so small that it is hard to imagine their size. An individual carbon atom is roughly 0.2 nm in diameter, so it would take about 5 billion of them, laid out in a straight line, to span one metre. A count of 6.022×10^{23} atoms or molecules is called Avogadro's number. It is the key scale factor relating everyday quantities to quantities measured in individual atoms or molecules. If a substance has a molecular weight of X, then X grams of the substance contain 6.022×10^{23} molecules; this quantity is called one mole of the substance.

Thermodynamics and Bioenergetics

LEARNING OBJECTIVES

5.1 Systems & the first law (internal energy, enthalpy)

5.2 The second & third laws (entropy)

5.3 Gibbs free energy & spontaneity

5.4 ΔG , ΔG° and the equilibrium constant

5.5 Coupled reactions & group transfer

5.6 ATP and high-energy compounds

Thermodynamics deals with energy transformations in the universe — with heat, temperature and their relation to energy and work. It treats the bulk, macroscopic properties of a system rather than its microscopic constituents, and for the biologist it is chiefly concerned with the conditions under which processes occur spontaneously. A primary goal is to determine the heat exchanged between a system and its surroundings. The system is the part of the universe under study — as large as the whole Earth or as small as a mitochondrion or a single protein — and the surroundings are the rest of the universe that interacts with it. There are three basic kinds of system (Figure 5.1):

- An isolated system exchanges neither matter nor energy with its surroundings; the universe as a whole is an isolated system.
- A closed system may exchange energy but not matter — for example a tightly capped bottle of hot water, which loses heat but keeps its mass constant.
- An open system may exchange matter, energy or both. Living things are typically open systems, exchanging nutrients, waste and heat with their surroundings.

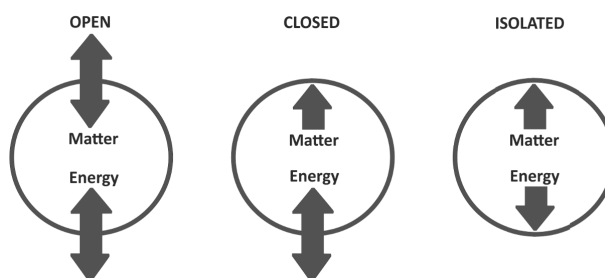


Figure 5.1. Open, closed and isolated systems, defined by what they can exchange with their surroundings.

5.1 The First Law: Conservation of Energy

The first law of thermodynamics states that the total energy of an isolated system, or of the universe, is constant: energy can be transferred from place to place or converted between forms, but it can be neither created nor destroyed. Light bulbs turn electrical energy into light, gas stoves turn chemical energy into heat, and plants perform one of the most important transformations on Earth — converting the energy of sunlight into the chemical energy of organic molecules.

Internal energy

The internal energy (U or E) is the function used to track heat transfers and work in a system. It is a state function: it depends only on the present state of the system, not on the path by which that state was reached, so if the system is taken through any cycle back to its starting state, U is unchanged. The internal energy can change only if energy flows in or out as heat or work.

For a process converting state 1 into state 2:

$$\Delta U = U_2 - U_1 = q + w \quad (1)$$

where q is the heat absorbed by the system from the surroundings and w is the work done on (+) or by (–) the system. In physical systems, work is force acting through a distance, $w = F \times d$, measured in joules. In chemical and biochemical systems the relevant work is usually pressure–volume work:

$$w = P \Delta V \quad (2)$$

where P is the pressure and $\Delta V = V_2 - V_1$ the volume change. If only mechanical work is considered and the volume is held constant, no work is done, so $\Delta U = q$ — the internal-energy change equals the heat exchanged at constant volume.

Illustrative Question 1. *The internal energy of a gas increases by 1 J when it is compressed by a force of 1 N through 2 m. What is the heat change of the system?*

Solution. From the first law $\Delta U = q + w$. The work done on the system is:

$$w = F \times d = 1 \text{ N} \times 2 \text{ m} = 2 \text{ J}$$

With $\Delta U = +1 \text{ J}$, solve for the heat q :

$$q = \Delta U - w = 1 \text{ J} - 2 \text{ J} = -1 \text{ J}$$

So the system releases 1 J of heat to the surroundings.

