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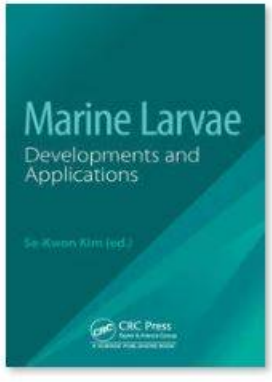

Chapter

Chapter 13 Potential Secondary Metabolites and Medical Application of Marine Larvae

By J. Jeyasrree, J. Jasmine Buelah, Abdul Bakrudeen Ali Ahmed, Sumathy Rengarajan

Book Marine Larvae

Edition	1st Edition
First Published	2024
Imprint	CRC Press
Pages	13
eBook ISBN	9781003359388

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A HAND BOOK OF TECHNIQUES IN BIOCHEMISTRY

Edited by
DR. A. SUNDARESAN

978-93-6255-994-4

A Hand Book of Techniques in Biochemistry

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ISBN: 978-93-6255-994-4

Publisher: Ponnaiyah Ramajayam Institute of Science and Technology (PRIST)

Contents

SECTION A : CHEMICAL BASIS OF LIFE

CHAPTER
1

Biochemical Perspective to Medicine

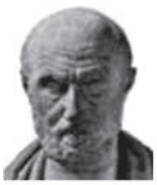
CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

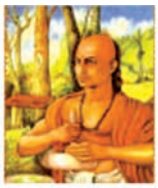
1. History of biochemistry
2. Biomolecules and metabolism
3. Ionic bonds
4. Hydrogen bonding
5. Hydrophobic interactions
6. Principles of thermodynamics
7. Donnan membrane equilibrium

Biochemistry is the language of biology. The tools for research in all the branches of medical science are based on principles of biochemistry. The study of biochemistry is essential to understand basic functions of the body. This will give information regarding the functioning of cells at the molecular level. How the food that we eat is digested, absorbed, and used to make ingredients of the body? How does the body derive energy for the normal day to day work? How are the various metabolic processes interrelated? What is the function of genes? What is the molecular basis for immunological resistance against invading organisms? Answer for such basic questions can only be derived by a systematic study of medical biochemistry.

Modern day medical practice is highly dependent on the laboratory analysis of body fluids, especially the blood. The disease manifestations are reflected in the composition of blood and other tissues. Hence, the demarcation of abnormal from normal constituents of the body is another aim of the study of clinical biochemistry.



Hippocrates
460-377 BC



Charaka
400 BC

Sushrutha
500 BC

The word chemistry is derived from the Greek word "chemi" (the black land), the ancient name of Egypt. Indian medical science, even from ancient times, had identified the metabolic and genetic basis of diseases. Charaka, the great master of Indian Medicine, in his treatise (circa 400 BC) observed that *madhumeha* (diabetes mellitus) is produced by the alterations in the metabolism of carbohydrates and fats; the statement still holds good.

Biochemistry has developed as an offshoot of organic chemistry, and this branch was often referred as "physiological chemistry". The term "Biochemistry" was coined by Neuberg in 1903 from Greek words, bios (= life) and chymos (= juice). One of the earliest treatises in biochemistry was the "Book of Organic Chemistry and its Applications to Physiology and Pathology", published in 1842 by Justus von Liebig (1803-73), who introduced the concept of metabolism. The "Textbook of Physiological Chemistry" was published in 1877 by Felix Hoppe-Seyler (1825-95), who was professor of physiological chemistry at Strausbourge University, France. Some of the milestones in the development of science of biochemistry are given in Table 1.1.

The practice of medicine is both an art and a science. The word "doctor" is derived from the Latin root, "docere", which means "to teach". Knowledge devoid of ethical background may sometimes be disastrous! Hippocrates (460 BC to 377 BC), the father of modern medicine articulated "the Oath". About one century earlier, Sushrutha (500 BC), the great Indian surgeon, enunciated a code of conduct to the medical practitioners, which is still valid. He proclaims: "You must speak only truth; care for the good of all living beings; devote yourself to the healing of the sick even if your life be lost by your work; be simply clothed and drink no intoxicant; always seek to grow in knowledge; in face of God, you can take upon yourself these vows."

Biochemistry is perhaps the most rapidly developing subject in medicine. No wonder, the major share of Nobel prizes in medicine has gone to research workers engaged in biochemistry. Thanks to the advent of DNA-recombination technology, genes can now be transferred from one person to another, so that many of the genetically determined diseases are now amenable to gene therapy. Many genes, (e.g. human insulin gene) have already been transferred to microorganisms for large scale production of human proteins. Advances in genomics like RNA interference for silencing of genes and creation of transgenic animals by gene targeting of embryonic stem cells are opening up new vistas in therapy of diseases like cancer and AIDS. It is hoped that in future, physician will be able to treat the patient, understanding his genetic basis, so that very efficient "designer medicine" could cure the diseases. The large amount of data, especially with regard to single

CHAPTER
2

Subcellular Organelles and Cell Membranes

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Nucleus
2. Endoplasmic reticulum
3. Golgi apparatus
4. Lysosomes
5. Mitochondria
6. Plasma membrane
7. Transport mechanisms
8. Simple and facilitated diffusion
9. Ion channels
10. Active transport
11. Uniport, symport and antiport

