P.S.R. ENGIN04RING COLLEGE, SIVAKASI – 626 140
(An Autonomous Institution, Affiliated to Anna University, Chennai)
DEPARTMENT OF ELECTRICAL AND ELECTRONICS ENGINEERING

COURSE MATERIAL

STAFF NAME	**: Mrs. M. Kanimozhi**
SUBJECT CODE	**: 191BS31**
SUBJECT NAME	**: BIOLOGY FOR ENGINEERS**
BRANCH	**: ELECTRICAL AND ELECTRONICS ENGINEERING**
SEMESTER	**: III**
YEAR	**: II**
DURATION	**: August - 2023 to November - 2024**

Institute Vision and Mission

Vision

To contribute to the society through excellence in technical education with societal values and thus a valuable resource for industry and the humanity.

Mission

- To create an ambience for quality learning experience by providing sustained care and facilities.
- To offer higher level training encompassing both theory and practices with human and social values.
- To provide knowledge based services and professional skills to adapt tomorrow's technology and embedded global changes.

Department Vision and Mission

Vision

To be a technical hub of creating Electrical and Electronics Engineers with superior quality, human values and ethical views

Mission

- To provide an excellent, innovative and comprehensive education in electrical and electronics engineering.
- To create a conducive learning environment and train the students in the latest technological development domain to enhance carrier opportunities
- To produce competent and disciplined engineers suitable for making a successful career in industry/research.

Programme Educational Objectives (PEOs)

1. Lead a professional career by acquiring the basic knowledge in the field of specialization and allied Engineering.
2. Assess the real life problems and deal with them confidently relevance to the society.
3. Engage in lifelong learning by pursuing higher studies and participating in professional organizations.
4. Exhibit interpersonal skills and able to work as a team for success.

Programme Outcomes (PO'S)

A graduate of Electrical and Electronics Engineering Program will attain

- **PO1: Engineering Knowledge**: Apply the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.

- **PO2: Problem Analysis**: Identify, formulate, review research literature, and analyze complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.
- **PO3: Design / Development of Solutions**: Design solutions for complex engineering problems and design system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations.
- **PO4: Conduct Investigations of Complex Problems**: Use research-based knowledge and research methods including design of experiments, analysis and interpretation of data, and synthesis of the information to provide valid conclusions.
- **PO5: Modern Tool Usage**: Create, select, and apply appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.
- **PO6: The Engineer and Society**: Apply reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
- **PO7: Environment and Sustainability**: Understand the impact of the professional engineering solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
- **PO8: Ethics**: Apply ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
- **PO9: Individual and Team Work**: Function effectively as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
- **PO10: Communication**:Communicate effectively on complex engineering activities with the engineering community and with society at large, such as, being able to comprehend and write effective reports and design documentation, make effective presentations, and give and receive clear instructions.
- **PO11: Project Management and Finance**: Demonstrate knowledge and understanding of the engineering and management principles and apply these to one's own work, as a member and leader in a team, to manage projects and in multidisciplinary environments.
- **PO12: Life-long Learning**:Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change.

Programme Specific Outcomes (PSO'S)

- **PSO1:**Skilled to analyze, design and test various electrical and electronic circuits, control systems, instrumentation systems, computer systems, microprocessor and microcontroller based systems.
- **PSO2:**Exhibit knowledge and hands-on competence in the application of Electrical machines and Power Electronics based drives systems.
- **PSO3:**Design and investigate problems in power system network along with protection schemes and effective utilization of electrical energy.
- **PSO4:**Develop a project management tool for solving complex electrical / electronic problems by applying the knowledge of basic sciences, mathematics and engineering fundamentals.

191BS31 **BIOLOGY FOR ENGINEERS** **L-T-P C**
3-0-0 3

Programme: **B.E./B.Tech. (Common to all Branches)** **Sem: III** **Category: BSC**

Prerequisites: Basic science

Aim: To understand basic and fundamental engineering knowledge from biology.

Course Outcomes:

The Students will be able to

CO1: Understand various biochemical interactions and the structure and function of various biological molecules

CO2: Explain basic concepts of thermodynamics and energy transactions.

CO3: Discuss different aspects of molecular computing

CO4: Demonstrate an understanding of Mendelian laws of inheritance.

CO5: Describe cellular architecture and utilize these concepts to design an engineering system.

CO6: Understand fundamental concepts in sensory physiology analogy with communication systems.

INTRODUCTION. **9**

Biological analogy in engineering science, Biological elements-Carbohydrate, protein, amino acids, lipids and nucleic acids structure and function. Primary, secondary, tertiary and quaternary structure of protein. Protein as enzymes, transporter, receptors and structural elements.

METABOLISM AND ENGINEERING **9**

Engineering aspects in thermodynamics of energy transactions, exothermic and endothermic versus endergonic and exergonic reactions. ATP as an energy source, glycolysis, Krebs cycle and photosynthesis. Energy yielding and energy consuming reactions. Enzymes classification, mechanism of enzyme action, enzyme kinetics and kinetic parameters

GENETICS AND TRANSFORMATION TECHNOLOGY **9**

Molecular basis of information transfer. DNA as a genetic material. Concept of genetic code. Mendal's laws, concept of segregation and independent assortment. Concept of allele, Gene mapping, Gene interaction, Epistasis, concepts of recessiveness and dominance and their relativeness to programming. Cell multiplication. Phenotype and genotype. Single gene disorders in humans and human genetics..

CLASSIFICATION AND SYSTEM ENGINEERING **9**

Structure, function and relativeness to engineering of prokaryotes and eukaryotes. Habitats- aquatic or terrestrial. Molecular taxonomy-three major kingdoms. Microbial species and strains. Identification and classification of microorganisms. Industrial application of microorganisms. Sterilization and media compositions. Growth kinetics.

SENSOR BIOLOGY AND COMMUNICATION SYSTEMS **9**

Sensory system, circulatory system and excretory system and their relativeness to communication engineering. Hormonal regulation. General defense mechanism in human. Major human disorder and diseases.

Total Periods: 45

Text Book

1. Arthur T. Johnson, CRC Press, New York2011

2. ThyagaRajan.S., Selvamurugan. N., Rajesh.M.P., Nazeer.R.A., Richard W. Thilagaraj, Barathi.S., and Jaganthan.M.K., "Biology for Engineers", Tata McGraw-Hill, New Delhi, 2012

References

1. Rajiv Singal, Gaurav Agarwal, Ritu Bir, Biology for Engineers, CBS Publisher,2019
2. Charles Molnar and Jane Gair, Concepts of Biology-1st Canadian Edition, OpenStax Publication, 2013.
3. Raven Johnson, Biology, 11th Edition, Mc Graw Hill Publication, 2017

Course Outcomes	Program Outcomes (POs)												Program Specific Outcomes (PSOs)			
	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12	PSO1	PSO2	PSO3	PSO4
CO1		2											1			1
CO2	2												1			1
CO3			1										1			1
CO4						2							1			1
CO5					1		2						1			1
CO6										2			1			1

1: Slight (Low) 2: Moderate (Medium) 3: Substantial (High)

P.S.R. ENGINEERING COLEGE, SIVAKASI.
DEPARTMENT OF ELECTRICAL AND ELECTRONICS ENGINEERING
COURSE PLAN

191BS31	**BIOLOGY FOR ENGINEERS**			**L-T-P**	**C**
				3-0-0	**3**
Programme:	B.E. Electrical and Electronics Engineering	**Sem:**	**III**	**Category:**	**BSC**
Faculty Name	Ms.M.Kanimozhi, Assistant Professor/EEE	**Academic Year:**		**2023-24**	
AIM:	To understand basic and fundamental engineering knowledge from biology.				

Course Outcomes: The Students will be able to

CO1. Understand various biochemical interactions and the structure and function of various biological molecules.
CO2. Explain basic concepts of thermodynamics and energy transactions.
CO3. Discuss different aspects of molecular computing.
CO4. Demonstrate an understanding of Mendelian laws of inheritance.
CO5. Describe cellular architecture and utilize these concepts to design an engineering system.
CO6. Understand fundamental concepts in sensory physiology analogy with communication systems.

S.No	Topics	Hours	Cumm. Periods	Ref.Books
	UNIT – I INTRODUCTION			
1.	Biological analogy in engineering science	1	1	T1,R1,R3
2.	Biological elements-Carbohydrate, protein	1	2	T1,R1,R3
3.	Amino acids, lipids	1	3	T1,T2
4.	Nucleic acids structure and function	2	5	T1,R1,R2
5.	Primary, secondary structure of protein	1	6	T2,R2
6.	Tertiary and quaternary structure of protein	1	7	T2,R2
7.	Protein as enzymes transporter, receptors and structuralelements.	2	9	T1,T2,R2,R3
	UNIT – II DC MACHINES			
8.	Engineering aspects in thermodynamics of energy transactions	1	10	T2,R2
9.	Exothermic and endothermic versus endergonic and exergonic reactions.	1	11	T1,R1,R2
10.	ATP as an energy source	1	12	T1,T2
11.	Glycolysis, Krebs cycle and photosynthesis	2	14	T1,R1,R3
12.	Energy yielding and energy consuming reactions	1	15	T1,R1,R3
13.	Enzymes classification	1	16	T1,T2,R2,R3
14.	Mechanism of enzyme action	1	17	T1,T2
15.	Enzyme kinetics and kinetic parameters	1	18	T2,R2
	UNIT –III TRANSFORMERS			
16.	Molecular basis of information transfer	1	19	T1,R2
17.	DNA as a genetic material	1	20	T1,T2,R2
18.	Concept of genetic code. Mendal's laws,	1	21	T2,R2
19.	Concept of segregation and independent assortment	1	22	T1,T2
20.	Concept of allele, Gene mapping, Gene interaction, Epistasis,	1	23	T1,R1,R3
21.	Concepts of recessiveness and dominance and their relativenessto programming.	2	25	T1,R1,R2
22.	Cell multiplication, Phenotype and genotype	1	26	T1,T2
23.	Single gene disorders in humans and human genetics	1	27	T1,T2,R2,R3
	UNIT – IV INDUCTION MOTORS			
24.	Structure, function and relativeness to engineering of prokaryotes and eukaryotes.	2	28	T1,T2,R2,R3
25.	Habitats- aquatic or terrestrial.	1	30	T1,R1,R2
26.	Molecular taxonomy- Three major kingdoms.	1	31	T2,R2
27.	Microbial species and strains	1	32	T1,T2,R2
28.	Identification and classification of microorganisms	1	33	T1,R2
29.	Industrial application of microorganisms	1	34	T1,R1,R2
30.	Sterilization and media compositions. Growth kinetics	2	36	T1,T2
	UNIT – V SYNCHRONOUS MACHINES			
31.	Sensory system, circulatory system	2	38	T1,R1,R2
32.	Excretory system and their relativeness to communication engineering.	2	40	T1,R2
33.	Hormonal regulation	1	41	T1,T2,R2, R3

34.	General defense mechanism in human	2	43	T1,R2
35.	Major human disorder and diseases	2	45	T1,R1,R2

Text Books

1. Arthur T. Johnson, CRC Press, New York 2011.
2. ThyagaRajan.S., Selvamurugan. N., Rajesh.M.P., Nazeer.R.A., Richard W. Thilagaraj, Barathi.S., and Jaganthan.M.K., "Biology for Engineers", Tata McGraw-Hill, New Delhi, 2012.

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1. Rajiv Singal, Gaurav Agarwal, Ritu Bir, Biology for Engineers, CBS Publisher,2019
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3. Raven Johnson, Biology, 11th Edition, Mc Graw Hill Publication, 2017.