Enthalpy

Biochemical processes usually occur at constant pressure, where ΔU is not necessarily equal to the heat transferred. For such processes a more suitable state function, the enthalpy H , is defined:

$$H = U + P V \quad (3)$$

Being a combination of state functions, H is itself a state function. At constant pressure, combining the definitions above gives $\Delta H = \Delta U + P \Delta V = (q - w) + P \Delta V = q - P \Delta V + P \Delta V$, so:

$$\Delta H = q \quad (4)$$

Thus ΔH equals the heat transferred at constant pressure. Because the volume change in most biochemical processes is negligible, enthalpy and internal energy are often essentially equal. All forms of energy can be expressed in calories or joules: one calorie is the heat needed to raise 1 g of water from 14.5 °C to 15.5 °C, and 1 calorie = 4.18 J (1 kcal = 4.18 kJ).

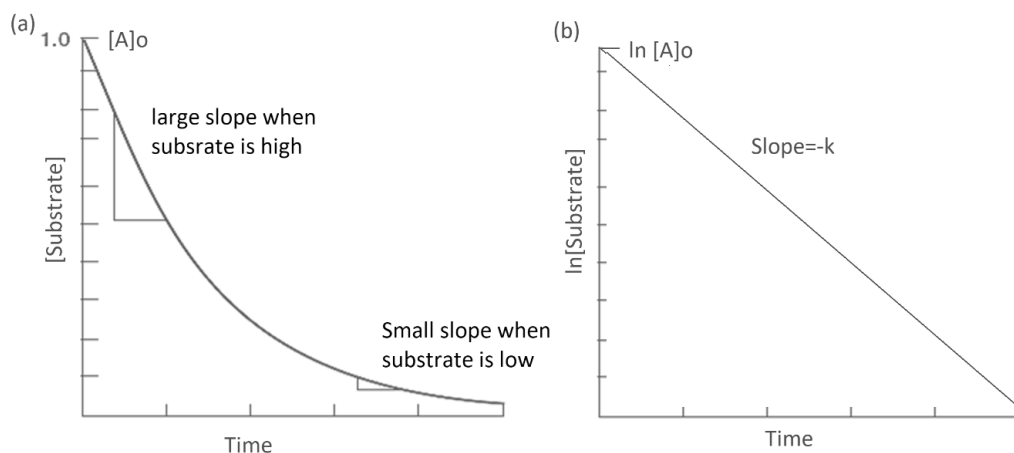
5.2 The Second and Third Laws: Entropy

The second law of thermodynamics explains why no energy transfer or transformation is completely efficient: in every transfer some energy is lost in an unusable form, usually as heat, increasing the disorder, or entropy, of the surroundings. Living systems require constant energy input to maintain their highly ordered, low-entropy state; the processes that keep them ordered increase the entropy of their surroundings, so the entropy of the universe always increases. The second law can be stated in several equivalent ways:

- Systems tend to proceed from ordered (low-entropy) to disordered (high-entropy) states.
- The entropy of system plus surroundings is unchanged by a reversible process, but increases for any irreversible process.
- All naturally occurring processes proceed toward equilibrium.

Entropy (S) is a measure of disorder and randomness. In a biochemical system it takes three forms: the freedom of a molecule to vibrate, twist and rotate; the random versus ordered arrangement of vast numbers of molecules; and changes in the number of individual molecules or ions during a reaction — the greater the number of particles, the greater the randomness. For any spontaneous process the overall ΔS must be positive. A reaction can still show a negative ΔS in the system if it releases enough heat (a large negative ΔH) to raise the entropy of the surroundings by more than the system's entropy falls, so that the total ΔS (system + surroundings) remains positive.