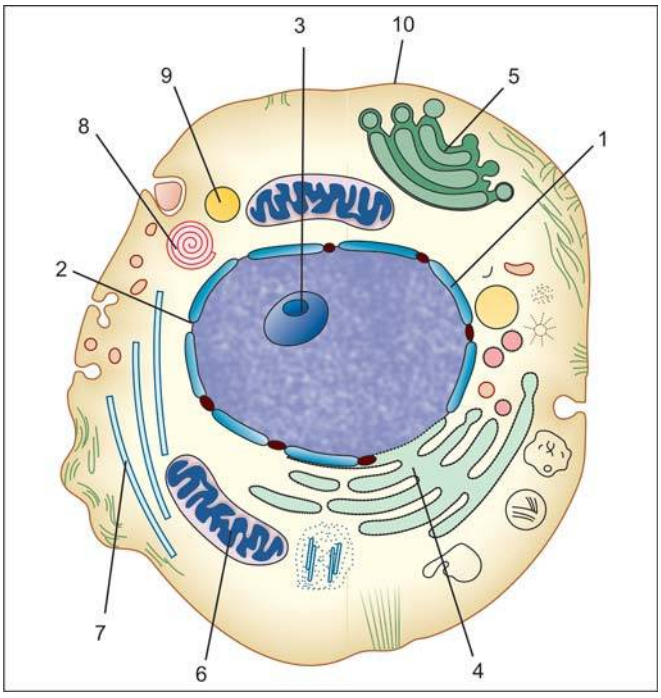


Fig. 2.1. Typical cell

1= Nuclear membrane; 2= Nuclear pore; 3= Nucleolus; 4= endoplasmic reticulum; 5= Golgi body; 6= Mitochondria; 7= Microtubule; 8= Lysosome; 9= Vacuole; 10= Plasma membrane

SUBCELLULAR ORGANELLES

Cells contain various organized structures, collectively called as cell organelles (Fig. 2.1). When the cell membrane is disrupted, either by mechanical means or by lysing the membrane by Tween-20 (a lipid solvent), the organized particles inside the cell are homogenised. This is usually carried out in 0.25 M sucrose at pH 7.4. The organelles could then be separated by applying differential centrifugal forces (Table 2.1). Albert Claude got Nobel prize in 1974 for fractionating subcellular organelles.

Marker Enzymes

Some enzymes are present in certain organelles only; such specific enzymes are called as marker enzymes (Table 2.1). After centrifugation, the separated organelles are identified by detection of marker enzymes in the sample.

NUCLEUS

1. It is the most prominent organelle of the cell. All cells in the body contain nucleus, except mature RBCs in circulation. The uppermost layer of skin also may not possess a readily identifiable nucleus. In some cells, nucleus occupies most of the available space, e.g. small lymphocytes and spermatozoa.
2. Nucleus is surrounded by two membranes: the inner one is called perinuclear membrane with

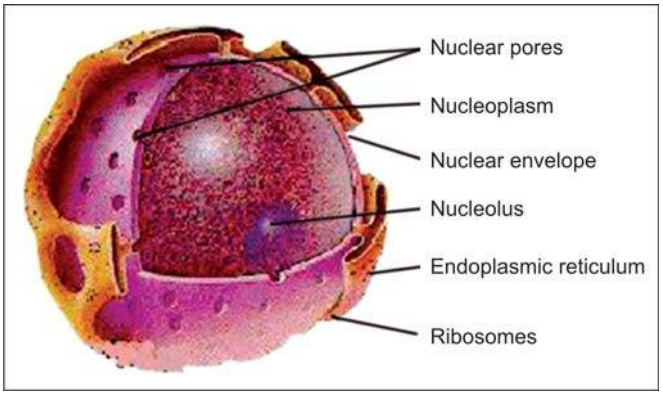


Fig. 2.2. Nucleus

CHAPTER
3

Amino Acids: Structure and Properties

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Classification of amino acids based on structure
2. Based on side chain character
3. Based on metabolic fate
4. Based on nutritional requirements
5. Iso electric point
6. Reactions due to carboxyl group
7. Reactions due to amino group
8. Reactions of SH group
9. Peptide bond formation

Proteins are of paramount importance for biological systems. All the major structural and functional aspects of the body are carried out by protein molecules. All proteins are polymers of amino acids. Proteins are composed of a number of amino acids linked by peptide bonds.

Although about 300 amino acids occur in nature, only 20 of them are seen in human body. Most of the amino acids (except proline) are **alpha amino acids,** which means that the amino group is attached to the same carbon atom to which the carboxyl group is attached (Fig. 3.1).

CLASSIFICATION OF AMINO ACIDS

1. Based on Structure

1-A. Aliphatic amino acids

a. Mono amino mono carboxylic acids:

- Simple amino acids: Glycine, Alanine (Fig. 3.2)
- Branched chain amino acids: Valine, Leucine, Isoleucine (Fig. 3.3)
- Hydroxy amino acids: Serine, Threonine (Fig. 3.4.)
- Sulphur containing amino acids: Cysteine, Methionine (Fig. 3.5)
- Amino acids with amide group: Asparagine, Glutamine (Fig. 3.6)

b. Mono amino dicarboxylic acids: Aspartic acid, Glutamic acid (Fig. 3.7)