Items	Conventional Chalk and Board/OHP	ICT Tools (Video/Animation/PPT/Google Class rooms)	If Industrial visit planned fill out the details
Total No. of hours planned	12	33	Aarthi Scan Center, Sivakasi

Course Outcomes	Program Outcomes (POs)												Program Specific Outcomes (PSOs)			
	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12	PSO1	PSO2	PSO3	PSO4
CO1		2											1			1
CO2	2												1			1
CO3			1										1			1
CO4						2							1			1
CO5					1		2						1			1
CO6										2			1			1

Evaluation Criteria , Marks	Continuous Assessment (30)			End Semester Examination	Total Marks
	Assessment Test (60%)	Assignment/Seminar/ Mini Project (30%)	Attendance (10%)		
	18	9	3	70 [Min Pass:35]	100 [Min Pass:50]

Attendance Mark	91% and above -10, 86-90%-8, 81-85-6, 76-80%-4, 75%-2, Below 75%-0
Grade Criteria	O(90-100), A+(80-89), A(70-79),B+(60-69), B(50-59),RA(<50)-Fail

UNIT – I
Introduction

1.1 Why should engineers know biology?

There are a numerous of options for undergraduate study in our nation that currently give the impression satisfactory to the society and are considered well-intentioned enough to pursue. This is a healthy state and the social acceptance of several undergraduate study choices requests to improve further. Not so long ago in our country, there were only two socially preferred undergraduate streams for study—engineering and medicine. The usual groups (streams) for study at the higher secondary school level that led to the above preferred undergraduate streams were either mathematics—physics—chemistry—biology, mathematics—physics—chemistry—computer science, or physics—chemistry—biology. For engineering, mathematics is required and biology is not. Hence, only the first two groups are suitable for pursuit of an engineering degree. The relevant groups at the higher secondary school level that lead to undergraduate study in engineering or medicine have not changed much until now. Therefore, the students who enter engineering undergraduate studies may or may not have studied biology after their 10th standard. Another aspect is the innate interest of the student in biology. Irrespective of the group they choose to pursue at the higher secondary level, some students have an innate interest in biology, whereas others do not. The students who do not have an interest in biology or are neutral, when exposed to uninspired teaching of biology as a part of science at the high school (sixth to 10th standard), most likely develop a hatred toward it. The frequency of occurrence of inspired, good teachers is low in any population, and thus, the above probably leads to a large number of high school students to hate biology. In some undergraduate engineering institutions in the country, a significant majority of students have not studied biology at the higher secondary level. For example, class surveys done at IIT Madras among all undergraduates who take the author's introductory course on Life Sciences or another related course show that usually, more than 90 per cent of the engineering undergraduates have not studied biology at the higher secondary stage. Among them, a disturbingly large majority (> 98%) hate biology.

1.1.1 Need for Biology

The previous few centuries saw a better fundamental understanding of the physical and chemical world through advances in physics and chemistry. The better

understanding and advances gave rise to technologies and products, such as computers, communication devices, aircraft, and others that revolutionized life. Since this is the century of biology, a similar phenomenon is expected, which will lead to probably another revolution. Many engineers are expected to contribute to a biological aspect to fuel this revolution. Therefore, the engineering undergraduates need to be suitably exposed atleast to the very minimum biology, so that they would atleast be able to consider a biological system/aspect in which they could later make appropriate contributions, through their main expertise, say electrical engineering, mechanical engineering, computer science, materials engineering, or any other. Let us elaborate further in the following sections.

1.1.2 Shinkansen Sonic Beam

Many man-made things have significant scope for optimising their design. For example, Shinkansen, Japan's high-speed bullet train, plays an important role in Japan with a coverage of close to 3000 km. It has been operating for many decades now, from 1940. The earliest ones were operated by steam engines to reach peak speeds of 170 km h-1. The maximum speeds that are technologically possible and the operational speeds of bullet trains have steadily increased. In 1989, the operational speeds had reached high enough levels, say 270 km h-1, to cause sonic booms when they exited tunnels. In residential localities, this sonic boom caused significant difficulties to the public. To address the sonic boom problem, a team was assembled. The team had many departments and the head of one of the departments, Eiji Nakatsu, believed in learning from nature—his hobby was bird-watching. He was fascinated by the silent, smooth, and high-speed swoops of birds such as kingfishers and owls. The kingfisher's beak was noticed for its efficiency to slice through water to catch a fish. The owl's silent swoops were noticed and related to its serrated feather design. Based on those and other aspects with a parallel to nature's designs, the Shinkansen was re-designed with a pointed front similar to the beak of the kingfisher (Figure 1.1).

Figure.1.1 The nose of the re-designed Shinkanzen, shaped like a Kingfisher's beak

The pantograph, the part that connects the train to the electricity supply lines above, was designed based on owl's serrated feathers and other natural features. The redesigned train was not only acceptably quieter when it exited the tunnels in dense residential areas, it was also 10 per cent faster and 15 per cent more energy-efficient. Biological features were mimicked to solve engineering problems and the results exceeded expectations. In fact, an entire field called bio-mimetics is a study of these fascinating aspects. In bio-mimetics, there are many specialized areas such as:Bio-robotics. It refers to robots that are inspired by biological entities or the use of biological components in robots. We are possibly familiar with the concept of bio-robotics from many sci-fi movies and TV shows, which show robots with human like features (e.g. the character, Data, in the hugely popular, 'Star Trek'). In reality, there have been many initiatives such as 'artificial sensing skin', which can detect pressure changes upon touch, robots inspired by animal movements, and softer robots that interface with the body for various prosthetics, or to provide insights into the working of natural systems. These softer bio-robots provide distinct advantages over rigid robots that are traditionally used for various automated operations, for example, in the assembly line for cars. The bio-robots can be used to perform intricate tasks which the traditional ones cannot perform. More recently, a number of androids (human-like robots) have even passed the Turing test, a test devised by Alan Turing to differentiate between humans and robots.

1.1.2 Bio-robotics

It refers to robots that are inspired by biological entities or the use of biological components in robots. We are possibly familiar with the concept of bio-robotics from many sci-fi movies and TV shows, which show robots with human like features (e.g. the character, Data, in the hugely popular, 'Star Trek'). In reality, there have been many

initiatives such as ‘artificial sensing skin’, which can detect pressure changes upon touch, robots inspired by animal movements, and softer robots that interface with the body for various prosthetics, or to provide insights into the working of natural systems. These softer bio-robots provide distinct advantages over rigid robots that are traditionally used for various automated operations, for example, in the assembly line for cars. The bio-robots can be used to perform intricate tasks which the traditional ones cannot perform. More recently, a number of androids (human-like robots) have even passed the Turing test, a test devised by Alan Turing to differentiate between humans and robots.

1.1.3 Muscular Bio-polymers

These refer to specialized biopolymer nano-composites that can be used for artificial muscles, and so on. These specialized materials aim to have properties that are close to biological muscles such as excitability, contractility, elasticity, and extensibility. The above example is only one advantage of engineers knowing biology, that is, inspiration or help with solutions to challenging problems. If we look further into history, it is well known that bird flight inspired the discovery of modern aeroplanes. Biology is full of solutions to current, highly significant challenges such as sustainability. Life has existed on earth for billions of years, whereas the so-called ‘evolved life forms’ such as primates have been on earth for only about 65 million years and humans for only about 5 million years, according to the scientific evidence. Life forms have evolved over billions of years and have harmoniously co-existed with their respective environments. Life has worked out highly sustainable ways over billions of years—life molecules are recycled in an effective fashion.

1.2 For Our Wellness—We Are All Biological Entities

The understanding of our biological selves is nowhere near complete. It is a challenge to ensure our physical and mental wellness. The brain, which controls most life processes in the human body, is yet to be substantially understood despite intense efforts. However, engineering appropriately coupled with biology has helped improve our biological shortcomings or partially overcome our biological losses.

1.2.1 Examples

Retinal Prosthetic Let us now consider one of the many examples where the inputs from many engineering fields were effectively harnessed along with biology to provide eye-sight to people who could see due to retinal diseases such as macular degeneration.

When an eye ‘sees’ something, its lens projects an image onto its retina and its photoreceptor cells, where the image gets converted to appropriate codes. The codes, in turn, get converted to corresponding electrical signals by appropriate cells located just behind the retina. The electrical signals are conveyed to the brain where the image is interpreted. All this happens in milliseconds or less that we are able to visually perceive the happenings around us in real time. When the retina gets diseased, the relevant cells die and the image no longer gets converted to appropriate codes. Scientists have developed a device, a prosthetic, that can convert optical images to codes and later to electrical signals, which can be transmitted to the brain. The prosthetic is small enough to be placed inside the eye at the retina and it is connected to the cells that transmit the signals to the brain. Thus, the person who is blind due to retinal disease can see again. Versions of this device are already approved by the U.S. Food and Drug Administration (FDA) for use on patients.

Let us consider the engineering fields that contributed to the above useful retinal prosthetic. Clearly, computer science principles were used to develop the relevant codes. Principles of electrical engineering, mechanical engineering, materials engineering, biological engineering, etc. were utilised to construct the device, to ensure its bio-compatibility and to facilitate its implantation for long-term use in the eye. All the engineering principles were appropriately used in combination with biological principles to make a successful prosthetic.

If one considers the other direction of information flow, from the brain to the body peripherals, principles of biology and many engineering fields have been combined effectively to create devices that can be operated by thought-generated electrical signals. Thus, people who have lost limb or part-body function due to accidents or diseases can move again.

Next, let us see some of the terms that seem to catch the fancy of the uninitiated. Bio-sensors, bio-chips, bio-pesticides, bio-fertilizers, etc. are some of the attractive terms that a student may have come across in their earlier reading, which have a biological base or significance.

Bio-sensors These are devices that are used to measure many different parameters such as analyte concentrations. They are used for diverse purposes such as analysis, toxicology, medical diagnosis (i.e., they can even be incorporated into digital plasters to monitor the healing progress of wound), environmental monitoring, and others. In

principle, they have miniaturised detection elements to measure, for example, the current or the voltage that are generated in proportion to the analyte concentrations or in relation to activities of different organs in the body such as the heart or the brain.

Bio-pesticides These are organisms that can be used instead of chemicals for pest control and thus they overcome the negative effects of chemical pesticides. In the same vein, Bio-fertilizers are fertilizers that are composed of appropriate microorganisms. Chemical fertilizers improve the fertility of soil by adding chemicals such as N, P, and K. On the contrary, bio-fertilizers incorporate into relevant plant parts and provide the needed nutrients through biological processes called fixing. They help the plant to grow better by better fixing nitrogen, by better solubilizing phosphorus, or by improving the production of growth promoting substances by the plant itself. Bio-pesticides and bio-fertilizers have been known for many decades now.

Concrete Self-heal Organisms can be used to make concrete self-heal its cracks due to wearand-tear. For example, some bacteria can catalyse the formation of calcium carbonate in their surroundings under appropriate conditions. When this happens in cracks that are formed in the concrete, the microscopic cracks are filled with the calcium carbonate formed with the help of the bacteria, which can effectively seal the cracks, and thus effect self-healing of the concrete.

Bio-filters These are essentially sand columns containing organisms that are used in wastewater treatment. They can be used on a large scale to provide clean water to large areas such as fields and ponds. They can also be used on smaller scales to purify water for home-use in challenging regions of the world. They are accessible, sustainable, and affordable. When contaminated water passes through the sand and the complex ecosystem inside it, the contaminants in the water are removed to yield potable water. More recently, it has been suggested that the fibres made by microorganisms themselves can be used as effective bio-filters to convert contaminated water potable. The fibres secreted by some microorganisms create a natural mesh with pore sizes less than a micron, and thus, they can operate as effective bio-filters.

Nanoparticles These are particles of various shapes with sizes in the range of a few to a hundred nano meters. They have specialized physical and chemical properties because of their small size, which make them suitable for use for specialized needs. For example, Nanoparticles for drug delivery have been developed, which are effective in delivering anti-cancer drugs to cancer cells, without affecting normal cells. Nanoparticles made of a

suitable, bio-compatible material can be coated with agents that recognise cancer cells. Another coating on the same material is the drug that can kill cancer cells. Thus, we have 'bullets' that can home in preferentially to cancer cells, attach to them or go into them, and destroy them using the attached anticancer drug.

Organ-on-a-chip It is a micro set-up to test the effect of say, new drugs or toxins on representative animal tissues without actual animal studies. Microfluidic devices with small membranes and other support structures that can hold specialized organ cells are used to create the organson-a-chip. Body fluid-like materials that can flow on either side of the membrane, provide a functional representation of the real organ. Drugs to be tested are dissolved in those body fluid-like materials that are contacted with the tissue to test their effects on the organ tissues. Many years ago, the relevant tissues of organs such as lung, kidney, and others were developed to test the effect of drugs. The claim is that these organs-on-a-chip are closer to the human situation than the animals are, and thus the predictions could be much better. In addition, they could end the use of animals for drug-testing, which is a desirable scenario.