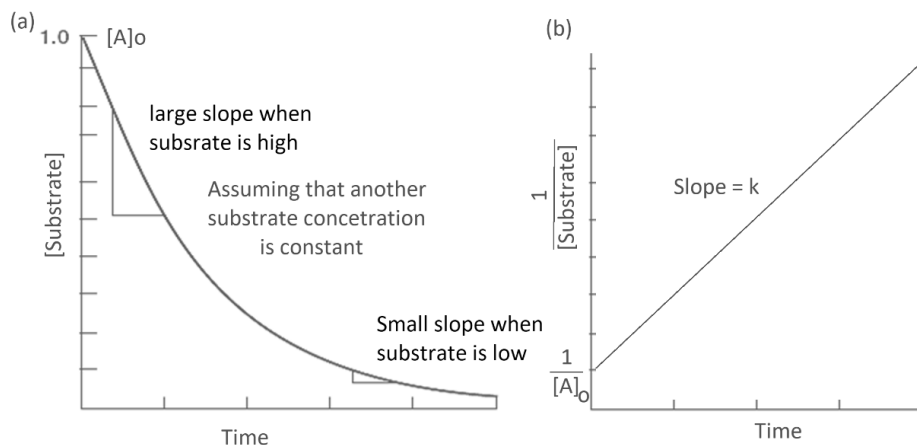
Figure 11.1. A first-order reaction: (a) velocity falls as substrate is consumed; (b) $\ln[A]$ versus time is linear with slope $-k$.



A second-order reaction depends on two concentrations ($2A \rightarrow \text{products}$ or $A + B \rightarrow \text{products}$), with rate $= k[A][B]$ and a rate constant in units of $M^{-1}s^{-1}$; if one reactant is held constant the reaction becomes pseudo-first-order in the other (Figure 11.2). Its integrated form gives a straight line of $1/[A]$ against time:

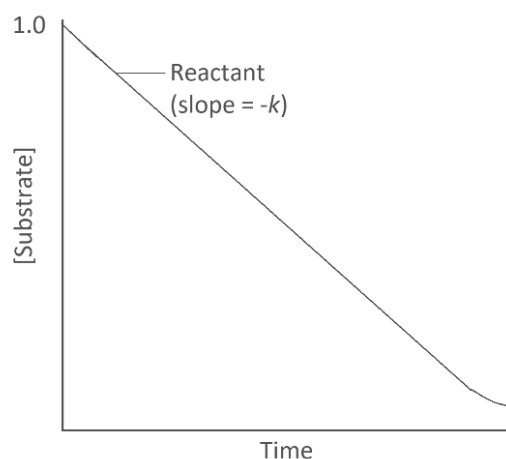
$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

Figure 11.2. A second-order reaction: (a) rate depends on both substrates; (b) $1/[A]$ versus time is linear with slope k .



A zero-order reaction has a rate independent of concentration (rate $= k$), so a plot of reactant against time is a straight line of slope $-k$ and the rate constant has units of $M \cdot s^{-1}$ (Figure 11.3). Enzyme-catalysed reactions become zero-order when the enzyme is saturated with substrate.

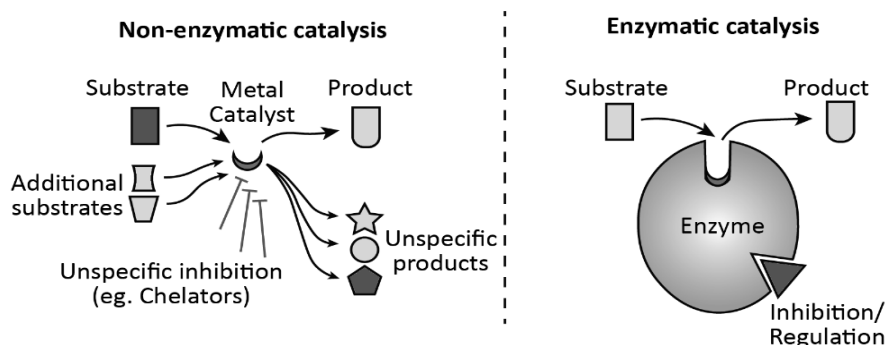
Figure 11.3. A zero-order reaction: the rate does not change as substrate concentration changes.



11.2 Enzymes: Nature and Classification

Enzymes are biological catalysts that speed up thermodynamically favourable reactions by thousands to millions of times, without themselves being consumed and without changing the reaction's equilibrium. Unlike ordinary chemical catalysts they are highly specific (Figure 11.4). Nearly all enzymes are proteins, but catalytic RNA molecules (ribozymes) show that RNA can also catalyse reactions and was probably an early biocatalyst. Enzymes work by binding substrates in an optimal orientation through many weak interactions and by stabilising the high-energy transition state, thereby selecting which of several possible reactions occurs.