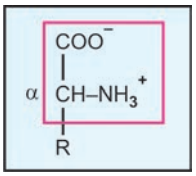


Fig. 3.1. General structure

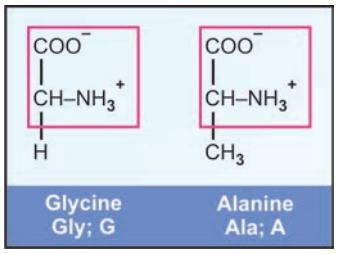


Fig. 3.2 Simple amino acids

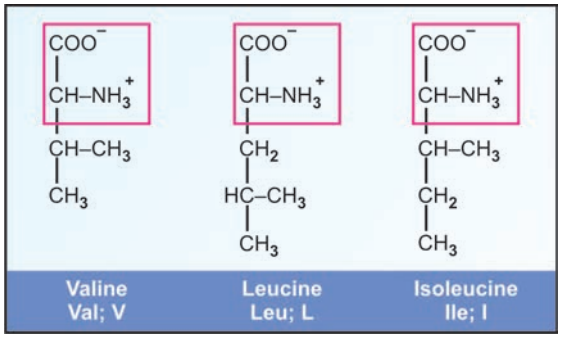


Fig. 3.3. Branched chain amino acids

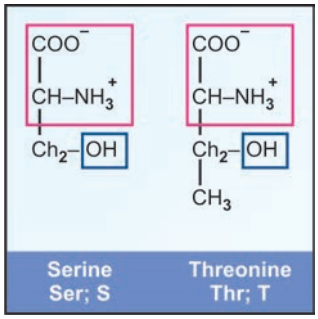


Fig. 3.4. Hydroxy amino acids

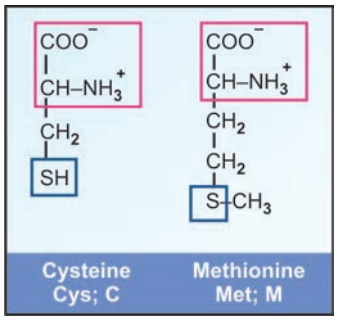


Fig. 3.5. Sulphur containing amino acids

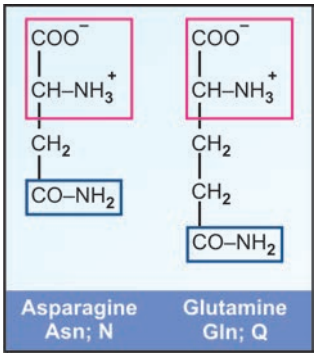


Fig. 3.6. Amino acids with amide groups

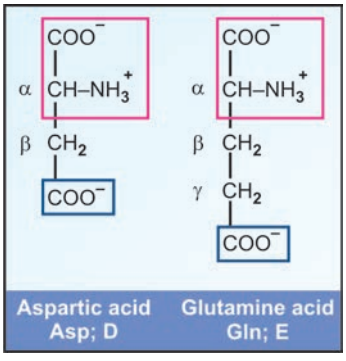


Fig. 3.7. Dicarboxylic amino acids

CHAPTER
4

Proteins:
Structure and Function

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Peptide bonds
2. Primary structure of proteins
3. Secondary structure
4. Tertiary structure
5. Quaternary structure
6. Sequence analysis (study of primary structure)
7. Iso-electric pH of proteins
8. Precipitation reactions of proteins
9. Classification of proteins
10. Quantitative estimation of proteins

The word protein is derived from Greek word, "proteios" which means primary. As the name shows, the proteins are of paramount importance for biological systems. Out of the total dry body weight, 3/4ths are made up of proteins. Proteins are used for body building; all the major structural and functional aspects of the body are carried out by protein molecules. Abnormality in protein structure will lead to molecular diseases with profound alterations in metabolic functions.

Proteins contain Carbon, Hydrogen, Oxygen and Nitrogen as the major components while Sulphur and Phosphorus are minor constituents. Nitrogen is characteristic of proteins. **On an average, the nitrogen content of ordinary proteins is 16% by weight.** All proteins are polymers of amino acids.

Amino Acids are Linked by Peptide Bonds

Alpha carboxyl group of one amino acid reacts with alpha amino group of another amino acid to form a peptide bond or CO-NH bridge (Fig. 4.1A).

Proteins are made by polymerisation of amino acids through peptide bonds. Two amino acids are combined to form a **dipeptide**; three amino acids form a **tripeptide**; four will make a **tetrapeptide**; a few amino acids together will make an **oligopeptide**; and combination of 10 to 50 amino acids is called as a **polypeptide.** By convention, big polypeptide chains containing more than 50 amino acids are called **proteins**.

In a tripeptide, there are 3 amino acids, but these 3 can be any of the total 20 amino acids. Thus 20^3 = 8000 different permutations and combinations are possible in a tripeptide. An ordinary protein having about 100 amino acids, will have 20^{100} different possibilities. This number is more than the total number of atoms present in the whole universe. Thus, even though there are only 20 amino acids, by changing the sequence of combination of these amino acids, nature produces enormous number of markedly different proteins.

STRUCTURE OF PROTEINS (Organisation of Proteins)

Proteins have different levels of structural organisation; primary, secondary, tertiary and quaternary.

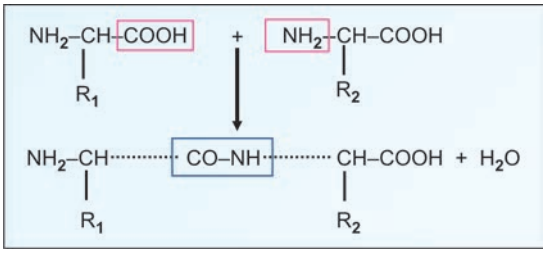


Fig. 4.1A. Peptide bond formation

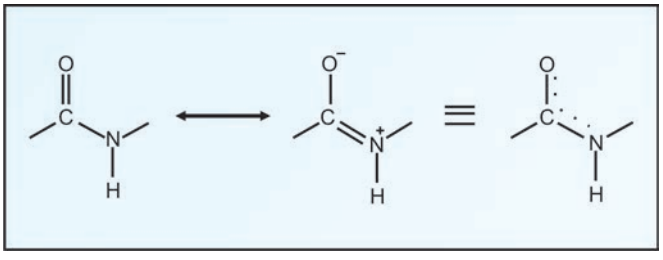


Fig. 4.1B. Peptide bond is a partial double bond

CHAPTER
5

Enzymology: General Concepts and Enzyme Kinetics

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Classification of enzymes
2. Co-enzymes
3. Mode of action of enzymes
4. Michaelis-Menten theory
5. Fischer's template theory
6. Koshland's induced fit theory
7. Michaelis constant, Km value, Vmax
8. Factors influencing enzyme activity
9. Enzyme activation
10. Inhibition, competitive, non-competitive
11. Allosteric inhibition, suicide inhibition
12. Covalent modification
13. Iso-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 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 division was possible; 1st son got one-half or 9 horses; 2nd son got 6 and 3rd son 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 which accelerate the rate of chemical reactions, but do not change the equilibrium.