Further, whole organs can potentially be 3-D bio-printed for various study purposes. It may be known that a 3-D printer is used to print many useful products. Even whole houses or parts of them can be 3-D printed and assembled together. However, 3-D bio-printing is a challenge because the biological cells need to be kept alive and vibrant during processing and printing. Cells need suitable nutrition and environment to be active, and techniques are being developed to maintain cells under desirable conditions during the 3-D printing process. The printing solution or gel is infused with the nutrients needed for the cell. The shear forces that the cells feel when they are extruded through the jets during the 3-D printing process also need to be minimised. A lot of research is currently underway to improve the success of 3-D printing. With further developments, the 3-D bio-printed organs can be used for organ transplants in the future. They could also eliminate the need to use animals for drug testing.

Artificial Neural Network The artificial neural network (ANN), a processing set-up, is supposedly inspired by the working of animal brains—they learn by example. ANNs are made up of a large number of interconnected elements, each of which work similar to a biological nerve cell. ANNs can be used to derive useful information from imprecise data. For example, they can be used to detect patterns or trends in complicated data that are difficult to detect by manual inspection. Genetic algorithm is an example of a

mathematical optimisation method that is inspired by biological processes—the recombination of genes in this case. We will learn about genes in detail in later chapters.

Bioinformatics, Systems Biology, and Computational Biology These are currently popular fields of study which are highly multi-disciplinary, and engineers can significantly contribute to those fields. Those fields of study computationally analyse very large data sets to draw insights into the working of the fundamental functional unit of life—the cell. As we will see later in the book, there are many thousands of reactions that simultaneously occur in the cell as a part of its normal function. We will also see that the genetic information is contained in a polymeric molecule that has 6 billion units in every single cell of the body. To make sense of such large data sets, people with backgrounds as diverse as computer science, biology, biochemistry, biological engineering, electronics engineering, and many others, need to come together.

1.3 Biological Elements

1.3.1 Six most abundant elements of life

Biological molecules, or biomolecules, are built by joining atoms through covalent bonds. Although more than 25 types of elements can be found in biomolecules, six elements are most common. These are called the CHNOPS elements; the letters stand for the chemical abbreviations of carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur.

1.3.2 Valence and Covalent Bonding

The ability of an atom to combine with other atoms depends on the number of electrons in the outer shells of the atoms. Some elements, the so-called noble gases, have complete outer shells and do not share electrons. Many elements will share electrons with other elements, such that each element completes its outer electron shell capacity.

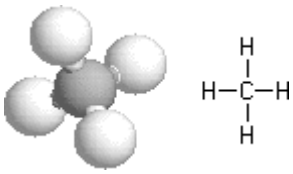
A shared electron pair is called a covalent bond. The number of covalent bonds that each element can form is called its valence.

1.3.3 Organic Molecules: Hydrocarbons

Hydrocarbons, the simplest organic molecules, contain only carbon and hydrogen atoms. Because carbon can form simultaneous covalent bonds with up to four partners,

an enormous number of carbon compounds are possible. Carbon chemistry is called organic chemistry, to distinguish it from the chemistry of all other elements.

Among the simplest organic compounds are those containing only hydrogen and carbon, or hydrocarbons. Hydrocarbons include methane gas (produced and discharged daily from our intestines as flatus), liquid gasoline, and solid paraffin wax in candles.



To the right is a molecule of methane, CH_4, in which one atom of carbon shares electrons with four atoms of hydrogen. Our two-dimensional screen can only represent a planar structure, but note that the shaded model portrays the actual three-dimensional structure of methane, in which the four hydrogen atoms are oriented at the vertices of a tetrahedron.

1.3.4 Isomers

Organic molecules exist in three-dimensional space, and the same set of atoms can be put together in many recognizably different ways, resulting in molecules called isomers. The atoms found in a simple sugar, with the structural formula $C_6H_{12}O_6$, can be arranged in over a dozen different ways.

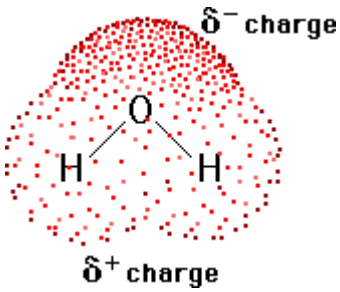
Even though many isomers can theoretically exist, cells are discriminating about which ones they will synthesize and recognize. For example, the sugar glucose ($C_6H_{12}O_6$) is very abundant and can be used by almost all organisms as a quick energy source. By contrast, an isomer of glucose called tagatose (also $C_6H_{12}O_6$) is rare and is not useful to most forms of life—same atoms, different shapes.

Three situations can lead to the existence of isomers:

1. **Structural isomers:** Variations in the position at which different atoms are joined together.
2. **Enantiomers:** Left-handed and right-handed variations resulting from the tetrahedral geometry of carbon.
3. **Geometric isomers:** Variations in the placement of atoms around carbon atoms joined by double covalent bonds.

1.3.5 Polarity

Many combinations of different elements result in unequal electron sharing, called polar bonding.



The sharing of electrons in covalent bonds is not always equal. In a covalent bond, atoms such as oxygen contain a higher localization of negative charge density than their atomic partners. As a result, the electron distribution is asymmetric, or polar, and the oxygen atom is said to be electronegative. This asymmetry results in regions of slight negative and positive charge in different regions of the molecule, denoted by the Greek symbol δ, for "partial" charge.

1.3.6 Functional Groups

The properties of different biological molecules depend on certain characteristic groupings of atoms called functional groups.

If you know the properties of some of the functional groups, you will be able to quickly look at many simple biological molecules and get some idea of their solubility and possible identity. The names of the six most important functional groups are:

- Hydroxyl - OH
- Carbonyl – C=O
- Carboxyl – C-O
- Amino – NH_3^+
- Sulfhydryl - SH
- Phosphate – PO_4^{2-}

1.4 Biomolecules

Biomolecules are molecules that occur naturally in living organisms. Biomolecules include macromolecules like proteins, carbohydrates, lipids and nucleic acids. It also includes small molecules like primary and secondary metabolites and natural products. Biomolecules consists mainly of carbon and hydrogen with nitrogen, oxygen, sulphur,

and phosphorus. Biomolecules are very large molecules of many atoms, which are covalently bound together.

1.4.1 Classes of biomolecules

There are four major classes of biomolecules

- Carbohydrates
- Proteins
- Nucleic acids
- Lipids

These molecules are produced by cells and living organisms.

1.4.1.1 Carbohydrates

Carbohydrates are good source of energy. Carbohydrates (polysaccharides) are long chains of sugars. Monosaccharides are simple sugars that are composed of 3-7 carbon atoms. They have a free aldehyde or ketone group, which acts as reducing agents and are known as reducing sugars. Disaccharides are made of two monosaccharides. The bonds shared between two monosaccharides are the glycosidic bonds. Monosaccharides and disaccharides are sweet, crystalline and water-soluble substances. Polysaccharides are polymers of monosaccharides. They are un-sweet and complex carbohydrates. They are insoluble in water and are not in crystalline form.

Examples: glucose, fructose, sucrose, maltose, starch, cellulose etc.

1.4.1.2 Lipids

Lipids are composed of long hydrocarbon chains. Lipid molecules hold a large amount of energy and are energy storage molecules. Lipids are generally esters of fatty acids and are building blocks of biological membranes. Most of the lipids have a polar head and non-polar tail. Fatty acids can be unsaturated and saturated fatty acids. Lipids present in biological membranes are of three classes based on the type of hydrophilic head present:

- Glycolipids are lipids whose head contains oligosaccharides with 1-15 saccharide residues.
- Phospholipids contain a positively charged head which are linked to the negatively charged phosphate groups.
- Sterols, whose head contain a steroid ring. Example steroid.

Examples: oils, fats, phospholipids, glycolipids, etc.

1.4.1.3 Nucleic acids

Nucleic acids are organic compounds with heterocyclic rings. Nucleic acids are made of polymer of nucleotides. Nucleotides consist of nitrogenous base, a pentose sugar and a phosphate group. A nucleoside is made of nitrogenous base attached to a pentose sugar. The nitrogenous bases are adenine, guanine, thymine, cytosine and uracil. Polymerized nucleotides form DNA and RNA which are genetic material.

1.4.1.4 Proteins

Proteins are heteropolymers of stings of amino acids. Amino acids are joined together by the peptide bond which is formed in between the carboxyl group and amino group of successive amino acids. Proteins are formed from 20 different amino acids, depending on the number of amino acids and the sequence of amino acids. There are four levels of protein structure:

(i) **Primary structure of Protein** - Here protein exist as long chain of amino acids arranged in a particular sequence. They are non-functional proteins.

(ii) **Secondary structure of protein** - The long chain of proteins are folded and arranged in a helix shape, where the amino acids interact by the formation of hydrogen bonds. This structure is called the pleated sheet. Example: silk fibres.

(iii) **Tertiary structure of protein** - Long polypeptide chains become more stabilizes by folding and coiling, by the formation of ionic or hydrophobic bonds or disulphide bridges, these results in the tertiary structure of protein.

(iv) **Quaternary structure of protein** - When a protein is an assembly of more than one polypeptide or subunits of its own, this is said to be the quaternary structure of protein. Example: Haemoglobin, insulin.

1.4.2 Function of Biomolecules

- **Carbohydrates** provide the body with source of fuel and energy, it aids in proper functioning of our brain, heart and nervous, digestive and immune system. Deficiency of carbohydrates in the diet causes fatigue, poor mental function.
- Each **protein** in the body has specific functions, some proteins provide structural support, help in body movement, and also defense against germs and infections. Proteins can be antibodies, hormonal, enzymes and contractile proteins.
- **Lipids**, the primary purpose of lipids in body are energy storage. Structural membranes are composed of lipids which form a barrier and controls flow of

material in and out of the cell. Lipid hormones, like sterols, help in mediating communication between cells.

- **Nucleic Acids** are the DNA and RNA; they carry genetic information in the cell. They also help in synthesis of proteins, through the process of translation and transcription.

Carbohydrates

Carbohydrate is an organic compound, it comprises of only oxygen, carbon and hydrogen. The oxygen: hydrogen ratio is usually is 2:1. The empirical formula being $Cn(H_2O)n$. Carbohydrates are hydrates of carbon; technically they are polyhydroxy aldehydes and ketones. Carbohydrates are also known as saccharides; the word saccharide which comes from Greek word **sakkron** that means sugar.

Classification of Carbohydrates

The carbohydrates are divided into three major classes depending upon whether or not they undergo hydrolysis and if they do, on the number of products formed.

Monosaccharides: The monosaccharides are polyhydroxy aldehydes or polyhydroxy ketones which cannot be decomposed by hydrolysis to give simpler carbohydrates. e.g. Glucose, fructose, Galactose etc.

Oligosaccharides: The oligosaccharides (Oligo: few) are carbohydrates which yield a definite number (2-9) of monosaccharide molecules on hydrolysis.

(i) Disaccharides - Which yield two monosaccharides molecules on hydrolysis. Which have molecular formula is $C_{12}H_{22}O_{11}$. e.g. Sucrose, maltose etc

(ii) Trisaccharide - Which yield three monosaccharides molecules on hydrolysis and have molecular formula is $C_{18}H_{32}O_{16}$.

(iii) Tetrasaccharides - Which yield four monosaccharides molecules on hydrolysis and have molecular formula is $C_{22}H_{42}O_{21}$. eg: Stachyose

Polysaccharides - The carbohydrates which have higher molecular weight, which yield many monosaccharide molecules on hydrolysis. E.g. Starch, glycogen, Dextrin, Cellulose etc.

In general monosaccharides and oligosaccharides are crystalline solids, soluble in water and sweet to taste, they are collectively known as sugars, the polysaccharides on the other hand are amorphous, insoluble in water and tasteless, they are called non-sugars.