Figure 11.4. Ordinary catalysts are non-specific, whereas enzymes are highly specific.



Historically, W. F. Kühne coined 'enzyme' (in yeast) in 1876; Eduard Buchner extracted active enzymes from yeast in 1897; J. B. Sumner crystallised urease from jack bean in 1926, proving enzymes are proteins; and Thomas Cech and Sidney Altman discovered catalytic RNA in 1982. Enzymes accelerate reactions up to a million-fold by lowering the activation energy through weak interactions with the substrate, without changing the equilibrium or the free-energy change. They are made by every cell (endoenzymes, largely mitochondrial, or secreted exoenzymes such as digestive enzymes), have high molecular weights (colloidal), and work best at an optimum pH and temperature (30–40 °C for most animals, with Q_{10} of 2–3). Compartmentalisation in organelles organises them into purposeful pathways.

Nomenclature and the six classes

The IUBMB names enzymes for the substrate and the reaction catalysed, reserving the -ase suffix for single catalytic entities and calling linked groups 'enzyme systems' (e.g. the pyruvate dehydrogenase system). Enzymes are grouped into seven classes, each given a four-part Enzyme Commission (EC) number — for example alcohol dehydrogenase is EC 1.1.1.1 and DNA ligase EC 6.5.1.1 (Figure 11.5).

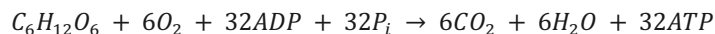
The oxidoreductases catalyse oxidation–reduction (dehydrogenases, oxidases, peroxidases, oxygenases; the largest class, with 19 subclasses). The transferases move chemical groups between substrates (including the polymerases and kinases). The hydrolases cleave bonds using water (esterases, phosphatases, glycosidases, peptidases, lipases, nucleases). The lyases break bonds without water, forming or adding to double bonds (decarboxylases, aldolases, dehydratases, some synthases). The isomerases interconvert isomers (racemases, epimerases, mutases). The ligases join two molecules at the expense of a nucleoside triphosphate or NAD^+ (DNA and RNA ligases, carboxylases, the CoA synthetases) — the term synthetase is reserved for this class. The translocases move ions or molecules across cell membranes, or separate them within membranes. They do not chemically change the transported molecule. Example include ATP-driven proton pumps.

11.3 Active Sites, Cofactors and Catalysis

The active site is the region that binds the substrate (and any cofactor) and contains the catalytic groups that make and break bonds (Figure 11.6). It is the part of the enzyme that most directly lowers the free energy of activation. Several generalisations hold: it is a three-dimensional cleft built from residues far apart in the sequence (in lysozyme, residues 35, 52, 62, 63, 101 and 108); it occupies only a small part of the enzyme, the rest serving as a scaffold, and only a few residues actually catalyse (the Asp-His-Ser triad of chymotrypsin); it is usually a non-polar crevice from which water is excluded; substrates bind by multiple weak interactions (never covalent peptide bonds), with dissociation constants of 10^{-2} to 10^{-8} M; and binding is specific.

Amphibolic role and the energetics of glucose oxidation

Cycle intermediates are drained for biosynthesis (cataplerotic reactions) — malate and oxaloacetate for gluconeogenesis, citrate for cytosolic acetyl-CoA and fatty-acid/cholesterol synthesis (via ATP-citrate lyase), oxaloacetate and α -ketoglutarate for aspartate and glutamate, and succinyl-CoA for porphyrins and heme (Figure 13.11) — and are replenished by anaplerotic reactions, chiefly pyruvate carboxylase making oxaloacetate. Overall, complete oxidation of one glucose through glycolysis and the cycle (with the malate–aspartate shuttle) yields 32 ATP:



Burning glucose in a calorimeter releases 2780 kJ; the 32 ATP capture about 976 kJ, roughly 35% of the energy — a high efficiency for a biological system.