Berzelius in 1835 showed hydrolysis of starch by malt extract and put forward the theory of enzyme catalysis (see Table 1.1). In 1878 Willy Kunhe coined the word enzyme, which in Greek means "in yeast". Edward Buchner (Nobel prize 1907) showed that cell-free extract of yeast could catalyse the fermentation of sucrose to ethanol. He named this active principle as Zymase. Sir Arthur Harden in 1897 (Nobel prize 1929) showed that Zymase is a complex mixture of enzymes, each catalysing a separate step in the degradation of sucrose. The rate of chemical reactions, chemical equilibrium and catalysis were studied by Ostwald

Edward Buchner NP 1907 1860-1917 | Arthur Harden NP 1929 1865-1940 | James Sumner NP 1946 1887-1955 | John Northrop NP 1946 1891-1987 | Wilhelm Ostwald NP 1909 1853-1932

(Nobel prize 1909). In 1926, James Sumner (Nobel prize 1946) was the first to crystallise the enzyme urease. In 1930, John Northrop (Nobel prize, 1946) crystallized a number of proteolytic enzymes from gastrointestinal tract and proved that they are all proteins.

Enzymes are biocatalysts

Life is possible due to the co-ordination 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. Enzyme catalysis is very rapid; usually 1 molecule of an enzyme can act upon about 1000 molecules of the substrate per minute. 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.

Characteristics of Enzymes

i. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
ii. They are heat labile.
iii. They are water-soluble.
iv. They can be precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid).
v. They contain 16% weight as nitrogen.

CLASSIFICATION OF ENZYMES

When early workers isolated certain enzymes, whimsical names were given. Some of these, such

CHAPTER
6

Chemistry of Carbohydrates

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Nomenclature and classification of sugars
2. Stereoisomers
3. Glucose, Mannose and Galactose
4. Fructose
5. Reactions of monosaccharides
6. Glycosides
7. Amino sugars and deoxy sugars
8. Pentoses
9. Sucrose, lactose and maltose
10. Starch, glycogen and cellulose
11. Heteroglycans, mucopolysaccharides

Functions of Carbohydrates

1. Carbohydrates are the main sources of **energy** in the body. Brain cells and RBCs are almost wholly dependent on carbohydrates as the energy source. Energy production from carbohydrates will be 4 k calories/g (16 k Joules/g).
2. Storage form of energy (starch and glycogen).
3. Excess carbohydrate is converted to fat.
4. Glycoproteins and glycolipids are components of cell membranes and receptors.
5. Structural basis of many organisms: Cellulose of plants; exoskeleton of insects, cell wall of microorganisms, mucopolysaccharides as ground substance in higher organisms.

The general molecular formula of carbohydrate is $Cn(H_2O)n$. For example, glucose has the molecular formula $C_6H_{12}O_6$. Carbohydrates are **polyhydroxy aldehydes or ketones** or compounds which yield these on hydrolysis (Fig. 6.1).

Fig. 6.1. Keto group and aldehyde group

NOMENCLATURE

Molecules having only one actual or potential sugar group are called **monosaccharides** (Greek, mono = one; saccharide = sugar). They cannot be further hydrolysed into smaller units. When two monosaccharides are combined together with elimination of a water molecule, it is called a **disaccharide** (e.g. $C_{12}H_{22}O_{11}$). **Trisaccharides** contain three sugar groups. Further addition of sugar groups will correspondingly produce tetrasaccharides, pentasaccharides and so on, commonly known as **oligosaccharides** (Greek, oligo = a few). When more than 10 sugar units are combined, they are generally named as **polysaccharides** (Greek, poly = many). Polysaccharides having only one type of monosaccharide units are called **homopolysaccharides** and those having different monosaccharide units are **heteropolysaccharides.**

Sugars having aldehyde group are called **aldoses** and sugars with keto group are **ketoses**. Depending on the number of carbon atoms, the monosaccharides are named as triose (C3), tetrose (C4), pentose (C5), hexose (C6), heptose (C7) and so on. Commonly occurring monosaccharides are given in Table 6.1.

STEREOISOMERS

Compounds having same structural formula, but differing in spatial configuration are known as stereoisomers. While writing the molecular formula of monosaccharides, the spatial arrangements of H and OH groups are important, since they contain asymmetric carbon atoms. Asymmetric carbon means that four different groups are attached to the same carbon. The reference molecule is glyceraldehyde (glycerose) which has a single asymmetric carbon atom (Fig. 6.2).

CHAPTER
7

Chemistry of Lipids

CHAPTER AT A GLANCE

The reader will be able to answer questions on the following topics:

1. Classification of lipids
2. Classification of fatty acids
3. Saturated and unsaturated fatty acids
4. Neutral fats or triacylglycerols
5. Phospholipids
6. Phosphatidyl choline or lecithin
7. Sphingomyelin
8. Non-phosphorylated lipids

Lipids constitute a heterogeneous group of compounds of biochemical importance. Lipids may be **defined as** compounds which are relatively insoluble in water, but freely soluble in nonpolar organic solvents like benzene, chloroform, ether, hot alcohol, acetone, etc. The functions of lipids are summarized in Box 7.1. The clinical applications are shown in Box 7.2.