Table. 1 Difference between Mono, oligo and polysaccharides

Character	Monosaccharide	Oligosaccharid	Polysacchari

		e	de
No of Sugar molecules	**1**	**2-9**	**More than 9**
Glycosidic bond	**Absent**	**Present**	**Present**
Molecular Weight	**Low**	**Moderate**	**High**
Taste	**Sweet**	**Minimally sweet taste**	**No taste**
Solubility	**Soluble**	**Soluble**	**Insoluble**
Nature	**Always reducing sugar**	**May or May not be**	**Always non reducing sugar**
Example	**Glucose, Fructose, Galactose**	**Sucrose, Maltose**	**Starch, Glycogen, Dextrin, Cellulose**

Properties of Carbohydrates

General properties of carbohydrates

Carbohydrates act as energy reserves, also stores fuels, and metabolic intermediates.

- Ribose and deoxyribose sugars forms the structural frame of the genetic material, RNA and DNA.
- Polysaccharides like cellulose are the structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
- Carbohydrates are organic compounds; they are aldehydes or ketones with many hydroxyl groups.

Physical properties of carbohydrates

- **Steroisomerism** - Compound shaving same structural formula but they differ in spatial configuration. Example: Glucose has two isomers with respect to penultimate carbon atom. They are D-glucose and L-glucose.
- **Optical Activity** - It is the rotation of plane polarized light forming (+) glucose and (-) glucose.
- **Diastereo isomeers** - It the configurational changes with regard to C2, C3, or C4 in glucose. Example: Mannose, galactose.
- **Anomerism** - It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

Chemical properties of carbohydrates

- Ozazone formation with phenylhydrazine
- Benedict's test
- Oxidation
- Reduction to alcohols

Structure of Carbohydrates

There are three types of structural representation

- ✓ Open chain structure
- ✓ Hemi-acetal structure
- ✓ Haworth structure

Functions of Carbohydrates

- ❖ Carbohydrates are chief energy source, in many animals; they are instant source of energy. Glucose is broken down by glycolysis/ kreb's cycle to yield ATP.
- ❖ Glucose is the source of storage of energy. It is stored as glycogen in animals and starch in plants.
- ❖ Stored carbohydrates act as energy source instead of proteins.
- ❖ Carbohydrates are intermediates in biosynthesis of fats and proteins.
- ❖ Carbohydrates aid in regulation of nerve tissue and are the energy source for brain.
- ❖ Carbohydrates get associated with lipids and proteins to form surface antigens, receptor molecules, vitamins and antibiotics.'
- ❖ They form structural and protective components, like in cell wall of plants and microorganisms.
- ❖ In animals they are important constituent of connective tissues.
- ❖ They participate in biological transport, cell-cell communication and activation of growth factors.
- ❖ Carbohydrates those are rich in fibre content help to prevent constipation.

- ❖ They help in modulation of immune system.

Example of Carbohydrates

- ➢ Monosaccharides - Glucose, galactose, glycerose, erythrose, ribose, ribulose, fructose
- ➢ Oligosaccharides - Maltose, lactose, sucrose, raffinose, stachyose.
- ➢ Polysaccharides - Starch, glycogen, cellulose, pectin, inulin, hyaluonic acid.

- Foods rich in carbohydrates are referred to as strachy foods. They are found in legumes, starchy vegetables, whole-grain breads and cereals. They also occur naturally with vitamins and minerals in foods like milk, fruits, and milk products. They are also found in refined and processed products like candy, carbonated beverages, and table sugar.

Examples of Polysaccharides

Name of the Polysaccharide	Composition	Occurrence	Functions
Starch	Polymer of glucose containing a straight chain of glucose molecules (amylose) and a branched chain of glucose molecules (amylopectin)	In several plant species as main storage carbohydrate	Storage of reserve food
Glycogen	Polymer of glucose	Animals (equivalent of starch)	Storage of reserve food
Inulin	Polymer of fructose	In roots and tubers (like Dahlia)	Storage of reserve food
Cellulose	Polymer of glucose	Plant cell wall	Cell wall matrix
Pectin	Polymer of galactose and its derivatives	Plant cell wall	Cell wall matrix
Hemi cellulose	Polymer of pentoses and sugar acids	Plant cell wall	Cell wall matrix
Lignin	Polymer of glucose	Plant cell wall (dead cells like sclerenchyma)	Cell wall matrix
Chitin	Polymer of glucose	Body wall of arthropods. In some fungi also	Exoskeleton Impermeable to water
Murein	Polysaccharide cross linked with amino acids	Cell wall of prokaryotic cells	Structural Protection
Hyaluronic acid	Polymer of sugar acids	Connective tissue matrix, Outer coat of mammalian eggs	Ground substance, protection
Heparin	Closely related to chrondroitin	Connective tissue cells	Anticoagulant

Gums and mucilages	Polymers of sugars and sugar acids	Gums - bark or trees. Mucilages - flower	Retain water in dry seasons

Lipids

Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by nonpolar solvents, because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles or on albumin. They are the major source of energy for the body, and they provide the hydrophobic barrier. They serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.

General characteristics of lipids:

- Lipids are relatively insoluble in water.
- They are soluble in non-polar solvents, like ether, chloroform, and methanol.
- Lipids have high energy content and are metabolized to release calories.
- Lipids also act as electrical insulators; they insulate nerve axons.
- Fats contain saturated fatty acids; they are solid at room temperatures. Example, animal fats.
- Plant fats are unsaturated and are liquid at room temperatures.
- Pure fats are colourless, they have extremely bland taste.
- The fats are sparingly soluble in water and hence are described are hydrophobic substances.
- They are freely soluble in organic solvents like ether, acetone and benzene.
- The melting point of fats depends on the length of the chain of the constituent fatty acid and the degree of unsaturation.
- Geometric isomerism, the presence of double bond in the unsaturated fatty acid of the lipid molecule produces geometric or cis-trans isomerism.
- Fats have insulating capacity; they are bad conductors of heat.
- Emulsification is the process by which a lipid mass is converted to a number of small lipid droplets. The process of emulsification happens before the fats can be absorbed by the intestinal walls.
- The fats are hydrolyzed by the enzyme lipases to yield fatty acids and glycerol.

- The hydrolysis of fats by alkali is called saponification. This reaction results in the formation of glycerol and salts of fatty acids called soaps.
- Hydrolytic rancidity is caused by the growth of microorganisms which secrete enzymes like lipases. These split fats into glycerol and free fatty acids.

Classification of Lipids:

- Simple lipids: Esters of fatty acids with various alcohols.
  - ✓ Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state.
  - ✓ Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.
- Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.
  - ✓ Phospholipids: Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingo phospholipids the alcohol is sphingosine.
  - ✓ Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine, and carbohydrate.
  - ✓ Other complex lipids: Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
- Precursor and derived lipids: These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins and hormones.

Essential Fatty acids:

Essential fatty acids, or EFAs, are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes but does not include the fats that only act as fuel.

Two fatty acids are dietary essentials in humans

- ✓ Linoleic acid, which is the precursor of arachidonic acid, the substrate for prostaglandin synthesis.
- ✓ α-linolenic acid is the precursor for growth and development.

Regulating Blood Cholesterol Level

Fats and cholesterol cannot dissolve in blood and are consequently packaged with proteins (to form lipoproteins) for transport. Hence LDLs raise blood cholesterol levels

('bad') while HDLs lower blood cholesterol levels ('good'). High intakes of certain types of fats will differentially affect cholesterol levels in the blood

- ✓ Low density lipoproteins (LDL) carry cholesterol from the liver to the rest of the body.
- ✓ High density lipoproteins (HDL) scavenge excess cholesterol and carry it back to the liver for disposal.
- ✓ Saturated fats increase LDL levels within the body, raising blood cholesterol levels.
- ✓ Trans fats increase LDL levels and decrease HDL levels within the body, significantly raising blood cholesterol levels.

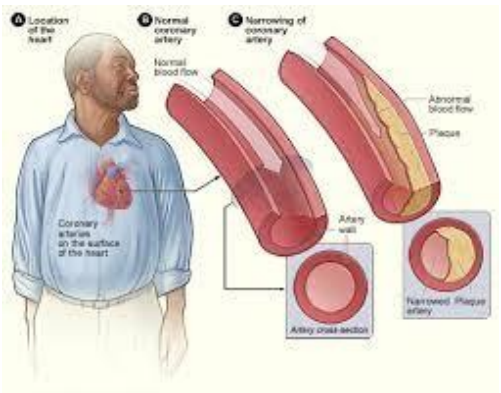
Lipid Health Claims

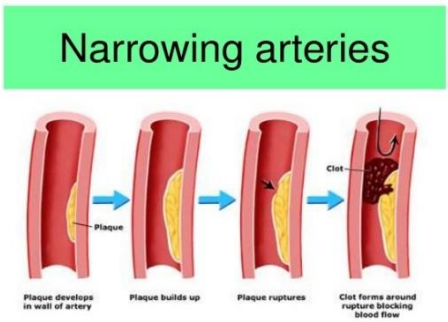
There are two main health claims made about lipids in the diet:

- ✓ Diets rich in saturated fats and trans fats increase the risk of CHD (Coronary Heart Disease)
- ✓ Diets rich in monounsaturated and polyunsaturated (cis) fats decrease the risk of CHD.

Health Risks of High Cholesterol:

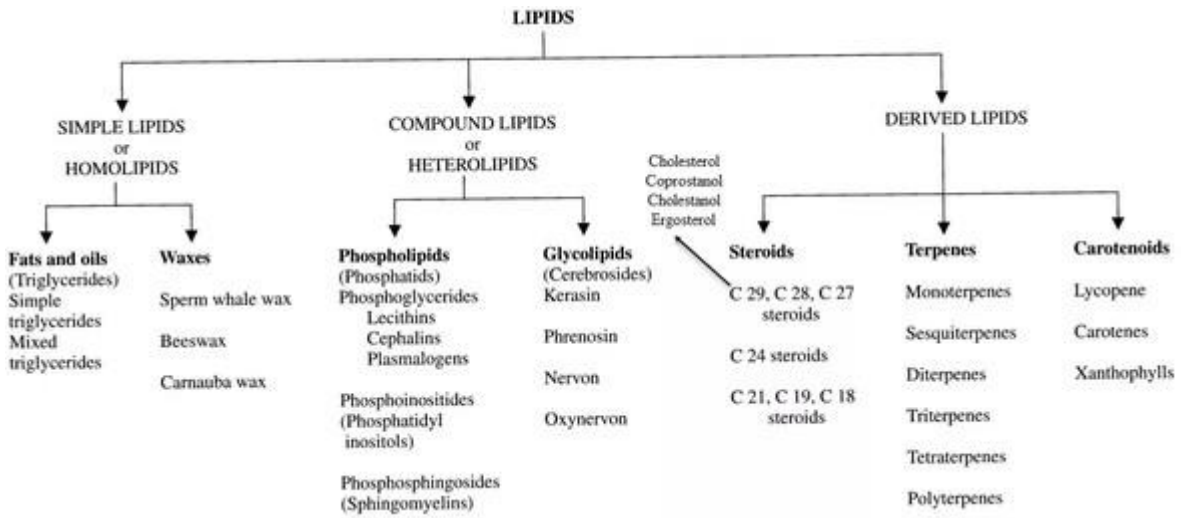
- ✓ High cholesterol levels in the bloodstream lead to the hardening and narrowing of arteries (atherosclerosis)
- ✓ When there are high levels of LDL in the bloodstream, the LDL particles will form deposits in the walls of the arteries
- ✓ The accumulation of fat within the arterial walls leads to the development of plaques which restrict blood flow
- ✓ If coronary arteries become blocked, Coronary Heart Disease (CHD) will result – this includes heart attacks and strokes.





Examples of Lipids

- Fatty acids - Oleic acid, Linoleic acid, Palmitoleic acid, Arachidonic acid.
- Fats and Oils - Animal fats - Butter, Lard, Human fat, Herring oil. Plant oils - Coconut oil, Corn, Palm, Peanut, Sunflower oil.
- Waxes - Spermacti, Beeswax, Carnauba wax.
- Phospholipids - Lecithins, Cephalins, Plasmoalogens, Phosphatidyl inositols, Sphingomyelins.
- Glycolipids - Kerasin, Phrenosin, Nervon, Oxynervon.
- Steroids - Cholesterol. - Terpenes - Monoterpenes, Sesquiterpenes, Diterpenes, Triterpenes.
- Carotenoids - Lycopene, Carotenes, Xanthophylls.