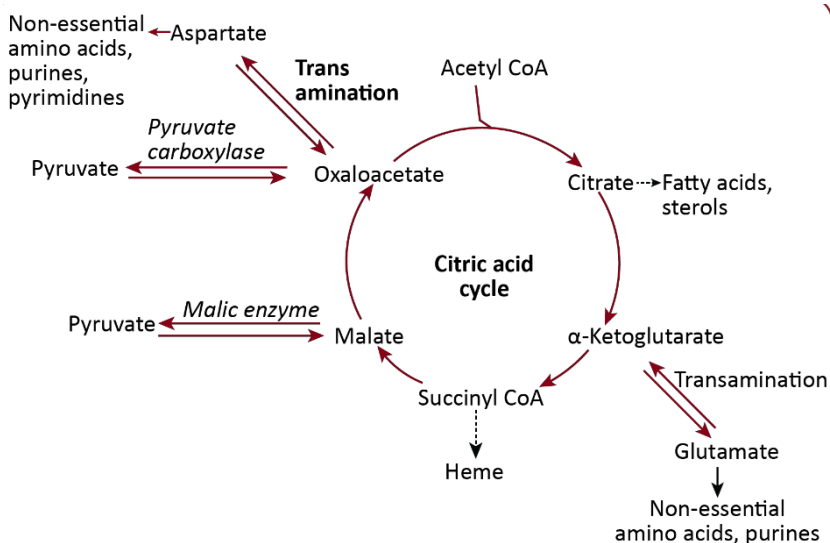


Figure 13.11. The amphibolic functions of the citric acid cycle.

TCA Cycle and Oncometabolites

The tricarboxylic acid (TCA) cycle is the central metabolic pathway that oxidizes acetyl-CoA to generate ATP through NADH and $FADH_2$ production. Besides energy generation, it supplies intermediates for the biosynthesis of amino acids, nucleotides, and lipids. Mutations in certain TCA cycle enzymes lead to the accumulation of abnormal metabolites called *oncometabolites*, which promote cancer by altering epigenetic regulation and activating hypoxia signaling.

Mutated gene	Enzyme	Oncometabolite	Associated cancers
<i>IDH1, IDH2</i>	Isocitrate dehydrogenase	D-2-Hydroxyglutarate (2-HG)	Glioma, AML, cholangiocarcinoma
<i>SDHA, SDHB, SDHC, SDHD</i>	Succinate dehydrogenase	Succinate	Paraganglioma, pheochromocytoma, GIST
<i>FH</i>	Fumarate hydratase	Fumarate	Hereditary leiomyomatosis and renal cell carcinoma

2-Hydroxyglutarate, succinate, and fumarate inhibit α -ketoglutarate-dependent dioxygenases, resulting in abnormal DNA and histone methylation, impaired cellular differentiation, and stabilization of hypoxia-inducible factor (HIF), thereby promoting tumor development.

Lactate as an Oncometabolite

Lactate, produced by enhanced glycolysis (Warburg effect), is now recognized as a non-classical oncometabolite. It acidifies the tumor microenvironment, promotes angiogenesis, suppresses anti-tumor immune responses, and facilitates cancer cell invasion and metastasis. Lactate also serves as an energy source for neighboring cancer cells and regulates gene expression through histone lactylation.

13.5 The Pentose Phosphate Pathway

The pentose phosphate pathway (hexose monophosphate shunt, or phosphogluconate pathway) is a cytosolic alternative to glycolysis that starts from glucose-6-phosphate and generates NADPH and pentose sugars without using or making ATP. It is most active in tissues that build fatty acids and steroids — liver, adipose tissue, adrenal cortex, mammary gland — and in red cells. It has an oxidative and a non-oxidative phase.

In the oxidative phase, glucose-6-phosphate dehydrogenase (the committed, rate-limiting enzyme) oxidises G6P to 6-phosphogluconolactone with NADPH; lactonase hydrolyses it to 6-phosphogluconate; and 6-phosphogluconate dehydrogenase oxidatively decarboxylates it to ribulose-5-phosphate, a second NADPH and CO₂ (Figure 13.12).