CLASSIFICATION OF LIPIDS

Detailed classification is shown in Table 7.1. Based on the chemical nature, lipids are classified as

1. **Simple lipids.** They are esters of fatty acids with glycerol or other higher alcohols (Table 7.1).
2. **Compound lipids.** They are fatty acids esterified with alcohol; but in addition they contain other groups. Depending on these extra groups, they are subclassified in Table 7.1.
   a. Phospholipids, containing phosphoric acid.
   b. Non-phosphorylated lipids (Table 7.1).
3. **Derived lipids.** They are compounds which are derived from lipids or precursors of lipids, e.g. fatty acids, steroids. For details of cholesterol and steroids, see Chapter 12.
4. **Lipids complexed to other compounds.**

Box 7.1. Functions of Lipids

1. **Storage form of energy (triglycerides)**
2. **Structural components of biomembranes (phospholipids and cholesterol)**
3. **Metabolic regulators (steroid hormones and prostaglandins)**
4. **Act as surfactants, detergents and emulsifying agents (amphipathic lipids)**
5. **Act as electric insulators in neurons**
6. **Provide insulation against changes in external temperature (subcutaneous fat)**
7. **Give shape and contour to the body**
8. **Protect internal organs by providing a cushioning effect (pads of fat)**
9. **Help in absorption of fat soluble vitamins (A, D, E and K)**
10. **Improve taste and palatability of food.**

FATTY ACIDS

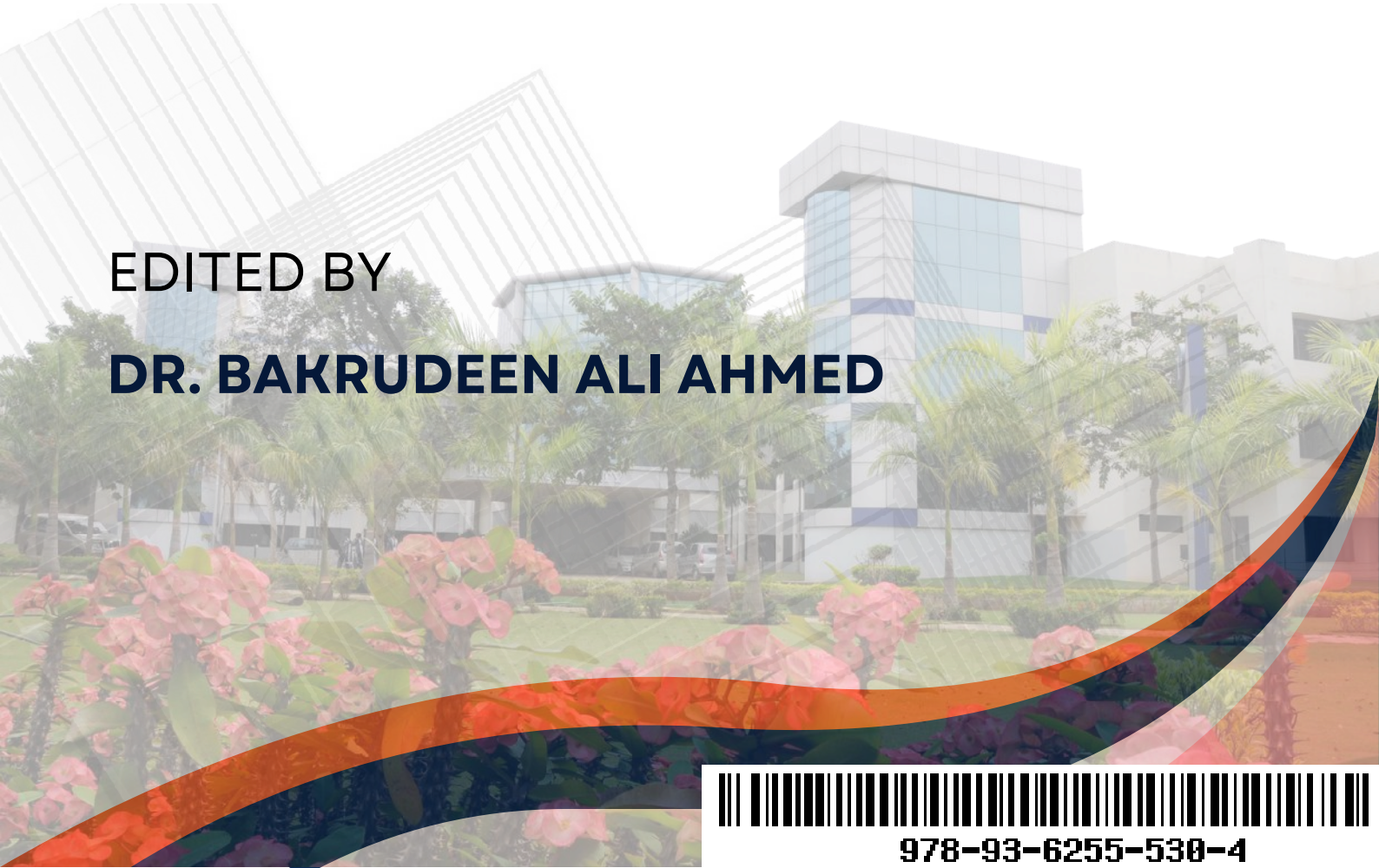
Fatty acids, are included in the group of derived lipids. It is the most common component of lipids in the body. They are generally found in ester linkage in different classes of lipids. In the human body free fatty acids are formed only during metabolism.

Fatty acids are **aliphatic carboxylic acids** and have the general formula, R—CO—OH, where COOH (carboxylic group) represents the functional group. Depending on the R group (the hydrocarbon chain), the physical properties of fatty acids may vary. Characteristics of common fatty acids are shown in Table 7.2. Classification of fatty acid is given in Table 7.3

Box 7.2. Clinical Applications

1. **Excessive fat deposits cause obesity. Truncal obesity is a risk factor for heart attack.**
2. **Abnormality in cholesterol and lipoprotein metabolism leads to atherosclerosis and cardiovascular diseases (Chapter 25).**
3. **In diabetes mellitus, the metabolisms of fatty acids and lipoproteins are deranged, leading to ketosis (Chapter 24).**

PRINCIPLE OF BIOCHEMISTRY



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978-93-6255-530-4

Principle of Biochemistry

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ISBN: 978-93-6255-530-4

Publisher: Ponnaiyah Ramajayam Institute of Science and Technology (PRIST)

· C O N T E N T S ·

· C H A P T E R · 1 ·

WHERE TO START

INSTRUCTIONS

Read for understanding. Read only what you don't know. Organize, organize, organize.