Biological Role of Lipids

- ✓ **Food material:** Lipids provide food, highly rich in calorific value. One-gram lipid produces 9.3 kilocalories of heat.
- ✓ **Food reserve:** Lipids provide are insoluble in aqueous solutions and hence can be stored readily in the body as a food reserve.
- ✓ **Structural component:** Lipids are an important constituent of the cell membrane.
- ✓ **Heat insulation:** The fats are characterized for their high insulating capacity. Great quantities of fat are deposited in the subcutaneous layers in aquatic mammals such as whale and in animals living in cold climates.
- ✓ **Fatty acid absorption**: Phospholipids play an important role in the absorption and transportation of fatty acids.

- ✓ **Hormone synthesis:** The sex hormones, adrenocorticoids, cholic acids and also vitamin D are all synthesized from cholesterol, a steroidal lipid.
- ✓ **Vitamin carriers:** Lipids act as carriers of natural fat-soluble vitamins such as vitamin A, D and E.
- ✓ **Blood cholesterol lowering:** Chocolates and beef, especially the latter one, were believed to cause many heart diseases as they are rich in saturated fatty acids, which boost cholesterol levels in blood and clog the arterial passage. But researches conducted at the University of Texas by Scott Grundy and Andrea Bonanome (1988) suggest that at least one saturated fatty acid stearic acid, a major component of cocoa butter and beef fat, does not raise blood cholesterol level at all. The researchers placed 11 men on three cholesterol poor liquid diets for three weeks each in random order. One formula was rich in palmitic acid, a known cholesterol booster; the second in oleic acid; and the third in stearic acid. When compared with the diet rich in palmitic acid, blood cholesterol levels were 14% lower in subjects put on the stearic acid diet and 10% lower in those on the oleic acid diet.
- ✓ **Antibiotic agent:** Squalamine, a steroid from the blood of sharks, has been shown to be an antibiotic and antifungal agent of intense activity. This seems to explain why sharks rarely contract infections and almost never get cancer.

Proteins

- ✓ Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues.
- ✓ Proteins are known as building blocks of life.
- ✓ Proteins are the most abundant intracellular macro-molecules. They provide structure, protection to the body of multicellular organism in the form of skin, hair, callus, cartilage, ligaments, muscles, tendons. Proteins regulate and catalyze the body chemistry in the form of hormones, enzymes, immunoglobulin's etc.

General Characteristics of Proteins

- ✓ Proteins are organic substances; they are made up of nitrogen and also, oxygen, carbon and hydrogen.
- ✓ Proteins are the most important biomolecules; they are the fundamental constituent of the cytoplasm of the cell.
- ✓ Proteins are the structural elements of body tissues.
- ✓ Proteins are made up of amino acids.

- ✓ Proteins give heat and energy to the body and also aid in building and repair.
- ✓ Only small amounts of proteins are stored in the body as they can be used up quickly on demand.
- ✓ Proteins are considered as the bricks, they make up bones, muscles, hair and other parts of the body.
- ✓ Proteins like enzymes are functional elements that take part in metabolic reactions.
- ✓ Antibodies, blood haemoglobin are also made of proteins.
- ✓ Proteins have a molecular weight of 5 to 300 kilo-Daltons

Physical Properties of Proteins

- ✓ Proteins are colorless and tasteless.
- ✓ They are homogeneous and crystalline.
- ✓ Proteins vary in shape, they may be simple crystalloid structure to long fibrilar structures.
- ✓ Protein structures are of two distinct patterns - Globular proteins and fibrilar proteins.
- ✓ Globular proteins are spherical in shape and occur in plants. Fibrilar proteins are thread-like, they occur generally in animals.
- ✓ In general proteins have large molecular weights ranging between 5 X 103 and 1 X 106.
- ✓ Due to the huge size, proteins exhibit many colloidal properties.
- ✓ The diffusion rates of proteins are extremely slow.
- ✓ Proteins exhibit Tyndall effect.
- ✓ Proteins tend to change their properties like denaturation. Many times, the process of denaturation is followed by coagulation.
- ✓ Denaturation may be a result of either physical or chemical agents. The physical agents include, shaking, freezing, heating etc. Chemical agents are like X-rays, radioactive and ultrasonic radiations.
- ✓ Proteins like the amino acids exhibit amphoteric property i.e., they can act as Acids and Alkalies.
- ✓ As the proteins are amphoteric in nature, they can form salts with both cations and anions based on the net charge.
- ✓ The solubility of proteins depends upon the pH. Lowest solubility is seen at isoelectric point, the solubility increases with increase in acidity or alkalinity.

- ✓ All the proteins show the plane of polarized light to the left, i.e., laevorotatory.

Chemical Properties of Proteins

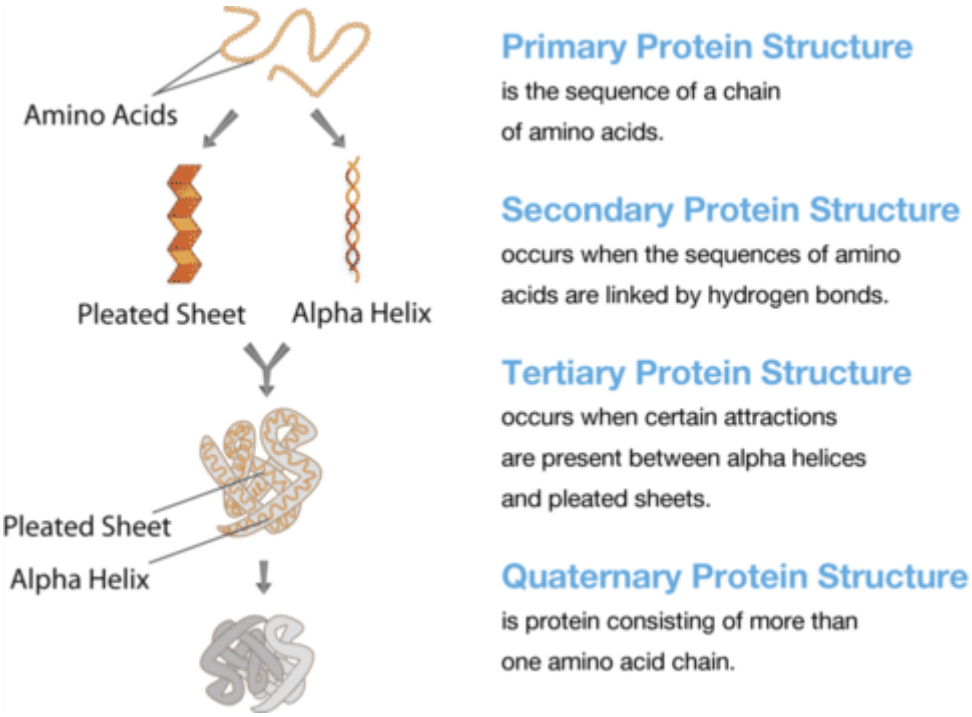
- ✓ Proteins when hydrolyzed by acidic agents, like conc.HCl yield amino acids in the form of their hydrochlorides.
- ✓ Proteins when are hydrolyzed with alkaline agents leads to hydrolysis of certain amino acids like arginine, cysteine, serine, etc., also the optical activity of the amino acids is lost.
- ✓ Proteins with reaction with alcohols give its corresponding esters. This process is known as esterification.
- ✓ Amino acid reacts with amines to form amides.
- ✓ When free amino acids or proteins are said to react with mineral acids like HCl, the acid salts are formed.
- ✓ When amino acid in alkaline medium reacts with many acid chlorides, acylation reaction takes place.
- ✓ Xanthoproteic test - On boiling proteins with conc. HNO3, yellow color develops due to presence of benzene ring.
- ✓ Folin's test - This is a specific test for tyrosine amino acid, where blue color develops with phosphomolybdotungstic acid in alkaline solution due to presence of phenol group**.**

Structure of Proteins

- ✓ Proteins are constructed by polymerization of only 20 different amino acids into linear chains.
- ✓ Proteins are the polymers of L-a-amino acids. The structure of proteins is rather complex which can be divided into 4 levels of organization.
    1. Primary structure:
        - ✓ The linear sequence of amino acids forming the backbone of proteins (polypeptides).
        - ✓ Examples of protein with a primary structure are Hexosaminidase, Dystrophin.
    2. Secondary structure:
        - ✓ The spacial arrangement of protein by twisting of the polypeptide chain.
        - ✓ Example of protein with a secondary structure is Myoglobin.
    3. Tertiary structure:

- ✓ The three-dimensional structure of a functional protein.
- ✓ Number of forces act to hold the polypeptide chain in this final configuration:
  - ➢ Polar/Nonpolar Interactions
  - ➢ Hydrogen Bonds
  - ➢ Van der Waals Forces
  - ➢ Ionic Interactions
  - ➢ Disulfide Bonds
- ✓ Examples of protein with a Tertiary structure are Globular Proteins (Enzymes) and Fibrous Proteins.

4. Quaternary structure:
   - ✓ Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spacial arrangement of these subunits is known as quaternary structure.
   - ✓ Examples of protein with a Quaternary structure are DNA polymerase, and ion channels.



Secondary Structure of Proteins

Shape:

- ✓ Alpha Helix: Alpha Helix is a right-handed coiled rod-like structure.
- ✓ Beta Pleated Sheet: Beta sheet is a sheet-like structure.

Formation:

- ✓ **Alpha Helix**: Hydrogen bonds form within the polypeptide chain in order to create a helical structure.
- ✓ **Beta Pleated Sheet:** Beta sheets are formed by linking two or more beta strands by H bonds.

Bonds

- ✓ **Alpha Helix:** Alpha helix has n + 4 H-bonding scheme. i.e. Hydrogen bonds form between N-H group of one amino residue with C=O group of another amino acid, which is placed in 4 residues earlier.
- ✓ **Beta Pleated Sheet:** Hydrogen bonds are formed in between the neighboring N-H and C=O groups of adjacent peptide chain - -R group
- ✓ **Alpha Helix:** -R groups of the amino acids are oriented outside of the helix.
- ✓ **Beta Pleated Sheet:** -R groups are directed to both inside and outside of the sheet. - Number
- ✓ **Alpha Helix:** This can be a single chain.
- ✓ **Beta Pleated Sheet:** This cannot exist as a single beta strand; there are must be two or more. - Type o Alpha Helix: This has only one type.
- ✓ **Beta Pleated Sheet:** This can be parallel, anti-parallel or mixed. - Qualities
- ✓ **Alpha Helix:** 100o rotation, 3.6 residues per turn and 1.5 Ao rise from one alpha carbon to the second
- ✓ **Beta Pleated Sheet:** 3.5 Ao rise between residues
- ✓ **Amino Acid o Alpha Helix:** Alpha helix prefers the amino acid side chains, which can cover and protect the backbone Hbonds in the core of the helix.
- ✓ **Beta Pleated Sheet:** The extended structure leaves the maximum space free for the amino acid side chains. Therefore, amino acids with large bulky side chains prefer beta sheet structure. - Preference
- ✓ **Alpha Helix:** Alpha helix prefers Ala, Leu, Met, Phe, Glu, Gln, His, Lys, Arg amino acids.
- ✓ **Beta Pleated Sheet:** Beta sheet prefers Tyr, Trp, (Phe, Met), Ile, Val, Thr, Cys.

Protein Classification

I. Classification of Proteins Based on Shape

i. Globular or Corpuscular Proteins:

- ✓ Globular proteins have axial ratio less than 10 but not below 3 or 4.
- ✓ They are compactly folded and coiled and possess a relatively spherical or ovoid shape.

- ✓ They are usually soluble in water and in aqueous media.
- ✓ Example: Insulin, plasma albumin, globulin enzymes.

ii. Fibrous or Fibrillar Proteins

- ✓ These proteins have axial ratio more than 10, hence, they resemble long ribbons or fibres in shape.
- ✓ They are mostly found in animals, and are not soluble in water or in solution of dilute acids.
- ✓ Fibrous proteins aid in protection and structural support.
- ✓ Example: Collagen, Keratin, Elastins, Fibroin.