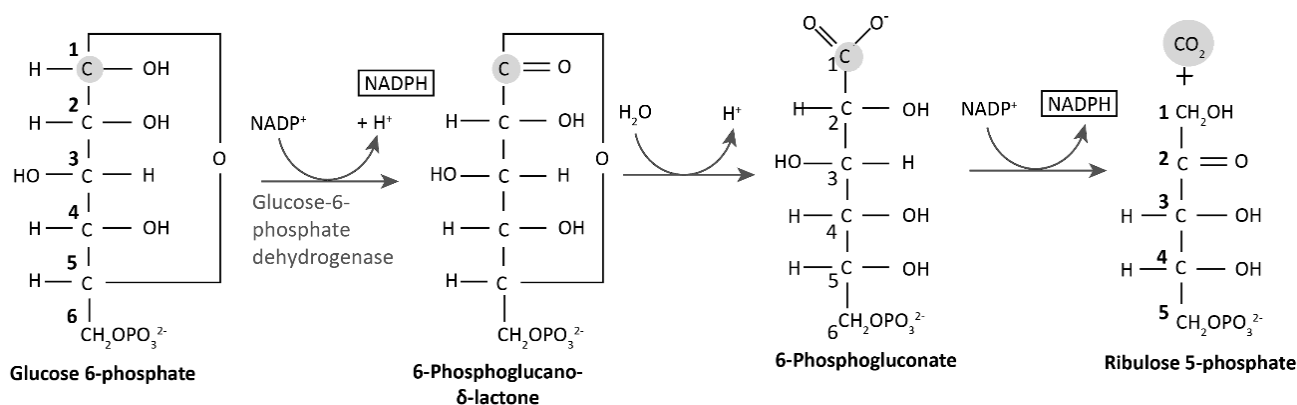
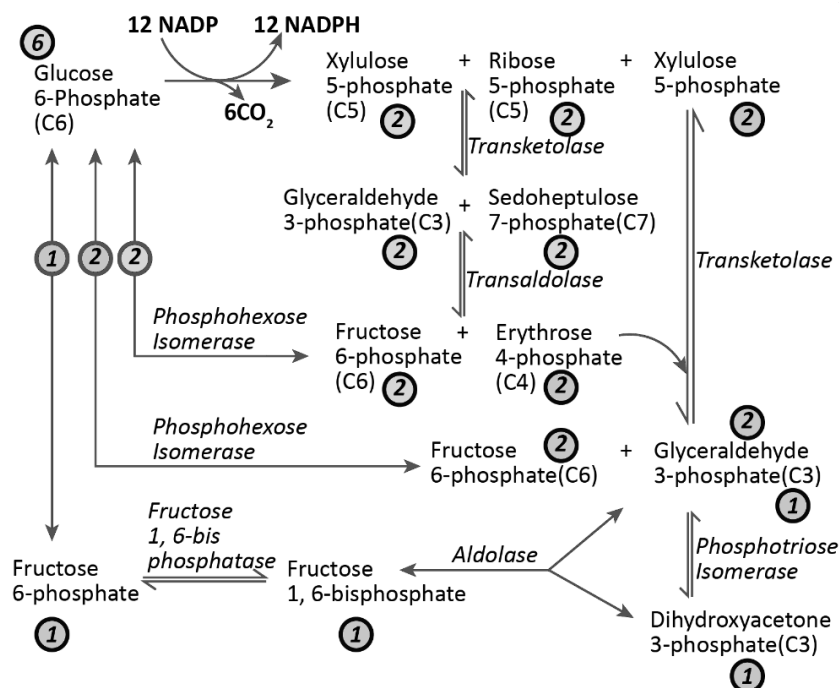


Figure 13.12. The oxidative phase of the pentose phosphate pathway.

In the non-oxidative phase, ribulose-5-phosphate is isomerised to ribose-5-phosphate and epimerised to xylulose-5-phosphate, and then transketolase (needing TPP) and transaldolase reshuffle carbons among these sugars to yield fructose-6-phosphate and glyceraldehyde-3-phosphate, linking the pathway back to glycolysis (Figure 13.13). Complete oxidation of six G6P regenerates five and gives twelve NADPH:

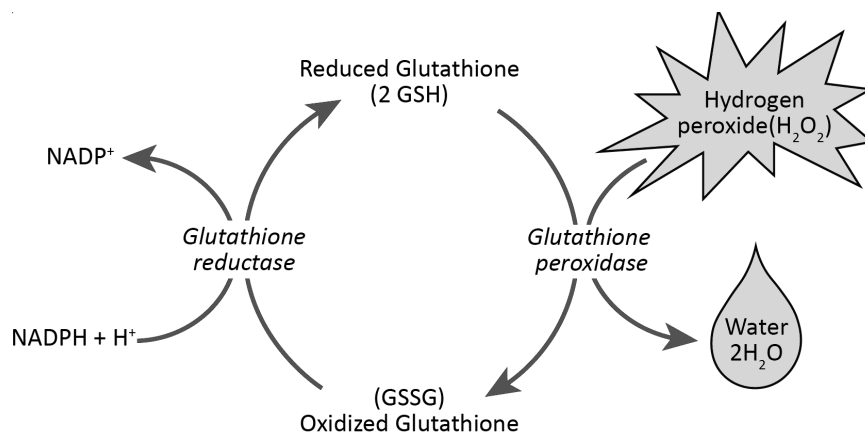
Figure 13.13. The complete pentose phosphate pathway, including the non-oxidative reactions.



Roles of NADPH and pentoses; G6PD deficiency

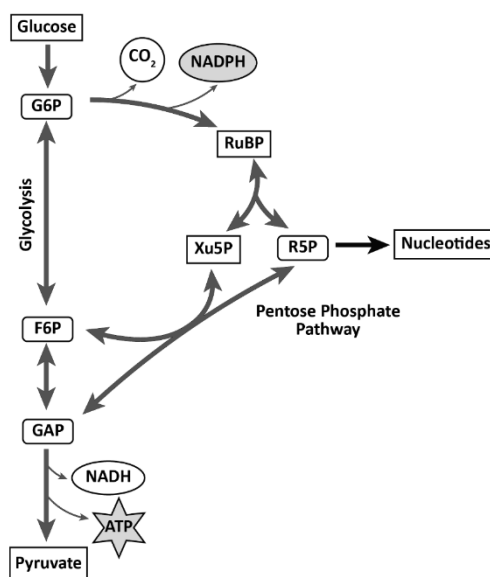
The pathway's two products serve distinct needs. Ribose-5-phosphate builds the nucleotides of RNA, DNA, ATP, NAD⁺, FAD and CoA. NADPH, chemically distinct from NADH, powers reductive biosynthesis of fatty acids and steroids, the glutathione defence against hydrogen peroxide (Figure 13.14), cytochrome-P450 detoxification, phagocytosis, and — in the red cell — the maintenance of reduced glutathione and of haemoglobin iron in the ferrous state.

Figure 13.14. The role of NADPH in the glutathione antioxidant system.



Because the shunt is the red cell's only source of NADPH, an inherited, X-linked deficiency of glucose-6-phosphate dehydrogenase causes haemolytic anaemia when oxidative stress rises — on eating fava beans (favism) or taking the antimalarial primaquine. The high frequency of G6PD deficiency in malarial regions reflects the resistance it confers to *Plasmodium falciparum*, a selective advantage like that of sickle-cell trait. The pathway is controlled by the NADPH/NADP⁺ ratio, NADPH competitively inhibiting G6PD, and transketolase and transaldolase balance the output of pentose and NADPH against demand (Figure 13.15).

Figure 13.15. The relationship between glycolysis and the pentose phosphate pathway.



13.6 Gluconeogenesis and the Glyoxylate Cycle

Gluconeogenesis makes glucose from non-carbohydrate precursors — lactate, pyruvate, glucogenic amino acids, glycerol and propionate — chiefly in the liver (and a little in the kidney). It is essentially the reverse of glycolysis, sharing seven reactions, but bypasses the three irreversible glycolytic steps with its own enzymes (Figure 13.16).

It is vital because the brain (about 120 g of glucose a day), red cells, testes and kidney medulla depend on glucose, and in fasting it maintains blood glucose and clears lactate, glycerol and propionate.