The first page of each chapter presents an index. A title-summary box for each section presents a short summary and memory jogger intended to be helpful for review. If you already know what the boxed terms mean and feel comfortable with them, don't bother to read the text section that follows—proceed until you find a heading you don't understand, and then read till you understand. The first rule (it may not really be the first rule, but it is a rule) is not to waste time reading things you already know.

Keep on not reading the text until you find something you don't understand—then read the text till you do. The sections are generally arranged in order of increasing complexity and build on previous sections. So if you screwed up and jumped in over your head, back up a section or two. Another option is just to look at the pictures. Pictures and diagrams, if extensively annotated and carefully designed (by you), can be an enormous review aid.

• C H A P T E R • 2 •

PROTEIN STRUCTURE

•

Amino Acid Structure

Interactions

Water

Hydrophobic Interaction

Van der Waals Interactions and London Dispersion Forces

Hydrogen Bonds

Secondary Structure

Protein Stability

Favorable (Good) Interactions

Unfavorable (Bad) Interactions

Temperature-Sensitive Mutations

Ligand-Binding Specificity

Global Conclusion

• • • • • • • • • • • •

Proteins start out life as a bunch of amino acids linked together in a head-to-tail fashion—the primary sequence. The one-dimensional information contained in the primary amino acid sequence of cellular proteins is enough to guide a protein into its three-dimensional structure, to determine its specificity for interaction with other molecules, to determine its ability to function as an enzyme, and to set its stability and lifetime.

AMINO ACID STRUCTURE

Remember a few of the amino acids by functional groups. The rest are hydrophobic.

· C H A P T E R · 3 ·

MEMBRANES AND MEMBRANE PROTEINS

GENERAL MEMBRANE FUNCTION

1. Separates one area of the cell from another
2. Provides a diffusion barrier
3. Concentrates membrane-associated molecules
4. Enables ion and concentration gradients

Membranes separate one part of the cell from the other. Proteins and other molecules can be localized in the membrane. Membrane localization concentrates the molecules and makes it easier for them to find each other (two-dimensional diffusion) than it is for two molecules in solution (three-dimensional diffusion). Because most molecules can't pass through the membrane by themselves, the cell machinery can create con-

CHAPTER 4

DNA-RNA STRUCTURE

DNA Structure

DNA Stability

RNA Secondary Structure

DNA STRUCTURE

Double helix
A = Adenine = purine
T = Thymine = pyrimidine (DNA only)
G = Guanine = purine
C = Cytosine = pyrimidine
U = Uracil = pyrimidine (RNA only)
AT/GC base pairs
Antiparallel strands
Major groove–minor groove
A-, B-, and Z-DNA

The two complementary strands of the DNA double helix run in antiparallel directions (Fig. 4-1). The phosphodiester connection between individual deoxynucleotides is directional. It connects the 5′-hydroxyl group of one nucleotide with the 3′-hydroxyl group of the next nucleotide. Think of it as an arrow. If the top strand sequence is written with the 5′ end on the left (this is the conventional way), the bottom strand will have a complementary sequence, and the phosphate backbone will run in the opposite direction; the 3′ end will be on the left. The antiparallel direc-

• C H A P T E R • 5 •

EXPRESSION OF GENETIC INFORMATION

INFORMATION METABOLISM

DNA → RNA → protein → structure.

Information metabolism provides a way to store and retrieve the information that guides the development of cellular structure, communication, and regulation. Like other metabolic pathways, this process is highly regulated. Information is stored by the process of DNA replication and meiosis, in which we form our germ-line cells. These processes are limited to specific portions of the cell cycle. Information is retrieved by the transcription of DNA into RNA and the ultimate translation of the signals in the mRNA into protein.

• C H A P T E R • 6 •

RECOMBINANT-DNA METHODOLOGY

•

Restriction Analysis

Gels and Electrophoresis

Blotting

Restriction Fragment-Length Polymorphism

Cloning

Sequencing

Mutagenesis

Polymerase Chain Reaction

• • • • • • • • • • • •

Much of what we know about the regulation of information flow (gene expression) has been made possible by the ability to manipulate the structures of DNA, RNA, and proteins and see how this affects their function. The ability to manipulate DNA (recombinant-DNA methods) has generated a new language filled with strange-sounding acronyms that are easy to understand if you know what they mean but impossible to understand if you don't. Understand?

RESTRICTION ANALYSIS

Restriction enzymes are sequence-specific endonucleases that cut double-stranded DNA at specific sites.

· C H A P T E R · 7 ·

ENZYME MECHANISM

Active Site

Transition State

Catalysis

Lock and Key

Induced Fit

Nonproductive Binding

Entropy

Strain and Distortion

Transition-State Stabilization

Transition-State Analogs

Chemical Catalysis

Enzymes do two important things: they recognize very specific substrates, and they perform specific chemical reactions on them at fantastic speeds. The way they accomplish all this can be described by a number of different models, each one of which accounts for some of the behavior that enzymes exhibit. Most enzymes make use of all these different mechanisms of specificity and/or catalysis. In the real world, some or all of these factors go into making a given enzyme work with exquisite specificity and blinding speed.

BASIS OF BIOCHEMISTRY

Edited by

DR. A. SOHNA CHANDRA

BACKIAVATHI

978-93-6255-586-1

Basis of Biochemistry

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ISBN: 978-93-6255-586-1

Publisher: Ponnaiyah Ramajayam Institute of Science and Technology (PRIST)

TABLE OF CONTENTS

Chapter 1

Biomolecules

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1.1 Strain classification

Early taxonomy distinguished agrobacteria on the basis of their pathogenic properties. Thus strains causing crown gall were classified as A. tumefaciens, those inducing cane gall on raspberry (Rubus idaeus) were described as A. rubi and hairy root-inducing isolates were allocated to A. rhizogenes. Non-pathogenic strains were called A. radiobacter (Allen and Holding, 1974). Later, strains were identified on the basis of their biochemical and physiological properties which led to the definition of three biotypes (Kerr and Panagopoulos, 1977; Süle, 1978). Species- and biotypebased taxonomies do not coincide (Kersters and De Ley, 1984). Biotype 3 strains were isolated almost exclusively from grapevine (Vitis vinifera) and allocated to A. vitis (Ophel and Kerr, 1990). Similarly, several isolates from weeping fig (Ficus benjamina) form a distinct group and were classified as A. larrymoorei (Bouzar and Jones, 2001).