II. Classification of Proteins Based on Composition and Solubility

i. Simple Proteins or Holoproteins:

- ✓ These proteins are made of only one type of amino acid, as structural component, on decomposition with acids, they liberate constituent amino acids. They are mostly globular type of proteins except for scleroproteins, which are fibrous in nature.
- ✓ Simple proteins are further classified based on their solubility.

a) Protamines and histones

- ✓ These proteins occur only in animals and are basic proteins.
- ✓ They possess simple structure and low molecular, are water soluble and are not coagulated by heat.
- ✓ They are strongly basic in character due to the high content of lysine, arginine.
- ✓ Example: Protamines - salmine, clupine, cyprinine; Histones - nucleoshistones, globin.

b) Albumins

- ✓ They are widely distributed in nature, mostly seen in seeds.
- ✓ They are soluble in water and dilute solutions of acids, bases and salts.
- ✓ Example: Leucosine, legumeline, serum albumin.

c) Globulins

- ✓ They are of two types, pseudoglobulins which are soluble in water,

- ✓ Other is euglobulins which are insoluble in water.
- ✓ They are coagulated by heat.
- ✓ Example: Pseudoglobulin, serum globulin, glycinine. etc.

d) Scleroproteins or Albuminoids

- ✓ These occur mostly in animals and are commonly known as animal skeleton proteins.
- ✓ They are insoluble in water, and in dilute solution of acids, based and salts.

ii. Conjugated or Complex Proteins or Heteroproteins:

- ✓ These are proteins that are made of amino acids and other organic compounds. The non-amino acid group is termed as prosthetic group.
- ✓ Complex proteins are further classified based on the type of prosthetic group present.

a) Metalloproteins:

- ✓ These are proteins linked with various metals.
- ✓ Example: casein, collagen, ceruloplasmin, etc.

b) Chromoproteins

- ✓ These are proteins that are coupled with a colored pigment.
- ✓ Example: Myoglubin, hemocyanin, cytochromes, flavoproteins, etc.

c) Glycoproteins and Mucoproteins

- ✓ These proteins contain carbohydrates as the prosthetic group.
- ✓ Example: Glycoproteins - egg albumin, serum globulins, serum albumins; Mucoproteins - Ovomucoid, mucin etc.

d) Phosphoproteins

- ✓ These proteins are linked with phosphoric acid.
- ✓ Example: casein.

e) Lipoproteins

- ✓ Proteins forming complexes with lipids are lipoproteins.
- ✓ Example: lipovitellin, lipoproteins of blood.

f) Nucleoproteins

- ✓ These are compounds containing nucleic acids and proteins.
- ✓ Example: Nucleoproteins, nucleohistones, nuclein.

iii. Derived Proteins

- ✓ These are proteins that are derived from the action of heat, enzyme or chemical reagents.
- ✓ Derived proteins are of two types, primarily derived proteins and secondary derived proteins.
  - ➢ Primary derived proteins
    - Derivatives of proteins, in which the size of the protein molecule is not altered materially.,.
    - Primary derived proteins are classified into three types - Proteans, Infraproteins and Coagulated proteins.
    - Example: edestan, coagulated egg-white.
  - ➢ **Secondary derived proteins**
    - ✓ While in secondary derived proteins, hydrolysis occurs, as a result the molecules are smaller than the original proteins.
    - ✓ They are further classified into 3 types - Proteoses, Peptones and Polypeptides

III. Classification of Proteins on Biological Function

i. Enzymic Proteins - They are the most varied and highly specialized proteins with catalytic activity. Enzymes catalyze a variety of reactions. - Example: Urease, catalase, cytochrome C, etc.

ii. Structural Proteins - These proteins aid in strengthening or protecting biological structures. - Example: Collagen, elastin, keratin, etc.

iii. Transport or Carrier Proteins - These proteins help in transport of ions or molecules in the body. - Example: Myoglobin, hemoglobin, etc.

iv. Nutrient and Storage Proteins - These proteins provide nutrition to growing embryos and store ions.

v. Contractile or Motile Proteins - These proteins function in the contractile system. - Example: Actin, myosin, tubulin, etc.

vi. Defense Proteins - These proteins defend against other organisms. - Example: Antibodies, Fibrinogen, thrombin.

vii. Regulatory Proteins - They regulate cellular or metabolic activities. - Example: Insulin, G proteins, etc.

viii. Toxic Proteins - These proteins hydrolyze or degrade enzymes. - Example: snake venom, ricin.

Function of Proteins

- ✓ Proteins are seen in muscles, hair, skin and other tissues; they constitute the bulk of body's non-skeletal structure. Example: The protein keratin is present in nails and hair.
- ✓ Some proteins are hormones and regulate many body functions. Example: Insulin hormone is a protein and it regulated the blood sugar level.
- ✓ Some proteins act enzymes, they catalyze or help in biochemical reactions. Example: Pepsin and Trypsin.
- ✓ Some proteins act as antibodies; they protect the body from the effect of invading species or substances. - Proteins transport different substances in blood of different tissues. Example: Haemoglobin is an oxygen transport protein.
- ✓ Contractile proteins help in contraction of muscle and cells of our body. Example: Myosin is contractile protein.
- ✓ Fibrinogen a glycoprotein helps in healing of wounds. It prevents blood loss and inhibits passage of germs.

Table 5.1 An Overview of Protein Functions

Type of Protein	Function	Examples
Structural proteins	Support	Insects and spiders use silk fibers to make their cocoons and webs, respectively. Collagen and elastin provide a fibrous framework in animal connective tissues. Keratin is the protein of hair, horns, feathers, and other skin appendages.
Storage proteins	Storage of amino acids	Ovalbumin is the protein of egg white, used as an amino acid source for the developing embryo. Casein, the protein of milk, is the major source of amino acids for baby mammals. Plants have storage proteins in their seeds.
Transport proteins	Transport of other substances	Hemoglobin, the iron-containing protein of vertebrate blood, transports oxygen from the lungs to other parts of the body. Other proteins transport molecules across cell membranes.
Hormonal proteins	Coordination of an organism's activities	Insulin, a hormone secreted by the pancreas, helps regulate the concentration of sugar in the blood of vertebrates.
Receptor proteins	Response of cell to chemical stimuli	Receptors built into the membrane of a nerve cell detect chemical signals released by other nerve cells.
Contractile proteins	Movement	Actin and myosin are responsible for the movement of muscles. Other proteins are responsible for the undulations of the organelles called cilia and flagella.
Defensive proteins	Protection against disease	Antibodies combat bacteria and viruses.
Enzymatic proteins	Selective acceleration of chemical reactions	Digestive enzymes catalyze the hydrolysis of the polymers in food.

Primary structure of a Protein

Sequence of Amino Acids in Proteins

Protein structure is studied as the primary, secondary, tertiary and quaternary levels. Primary structure denotes the number and sequence of amino acids in the protein. The higher levels of organization are decided by the primary structure. Each polypeptide chain has a unique amino acid sequence decided by the genes. The primary structure is maintained by the covalent bonds of the peptide linkages (Fig. 4.1). Students should have a clear concept of the term “sequence”. See the following example:

Gly - Ala - Val (1)

Gly - Val - Ala (2)

Both the tripeptides shown above contain the same amino acids; but their sequence is altered. When the sequence is changed, the peptide is also different.

Characteristics of a Peptide Bond

The peptide bond is a partial double bond. The C–N bond is ‘trans’ in nature and there is no freedom of rotation because of the partial double bond character (Fig. 4.2). The distance is 1.32Aº which is midway between single bond (1.49Å) and double bond

(1.27Aº). The side chains are free to rotate on either side of the peptide bond. The angles of rotation, known as Ramachandran angles, therefore determine the spatial orientation of the peptide chain.

Numbering of Amino Acids in Proteins

In a polypeptide chain, at one end there will be one free alpha amino group. This end is called the amino terminal (N-terminal) end and the amino acid contributing the alpha-amino group is named as the first amino acid. (Fig. 4.3). Usually the N-terminal amino acid is written on the left-hand side when the sequence of the protein is denoted. Incidentally, the biosynthesis of the protein also starts from the amino terminal end. The other end of the polypeptide chain is the carboxy terminal end (C-terminal), where there is a free alpha carboxyl group which is contributed by the last amino acid (Fig. 4.3). All other alpha amino and alpha carboxyl groups are involved in peptide bond formation. Amino acid residues in polypeptides are named by changing the suffix “-ine” to “-yl”, e.g. Glycine to Glycyl.

NH2-Gly-Ala-Val-COOH

In the above example, the amino group of glycine is free; but carboxyl group of glycine is bonded with amino group of alanine; the carboxyl group of alanine is, in turn, bonded with the amino group of valine; while the carboxyl group of valine is free. Therefore, this peptide is named as glycyl-alanyl-valine. It is abbreviated as GlyAla-Val, or. simply as GAV.

Branched and Circular Proteins

Generally, the polypeptide chains are linear. However branching points in the chains may be produced by interchain disulphide bridges. The covalent disulphide bonds between different polypeptide chains in the same protein (interchain) or portions of the same polypeptide chain (intrachain) are also part of the primary structure.

Rarely, instead of the alpha COOH group, the gamma carboxyl group of glutamic acid may enter into peptide bond formation, e.g. Glutathione (gammaglutamyl-cysteinyl-glycine). The term pseudopeptide is used to denote such a peptide bond formed by carboxyl group, other than that present in alpha position. Very rarely, protein may be in a circular form, e.g. Gramicidin.

Primary Structure of Insulin

As an example of the primary structure of a protein, that of insulin is shown in Figure 4.4. This was originally described by Sanger in 1955 who received the Nobel Prize in 1958. Insulin has two polypeptide chains. The A chain (Glycine chain) has 21 amino

acids and B (Phenylalanine) chain has 30 amino acids. They are held together by two interchain disulfide bonds (Fig. 4.4). The 7th cysteine in A chain and the 7th cysteine in B chain are connected. Similarly, A chain 20th cysteine and B chain 19th cysteine is connected. There is another intrachain disulfide bond between 6th and 11th cysteine residues of A chain. The species variation is restricted to amino acids in position 8, 9 and 10 in A chain and in C-terminal of B chain (Fig. 4.4). The amino acid sequence has been conserved to a great extent during evolution.

Proinsulin

Beta cells of pancreas synthesize insulin as a prohormone. Proinsulin is a single polypeptide chain with 86 amino acids. Biologically active insulin (2 chains) is formed by removal of the central portion of the proinsulin before release. The C-peptide (connecting peptide) is also released into the circulation.

Primary Structure Determines Biological Activity

A protein with a specific primary structure will automatically form its natural three-dimensional shape. So, the higher levels of organization are dependent on the primary structure.

Even a single amino acid change (mutation) in the linear sequence may have profound biological effects on the function, e.g. in HbA (normal hemoglobin) the 6th amino acid in the beta chain is glutamic acid; it is changed to valine in HbS (sickle cell anemia).

Secondary Structure of Proteins

The term “secondary structure” denotes the configurational relationship between residues, which are about 3–4 amino acids apart in the linear sequence (Box 4.2).

Secondary and tertiary levels of protein structure are preserved by noncovalent forces or bonds like hydrogen bonds, electrostatic bonds, hydrophobic interactions and van der Waals forces.

Alpha Helix

Pauling (Nobel prize, 1954) and Corey described the alpha helix and beta-pleated sheet structures of polypeptide chains in 1951.

The alpha helix is the most common and stable conformation for a polypeptide chain. In proteins like hemoglobin and myoglobin, the alpha helix is abundant, whereas it is virtually absent in chymotrypsin.

The alpha helix is a spiral structure (Fig. 4.6). The polypeptide bonds form the back-bone and the side chains of amino acids extend outward. The structure is stabilized by hydrogen bonds between NH and C=O groups of the main chain.

Each turn is formed by 3.6 residues. The distance between each amino acid residue (translation) is 1.5 Aº. The alpha helix is generally right-handed. Left-handed alpha helix is rare, because amino acids found in proteins are of L-variety, which exclude left handedness. Proline and hydroxyproline will not allow the formation of alpha helix.