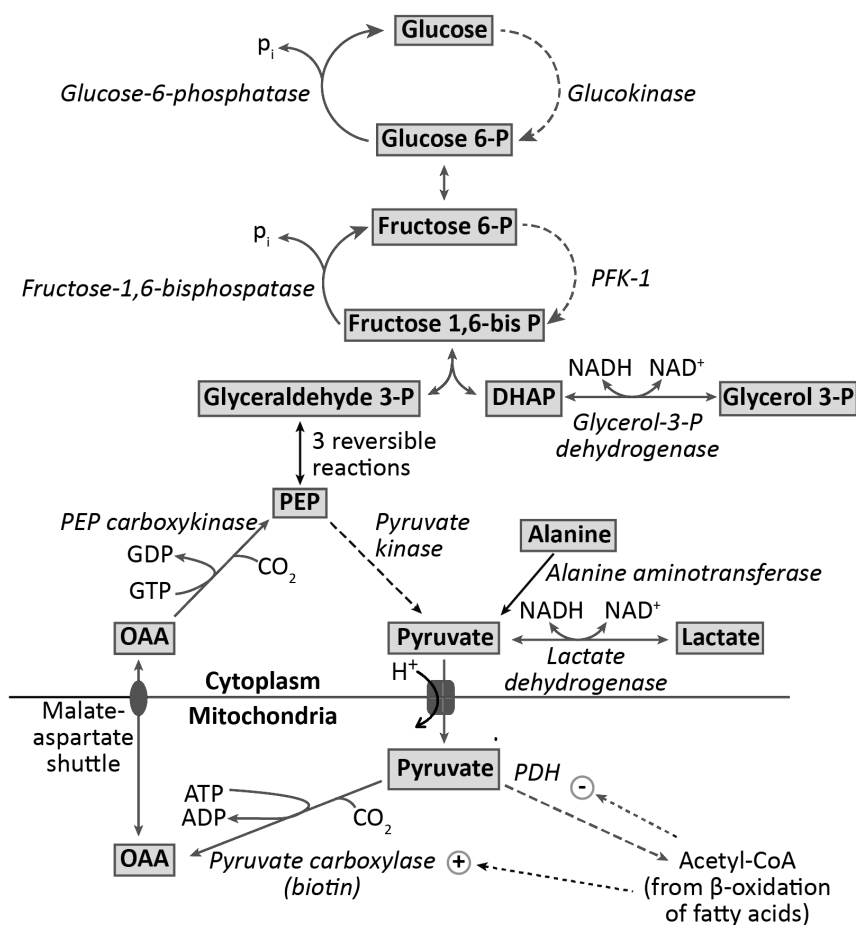
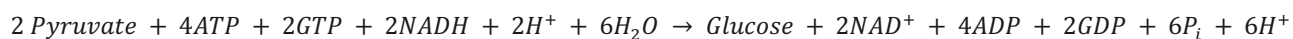


Figure 13.16. The gluconeogenesis pathway and its relationship to glycolysis.

The three bypasses are:

- (1) pyruvate is carboxylated to oxaloacetate by mitochondrial pyruvate carboxylase (using ATP and biotin, and activated by acetyl-CoA — an anaplerotic step), oxaloacetate is exported as malate, and cytosolic PEP carboxykinase then converts it to phosphoenolpyruvate using GTP (so two high-energy phosphates are spent where glycolysis gained one);
- (2) fructose-1,6-bisphosphatase (Mg^{2+} -dependent, regulatory) hydrolyses fructose-1,6-bisphosphate to fructose-6-phosphate; and
- (3) glucose-6-phosphatase, bound to the endoplasmic reticulum and present only in liver and kidney, hydrolyses G6P to free glucose for export.

Making one glucose from two pyruvate costs six high-energy phosphates (2 ATP at pyruvate carboxylase, 2 GTP at PEP carboxykinase, 2 ATP at phosphoglycerate kinase):



Eighteen of the twenty amino acids are glucogenic (only leucine and lysine, purely ketogenic, are not, since they give only acetyl-CoA); glycerol enters as DHAP; and propionate (from odd-chain fatty acids and some amino acids) enters as succinyl-CoA via the citric acid cycle, an important route in ruminants.

Lactate from muscle is carried to the liver, remade into glucose and returned — the Cori cycle (Figure 13.17) — and muscle alanine is used similarly in the glucose–alanine cycle. Glycolysis and gluconeogenesis are reciprocally regulated to avoid a futile cycle (Figure 13.18): AMP activates PFK and inhibits fructose-1,6-bisphosphatase, while ATP and citrate do the reverse; and F-2,6-BP, set by the glucagon/insulin balance, stimulates glycolysis and inhibits gluconeogenesis in the fed state and the reverse in fasting. In the liver, glucagon phosphorylates and inhibits pyruvate kinase to spare glucose, while acetyl-CoA from fat breakdown activates pyruvate carboxylase.