1.2 The infection process

During the infection process a segment of the Ti (tumor-inducing) plasmid, called T(transferred)-DNA, is exported from Agrobacterium to the plant cell nucleus where it is integrated into the chromosomal DNA and expressed. Hairy root is caused in a similar way by a root-inducing or Ri plasmid. The T-DNA transfer and integration processes involve a large number of bacterial and host factors, and finally results in genetically transformed plant cells. Details of this unique natural example of interkingdom DNA transfer have been reviewed (Zhu et al., 2000; Zupan et al., 2000; Gelvin, 2003; Tzfira et al., 2004 and other chapters in this book). During the infection process agrobacteria suppress plant defense mechanisms via the chromosomally encoded degradation of hydrogen peroxide (Xu and Pan, 2000) and by Ti plasmid-related functions. Transformation of plant cells results in elevated hormone (auxin and cytokinin) production and sensitivity. Both trigger abnormal proliferation leading to tumorous growth or abnormal rooting (Petersen et al., 1989; Gaudin et al., 1994; Costacurta and Vanderleyden, 1995). Tumors and hairy roots produce and

Chapter 2

Carbohydrates

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2.1 Early studies

As described in the first chapter of this volume, the "crown gall" disease of higher plants was a particular problem in orchards and vineyards, though a wide variety of plants were known to develop distinct 'galls'. The earliest work identifying bacteria as the cause of these galls, in contrast to the then known limited galls produced as a result of insect or nematode infection, was published by Cavara (1987) who isolated 'white bacteria' that would give rise to galls when inoculated on plants. A much more thorough (and apparently independent – see Braun, 1982) characterization of the causal agent of the crown gall disease was published by Smith and Townsend (1907) in which many of the characteristics of the inciting bacterium (named then as Bacterium tumefaciens) were described including its rod shape, size, polar flagella and inability to grow well at 37°C ('blood temperature'). The debate over the nomenclature of Agrobacterium species still exist (Box 2-1 and Chapter 5), and for simplicity, I will refer to Agrobacterium tumefaciens as the causal agent of crown gall tumors and Agrobacterium rhizogenes as the causal agent of the hairy root disease throughout the course of this chapter.

Through the next thirty years studies on the crown gall disease described the responses of many plants to various different field isolates, generally concurring with the observations of Smith and Townsend. Of particular interest amongst these early papers were the descriptions by Smith (1916) and later Levin and Levine (Levin and Levine, 1918; Levine, 1919) of 'teratomas' – spontaneously shoot forming tumors – that could be isolated on certain plants by certain bacterial isolates (see below). Nevertheless, despite a good deal of speculation about the relationship of crown gall tumors of plants to neoplasias of animals, no particular insights into the mechanism whereby A. tumefaciens might be inducing tumors were developed. The prospects for progress improved as physiological and genetic tools in both

Chapter 3

Proteins

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1 INTRODUCTION

Plant biotechnology has had a dramatic impact on agriculture, and on public awareness of the role of the private sector in industrial-scale farming in developed countries. This chapter focuses on the seminal contributions of Agrobacterium tumefaciens to this technological revolution, and on the applications of genetic engineering that continue to expand the limits of plant productivity. Agrobacterium-mediated transformation has yielded a stunning array of transgenic plants with novel properties ranging from enhanced agronomic performance, nutritional content, and disease resistance to the production of pharmaceuticals and industrially important compounds. Many of these advances have been made possible by creative and elegant methodological innovations that have enabled gene stacking, targeted mutagenesis, and the transformation of previously recalcitrant hosts.

Transgenic plants are not a panacea for global food shortages, distributional failures, or other structural causes of poverty. They can, however, have a positive impact on both human and environmental health. Agricultural biotechnology's image has been tarnished by the perception that it fails to address the needs of the world's hungry, and indeed most of the commercial products to date represent technology that is inappropriate for subsistence farmers (Huang et al., 2002a). As this chapter documents, there is ample potential for genetically modified plants to ameliorate some of the constraints faced by resource-poor farmers. Even modest enhancements of agronomic traits have the potential to help farmers overcome endemic problems such as lack of food security, limited purchasing power, and inadequate access to balanced nutritional resources (Leisinger, 1999).

Many of these innovations will come from public sector research, and the vast majority of the applications described herein have in fact emanated from basic investigations and collaborative product-oriented research originating in the non-profit realm. As plant biotechnology research moves forward and outward to include more stakeholders in

Chapter 4

Vitamines

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1 INTRODUCTION

In 2001 the journal Science published two papers back-to-back on the genome of the Agrobacterium biovar I organism A. tumefaciens C58 (Goodner et al., 2001; Wood et al., 2001). Two different teams of scientists had raced to complete and publish this genome, only becoming aware of the other's efforts near the end of the projects. After contacting each other, and thanks to the vision of Science editors, both teams were able to publish their results simultaneously. An interesting account of this race was published several years later in Nature Biotechnology (Harvey and McMeekin, 2004). The principle members of both groups have now combined efforts and, in addition to authoring this chapter, have completed the genome sequences of representative Agrobacterium strains from biovars II and III (Wood D, Burr T, Farrand S, Goldman B, Nester E, Setubal J and Slater S, unpublished data).