Beta-Pleated Sheet

The polypeptide chains in beta-pleated sheet is almost fully extended. The distance between adjacent amino acids is 3.5Å. It is stabilized by hydrogen bonds between NH and C=O groups of neighboring polypeptide segments. Adjacent strands in a sheet can run in the same direction with regard to the amino and carboxy terminal ends of the polypeptide chain (parallel) or in opposite direction (anti-parallel beta sheet) (Fig. 4.7). Beta-pleated sheet is the major structural motif in proteins like silk Fibroin (anti-parallel), Flavodoxin (parallel) and Carbonic anhydrase (both). Beta bends may be formed in many proteins by the abrupt U-turn folding of the chain. Intrachain disulfide bridges stabilize these bends.

Collagen Helix

It is a triple helical structure found in collagen

Tertiary Structure

Secondary structure denotes the configurational relationship between residues which are about 3–4 amino acids apart; or secondary level defines the organization at immediate vicinity of amino acids. The tertiary structure denotes three-dimensional structure of the whole protein (Box 4.1 and Fig. 4.8). The tertiary structure defines the steric relationship of amino acids which are far apart from each other in the linear sequence, but are close in the three-dimensional aspect.

The tertiary structure is maintained by noncovalent interactions such as hydrophobic bonds, electrostatic bonds and van der Waals forces. The tertiary structure acquired by native protein is always thermodynamically most stable.

Quaternary Structure

Certain polypeptides will aggregate to form one functional protein (Box 4.1 and Fig. 4.8). This is referred to as the quaternary structure. The protein will lose its function when the subunits are dissociated. The forces that keep the quaternary structure are hydrogen bonds, electrostatic bonds, hydrophobic bonds and van der Waals forces.

Depending on the number of polypeptide chain, the protein may be termed as monomer (1 chain), dimer (2 chains), tetramer (4 chains) and so on. Each polypeptide chain is termed as subunit or monomer.

Homodimer contains two copies of the same polypeptide chain. Heterodimer contains two different types of polypeptides as a functional unit. For example, 2 alpha-chains and 2 beta-chains form the hemoglobin molecule. Similarly, 2 heavy chains and 2 light chains form one molecule of immunoglobulin G. Creatine kinase (CK) is a dimer. Lactate dehydrogenase (LDH) is a tetramer.

Structure-function Relationship

The functions of proteins are maintained because of their ability to recognize and interact with a variety of molecules. The three-dimensional structural conformation provides and maintains the functional characteristics. The three-dimensional structure, in turn, is dependent on the primary structure. So, any difference in the primary structure may produce a protein which cannot serve its function. To illustrate the structure function relationship, the following three proteins are considered; each belongs to a different class in the functional classification.

Enzymes

The first step in enzymatic catalysis is the binding of the enzyme to the substrate. This, in turn, depends on the structural conformation of the active site of the enzyme, which is precisely oriented for substrate binding. Carbonic anhydrase catalyses the reversible hydration of carbon dioxide. This enzyme makes it possible for the precise positioning of the CO_2 molecule and the hydroxyl (OH–) ion for the formation of bicarbonate ion. The enzyme has a zinc ion located at a deep cleft co-ordinated to histidine residues. The CO_2 binding residues are very near to the zinc ion. Water binds to zinc ion, gets ionized to hydroxyl ion and it binds to the CO_2 which is proximally located. The substrates are brought in close proximity for the reaction to proceed.

Transport Proteins

Hemoglobin, the transporter of oxygen is a tetrameric protein (alpha 2, beta 2), with each monomer having a heme unit. Binding of oxygen to one heme facilitates oxygen binding by other subunits. Binding of H^+ and CO_2 promotes release of O_2 from hemoglobin. This allosteric interaction is physiologically important, and is termed as Bohr effect. Even a single amino acid substitution alters the structure and thereby the function, e.g. in sickle cell anemia (HbS), the 6^{th} amino acid in the beta chain is altered, leading to profound clinical manifestations.

Structural Proteins

Collagen is the most abundant protein in mammals and is the main fibrous component of skin, bone, tendon, cartilage and teeth. Collagen forms a super helical cable where the 3 polypeptide chains are wound around itself. In collagen, every 3rd residue is a glycine. The only amino acid that can fit into the triple stranded helix is glycine. Replacement of the central glycine by mutations can lead to brittle bone disease. The triple helix of collagen is stabilized by the steric repulsion of the rings of hydroxyproline and also by the hydrogen bonds between them. In vitamin C deficiency, failure of hydroxylation of proline/lysine leads to reduced hydrogen bonding and consequent weakness of collagen. The quarter staggered triple helical structure of collagen is responsible for its tensile strength. Different arrangements of collagen fibrils in tissues are seen. Parallel bundles in tendons and sheets layered at many angles in skin. Heat denatured collagen is gelatin.

Unit – 2
Metabolism and Engineering

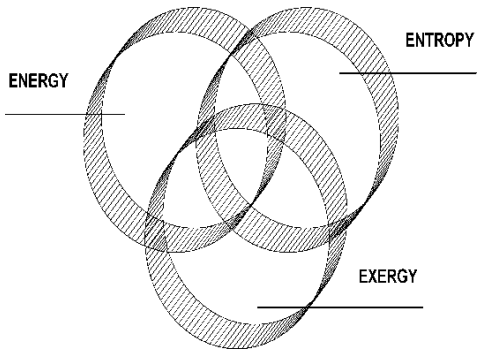
Engineering aspects in thermodynamics of energy transactions

Thermodynamics is broadly viewed as the science of energy, and thermal engineering is concerned with making the best use of available energy resources. The name thermodynamics stems from the Greek words therme (heat) and dynamics (force), which is most descriptive of the early efforts to convert heat into power. Today the same name is broadly interpreted to include all aspects of energy and energy transformations,

including power production, refrigeration, and relationships among the properties of matter.

The science of thermodynamics is built primarily on two fundamental natural laws, known as the first and the second laws. The first law of thermodynamics is simply an expression of the conservation of energy principle. It asserts that energy is a thermodynamic property, and that during an interaction, energy can change from one form to another but the total amount of energy remains constant. The second law of thermodynamics asserts that energy has quality as well as quantity, and actual processes occur in the direction of decreasing quality of energy. The high-temperature thermal energy is degraded as it is transferred to a lower temperature body. The attempts to quantify the quality or “work potential” of energy in the light of the second law of thermodynamics has resulted in the definition of the properties entropy and exergy.

The figure illustrated, where the domains of energy, entropy and exergy are shown. This paper focuses on the portion of the field of thermodynamics that intersects with the energy, entropy and exergy fields, and particularly emphasizes the intersection of all three domains. Note that entropy and exergy are also used in other fields (such as statistics and information theory), and therefore they are not subsets of energy. Also, some forms of energy (such as shaft work) are entropy-free, and thus entropy subtends only part of the energy field. Likewise, exergy subtends only part of the energy field as well since some systems (such as air at atmospheric conditions) possess energy but no exergy. Most thermodynamic systems (such as steam in a power plant) possess energy, entropy, and exergy, and thus appear at the intersection of these three fields.



Energy:

Thermodynamics plays a key role in the analysis of systems and devices in which energy transfer and energy transformation take place. Thermodynamics' implications are far-reaching, and its applications span the whole range of the human enterprise. All along our technological history, the development of sciences has enhanced our ability to

harness energy and use it for society's needs. The industrial revolution is a result of the discovery of how to exploit energy and how to convert heat into work. Nature allows the conversion of work completely into heat, but heat is taxed when converted into work. For this reason, the return on our investment of heat transfer is compared with the output work transfer and attempts are made to maximize this return.

Most of our daily activities involve energy transfer and energy change. The human body is a familiar example of a biological system in which the chemical energy of the food or body fat is transformed into other forms of energy such as heat transfer and work transfer. Our encounter with the environment also reveals a wide area of engineering applications. These include power plants to generate electricity, engines to run automobiles and aircraft, refrigeration and air conditioning systems, and so on.

In the hydroelectric power system, the potential energy of the water is converted into mechanical energy through the use of a hydraulic turbine. The mechanical energy is then converted into electric energy by an electric generator coupled to the shaft of the turbine. In a steam power generating plant, chemical or nuclear energy is converted into thermal energy in a boiler or a reactor. The energy is imparted to water, which vaporizes into steam. The energy of the steam is used to drive a steam turbine, and the resulting mechanical energy is used to operate a generator to produce electric power. The steam leaving the turbine is then condensed, and the condensate is pumped back to the boiler to complete the cycle. Breeder reactors use uranium-235 as a fuel source and can produce some more fuel in the process. A solar power plant uses solar concentrators (parabolic or flat mirrors) to heat a working fluid in a receiver located on a tower. The heated fluid then expands in a turbogenerator in a similar manner as in a conventional power plant. In a spark-ignition internal combustion engine, the chemical energy of the fuel is converted into mechanical work. An air-fuel mixture is compressed and combustion is initiated by a spark device. The expansion of the combustion gases pushes against the piston, which results in the rotation of the crankshaft. Gas turbine engines, commonly used for aircraft propulsion, convert the chemical energy of the fuel into thermal energy that is used to run a gas turbine. The turbine is directly coupled to a compressor that supplies the air required for combustion. The exhaust gases, upon expanding in a nozzle, create the necessary thrust. For power generation, the turbine is coupled to an electric generator and drives both the compressor and the generator. In a liquid-fuel rocket, a fuel and an oxidizer are combined, and the combustion gases expand in a nozzle creating a propulsive force (thrust) to propel the rocket. A typical nuclear

rocket propulsion engine offers a higher specific impulse when compared to chemical rockets. The fuel cell converts chemical energy into electric energy directly making use of an ion-exchange membrane. When a fuel such as hydrogen is ionized, it flows from the anode through the membrane toward the cathode. The released electrons at the anode flow through an external load. In a magnetohydrodynamic generator, electricity is produced by moving a high-temperature plasma through a magnetic field. The refrigeration system utilizes work supplied by the electric motor to transfer heat from a refrigerated space. Low-temperature boiling fluids such as ammonia and refrigerant-134a absorb energy in the form of heat transfer, as they vaporize in the evaporator causing a cooling effect in the region being cooled.

Concept of Energy

The concept of energy was first introduced in mechanics by Newton when he hypothesized about kinetic and potential energies. However, the emergence of energy as a unifying concept in physics was not adopted until the middle of the 19th century and was considered one of the major scientific achievements in that century. The concept of energy is so familiar to us today that it is intuitively obvious, yet we have difficulty in defining it exactly. Energy is a scalar quantity that cannot be observed directly but can be recorded and evaluated by indirect measurements. The absolute value of energy of system is difficult to measure, whereas its energy change is rather easy to calculate. In our life the examples for energy are endless. The sun is the major source of the earth's energy. It emits a spectrum of energy that travels across space as electromagnetic radiation. Energy is also associated with the structure of matter and can be released by chemical and atomic reactions. Throughout history, the emergence of civilizations has been characterized by the discovery and effective application of energy to society's needs.

Forms of Energy

- Macroscopic forms of energy
- Microscopic forms of energy

Macroscopic forms of energy

The **macroscopic forms of energy** are those where a system possesses as a whole with respect to some outside reference frame such as kinetic and potential energies. For example, the macroscopic energy of an upmoving object changes with velocity and elevation. The macroscopic energy of a system is related to motion and the influence of some external effects such as gravity, magnetism, electricity and surface

tension. The energy that a system possesses as a result of its motion relative to some reference frame is called kinetic energy. The energy that a system has as a result of its elevation in a gravitational field is called potential energy. Kinetic energy refers to the energy possessed by the system because of its overall motion, either translational or rotational. The word "overall" is italicized because the kinetic energy to which we refer is the kinetic energy of the entire system, not the kinetic energy of the molecules in the system. If the system is a gas, the kinetic energy is the energy due to the macroscopic flow of the gas, not the motion of individual molecules. The potential energy of a system is the sum of the gravitational, centrifugal, electrical, and magnetic potential energies. To illustrate using gravitational potential energy, a one-kilogram mass, 100 m above the ground, clearly has a greater potential energy than the same kilogram mass on the ground. That potential energy can be converted into other forms of energy, such as kinetic energy, if the mass is allowed to fall freely. Kinetic and potential energy depend on the environment in which the system exists. In particular, the potential energy of a system depends on the choice of a zero level. For example, if the ground level is considered to be at zero potential energy, then the potential energy of the mass at 100 m above the ground will have a positive potential energy equal to the mass (1 kg) multiplied by the gravitational constant ($g = 9.807\ m/s^2$ at sea level) and the height above the ground (100 m). Its potential energy will be 980.7 $(kgm^2)/s^2$ = 980.7 Newton-meters (Nm), that is, 980.7 J. The datum plane for potential energy can be chosen arbitrarily. If it had been chosen at 100 m above the ground level, the potential energy of the mass would have been zero. Of course, the difference in potential energy between the mass at 100 m and the mass at ground level is the same independent of the datum plane.