The two original Science papers, although covering a lot of common ground, were surprisingly complementary. Over 250 manuscripts have used the data from the original C58 genome sequences. The types of manuscripts fall into three basic categories: (i) those that use the sequence as part of genome-scale comparative analyses, (ii) those that simply cite the identification of an ortholog of their gene of interest in A. tumefaciens, and (iii) those that follow-up on specific genes in A. tumefaciens after identifying them in the genome sequence. The last category contains about 20% of these manuscripts. Here we present a description of the C58 genome that combines the findings of both teams, and summarizes many new results on A. tumefaciens biology that have been enabled by the A. tumefaciens C58 genome sequence. Table 4-1 lists all genes discussed herein and their designations by the original genome publications (Goodner et al., 2001; Wood et al., 2001). To harmonize nomenclature as we continue our annotation of the Agrobacterium genomes, we have chosen to use the gene designations and style of Wood et al. (2001). The 5.67-Mb

Chapter 5

Aminoacids

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1 INTRODUCTION

The classification of bacteria at generic and specific levels has been subject to repeated amendment, with frequent revisions made to keep nomenclature in line with contemporary taxonomic approaches. The genus Agrobacterium Conn 1942 is an exception. Although they had their origins in diverse genera, the plant pathogenic bacteria associated with oncogenic symptoms, commonly called ‘crown gall’ and ‘hairy root’, and other more rece ntly identified oncogenic pathogens, have been recognized as distinct species in the genus Agrobacterium since the genus was established (Kersters and De Ley, 1984).

Classification of the genus Agrobacterium and of its species has been based on its once-puzzling oncogene pathogenicity, which was the defining character of the genus (Kersters and De Ley, 1984). This was paralleled in the genus Rhizobium Frank 1889, originally reserved for bacteria with the capacity to form symbiotic nitrogen-fixing symbioses with legume species. For both genera, their distinctive generic characteristics are now known to be the result of the presence or absence of interchangeable conjugative plasmids that confer specific oncogenic or nodulating capabilities. However, a character that is the result of arbitrary acquisition or loss of a plasmid is obviously unstable and cannot form the basis of formal nomenclature. Although comparative phenotypic and genetic studies of Agrobacterium spp. and Rhizobium spp. have failed to confirm differentiation into separate genera based on oncogenicity and nitrogen-fixation respectively (Young et al., 2001), an element of the bacteriological community has continued to support a special-purpose nomenclature based on pathogenicity alone.

Pathogenicity was also used as the single defining character of individual Agrobacterium species (Kersters and De Ley, 1984) although, following comprehensive genetic and phenotypic studies, the genus has been revised with the recognition of natural species (Holmes and Roberts, 1981) to

Chapter 6

Primary Metabolites Applications

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1 INTRODUCTION

Transformation of plants by wild type strains of Agrobacterium tumefaciens results from the transfer of the Ti plasmid's T-DNA into host cells where it is ultimately integrated into chromosomal DNA and expressed (see other chapters in this volume). The virulence (vir) genes of the Ti plasmid required for virulence (Klee et al., 1983; Stachel and Nester, 1986) encode, for example, proteins involved in the processing, transport and ultimate integration of the T-DNA in the host (see other chapters). The resultant 'crown gall' tumors potentially yield great benefits to the infecting bacteria in the form of opines produced via enzymes encoded on the TDNA (De Greve et al., 1982), yet the process requires significant energyexpenditures by the bacterium and, accordingly, should be tightly regulated. In agreement with this hypothesis is the finding that the virulence genes are essentially silent unless the bacteria are exposed to a plant or plant derived molecules (Stachel et al., 1985b; Stachel et al., 1986). Activation of the genes in response to the host or host derived signals was first shown via experiments exploiting vir::lacZ fusions (Stachel et al., 1985a), and further experiments, importantly, showed that two virulence proteins encoded on the Ti plasmid, VirA and VirG, were required for the hostinduced expression of the vir genes (Stachel and Zambryski, 1986; Engstrom et al., 1987; Winans et al., 1988).

Early studies of VirA and VirG demonstrated that they were related to the just discovered class of bacterial regulatory 'two component' systems (TCS) (Winans, 1991; Charles et al., 1992). TCS are comprised, minimally, of a histidine autokinase (often called sensor kinase) that responds, either directly or indirectly to environmental input, and a response regulator that is phosphorylated by its cognate histidine kinase (Robinson et al., 2000; Stock et al., 2000; West and Stock, 2001). Often, but not exclusively, the response regulator controls transcription of sets of genes via binding to specific regions of promoters and recruiting the

Chapter 7

Lipids

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1 INTRODUCTION

The possesses a tightly linked cluster of genes, the jor histocompatibility complex (MHC), whose maproducts play roles in intercellular recognition and in discrimination between self and nonself. The MHC participates in the development of both humoral and cellmediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, because MHC molecules act as antigen-presenting structures, the particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual's TH and TC cells can respond. For this reason, the MHC partly determines the response of an individual to antigens of infectious organisms, and it has therefore been implicated in the susceptibility to disease and in the development of autoimmunity. The recent understanding that natural killer cells express receptors for MHC class I antigens and the fact that the receptor–MHC interaction may lead to inhibition or activation expands the known role of this gene family. The present chapter examines the organization and inheritance of MHC genes, the structure of the MHC molecules, and the central function that these molecules play in producing an immune response.

General Organization and Inheritance of the MHC The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules, now called histocompatibility antigens, originated from the work of Peter Gorer in the mid-1930s. Gorer was using inbred strains of mice to identify blood-group antigens. In the course of these studies, he identified four groups of genes, designated I through IV, that encoded blood-cell antigens. Work carried out in the 1940s and 1950s by Gorer and George Snell established that antigens encoded by the genes in the group designated II took part in the rejection of transplanted tumors and other tissue. Snell called these genes "histocompatibility genes"; their current designation as histocompatibility-2 (H-2) genes was in reference to Gorer's group II blood-group antigens. Although Gorer died before his