Microscopic forms of energy

The **microscopic forms of energy** are those related to the molecular structure of a system and the degree of the molecular activity, and they are independent of outside reference frames. The sum of all the microscopic forms of energy is called the internal energy of a system. The internal energy of a system depends on the inherent qualities, or properties, of the materials in the system, such as composition and physical form, as well as the environmental variables (temperature, pressure, electric field, magnetic field, etc.). Internal energy can have many forms, including, sensible and latent (i.e., thermal), chemical, nuclear, electrical, mechanical, magnetic, and surface energy. For example, a spring that is compressed has a higher internal energy (mechanical energy) than a spring that is not compressed, because the compressed spring can do some work on changing

(expanding) to the uncompressed state. As another example, consider two identical vessels, each containing hydrogen and oxygen. In the first, the gases are contained in the elemental form, pure hydrogen and pure oxygen in a ratio of 2:1. In the second, the identical number of atoms is contained, but in the form of water. One can appreciate that the internal energy of the first is different from the second. A spark set off in the first container will result in a violent release of energy. The same will not be true in the second. Clearly, the internal energy present differs in these two situations. Any energy balance will have to take this difference into account.

The First law of Thermodynamics

The FLT stands for the first law of the conservation of energy. This is stated as energy can be neither created nor destroyed; it just changes form. The FLT defines internal energy as a state function and provides a formal statement of the conservation of energy. However, it provides no information about the direction in which processes can spontaneously occur, that is, the reversibility aspects of thermodynamic processes. For example, it cannot say how cells can perform work while existing in an isothermal environment. It gives no information about the inability of any thermodynamic process to convert heat into mechanical work with full efficiency, or any insight into why mixtures cannot spontaneously separate or unmix themselves. An experimentally derived principle to characterize the availability of energy is required to do this. This is precisely the role of the second law of thermodynamics that we will explain later.

Entropy

Within the past 50 years our view of Nature has changed drastically. Classical science emphasized equilibrium and stability. Now we see fluctuations, instability, evolutionary processes on all levels from chemistry and biology to cosmology. Everywhere we observe irreversible processes in which time symmetry is broken. The distinction between reversible and irreversible processes was first introduced in thermodynamics through the concept of "entropy".

In the modern context, the formulation of entropy is fundamental for understanding thermodynamic aspects of self-organization, evolution of order and life that we see in Nature. When a system is isolated, energy increase will be zero. In this case the entropy of the system will continue to increase due to irreversible processes and reach the maximum possible value, which is the state of thermodynamic equilibrium. In the state of equilibrium, all irreversible processes cease. When a system begins to exchange entropy

with the exterior then, in general, it is driven away from equilibrium, and the entropy producing irreversible processes begins to operate. The exchange of entropy is due to exchange of heat and matter. The entropy flowing out of an adiabatic system is always larger than the entropy flowing into the system, the difference arising due to entropy produced by irreversible processes within the system. As we shall see in the following chapters, systems that exchange entropy with their exterior do not simply increase the entropy of the exterior, but may undergo dramatic spontaneous transformations to "self-organization." The irreversible processes that produce entropy create these organized states. Such self-organized states range from convection patterns in fluids to life. Irreversible processes are the driving forces that create this order.

Much of the internal energy of a substance is randomly distributed as kinetic energy at the molecular and sub molecular levels and as energy associated with attractive or repulsive forces between molecular and sub molecular entities, which are moving closer together or further apart in relation to their mean separation. This energy is sometimes described as being 'disordered' as it is not accessible as work at the macroscopic level in the same way as is the kinetic energy or gravitational potential energy that an entire system possesses owing to its velocity or position in the gravitational field. Although energy is the capacity to do work, it is not possible directly to access the minute quantities of disordered energy possessed at a given instant by the various modes of energy possession of the entities so as to yield mechanical shaft work on the macroscopic scale. The term 'disorder' refers to the lack of information about exactly how much energy is associated at any moment with each mode of energy possession of each molecular or sub molecular entity within the system.

At the molecular and sub molecular level there is also 'ordered energy' associated with attractive or repulsive forces between entities that have fixed mean relative positions. Part of this energy is, in principle, accessible as work at the macroscopic level under very special conditions, which are beyond the scope of this manuscript. Temperature is the property that determines whether a system that is in equilibrium will experience any decrease or increase in its disordered energy if it is brought into contact with another system that is in equilibrium. If the systems do not have the same temperature, disordered energy will be redistributed from the system at the higher temperature to the one at the lower temperature. There is then less information about precisely where that energy resides, as it is now dispersed over the two systems.

Heat transfer to a system increases the disordered energy of the system. Heat transfer from a system reduces the disordered energy. Reversible heat transfer is characterized by both the amount of energy transferred to or from the system and the temperature level at which this occurs. The property entropy, whose change between states is defined as the integral of the ratio of the reversible heat transfer to the absolute temperature, is a measure of the state of disorder of the system. This 'state of disorder' is characterized by the amount of disordered energy and its temperature level. When reversible heat transfer occurs from one system to another, both systems have the same temperature and the increase in the disorder of one is exactly matched by the decrease in disorder of the other. When reversible adiabatic work is done on or by a system its ordered energy increases or decreases by exactly the amount of the work and the temperature level changes in a way that depends on the substances involved. Reversible work is characterized by the amount of energy transferred to or from the system, irrespective of the temperature of the system. Irreversible work, such as stirring work or friction work, involves a change in the disorder of the system and, like heat transfer to a system, has the effect of increasing the entropy.

Entropy Aspects

It is now important to introduce a new thermodynamic property, entropy, that is simply a measure of the amount of molecular disorder within a system. In this regard, a system possessing a high degree of molecular disorder (such as a high temperature gas) has a very high entropy value and vice versa. It is important to note that numerical values for specific entropy are commonly listed in thermodynamic tables along with values for specific volume, specific internal energy, and specific enthalpy. Therefore, entropy is known as the core of the second law thermodynamics. Here, we have to highlight the following facts:

- The entropy of a system is a measure of the amount of molecular disorder within the system.
- A system can only generate, not destroy, entropy.
- The entropy of a system can be increased or decreased by energy transports across the system boundary.

Heat and work are mechanisms of energy transfer. They are measures of the change in the internal energy in one body as energy is transferred to it or from it to another. Work is accomplished by a force acting through a distance. Heat requires a difference in temperature for its transfer. The definition of heat energy can be broadened

to include the energy stored in a hot gas as the average kinetic energy of randomly moving molecules. This description enabled us to understand the natural flow of heat energy from a hot to a cooler substance. The concept of random motion was translated into a notion of order and disorder. The key linkage of order/disorder with probability followed. Energy transfers or conversions are changes of the state of a system. The natural direction of a change in state of a system is from a state of low probability to one of higher probability. That is what probability means. And disordered states are more probable than ordered ones. Thus, the natural direction of change of state of a system is from order to disorder. This is the "something" that is changing in all the energy transfers and conversions we have described. Finally, we gave that something a name-entropy. The entropy of a state of a system is proportional to (depends on) its probability. Thus, the SLT can be expressed more broadly in terms of entropy in the following way:

In any transfer or conversion of energy within a closed system, the entropy of the system increases. The consequences of the second law can thus be stated positively as the spontaneous or natural direction of energy transfer or conversion is toward increasing entropy, or negatively as all energy transfers or conversions are irreversible. Or, in keeping with our paraphrasing of the FLT as "You can't get something for nothing," the SLT asserts: You can't even get all you pay for.

It is low-entropy energy sources that are being used up, and low-entropy energy is "useful" energy. The energy sources in the universe were rated on an entropy/usefulness scale from the zero-entropy, highly useful mechanical forms such as gravitational potential energy, which are easily converted to work, to the high-entropy, unusable heat of our surroundings.

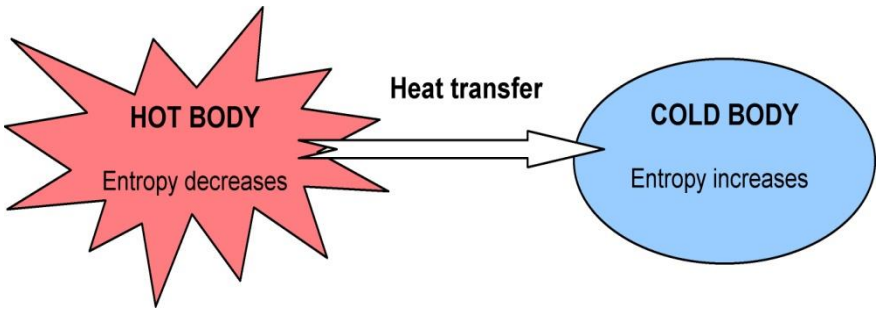
In summary, this broader interpretation of the SLT suggests that real "energy conservation" should include the practice of thermodynamic economy. Each energy transfer or conversion, all else being equal, should be arranged so that the total change in entropy (entropy generation) is a minimum. This requires that energy sources be matched in entropy to energy end use.

Significance of entropy

The "entropy" of the state of a system is a measure of the probability of its occurrence. States of low probability have low entropy; states of high probability have high entropy. With this definition we see, from the previous discussion and examples, that in any transfer or conversion of energy, because the spontaneous direction of the change of state of a closed system is from a less to a more probable state, the entropy of

the system must increase. That is the broader statement we have been seeking for the second law, "In any energy transfer or conversion within an isolated closed system, the entropy of that system increases."

Energy conversions can proceed so that the entropy of a part of a system is decreased. Charging a storage battery, freezing ice cubes, and even the processes of life and growth are examples. In each of these examples, order has been won from disorder and entropy has decreased. If the total system is considered, however, the total effect has been an increase in disorder. To charge a battery we must provide energy above and beyond that necessary to re-form the chemical combinations in the battery plates. Some of this low-entropy electrical energy is changed into high-entropy heat energy in the current-carrying wires. In freezing ice we increase the order and thus decrease the entropy of the water in the ice cube trays by removing heat from it. The heat energy removed, however, has to flow into a substance that is at a lower temperature than the surroundings. Thus, the entropy and the disorder of this gas are increased. Moreover, we put low-entropy electrical energy into the refrigerator through the motor, and this energy is degraded to heat. The overall change in entropy is positive. In the life process, highly ordered structures are built from the much simpler structures of various chemicals, but to accomplish this, life takes in relatively low-entropy energy-sunlight and chemical energy-and gives off high-entropy heat energy. The entropy of the total system again increases. The figure illustrates a heat transfer process from the entropy point of view.



Although a spontaneous process can proceed only in a definite direction, the FLT gives no information about direction; it merely states that when one form of energy is converted into another, identical quantities of energy are involved regardless of feasibility of the process. In this regard, events could be envisioned that would not violate the FLT, e.g., transfer of a certain quantity of heat from a low-temperature body to a high-temperature body, without expenditure of work. However, the reality shows that this is impossible and FLT becomes inadequate in picturizing the complete energy transfer.

Furthermore, experiments indicated that when energy in the form of heat is transferred to a system, only a portion of heat can be converted into work.

The Second Law of Thermodynamics

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The SLT establishes the difference in quality between different forms of energy and explains why some processes can spontaneously occur, whereas other cannot. It indicated a trend of change and is usually expressed as an inequality. The SLT has been confirmed by experimental evidences like other physical laws of nature.

The SLT defines the fundamental physical quantity entropy as a randomized energy state unavailable for direct conversion to work. It also states that all spontaneous processes, both physical and chemical, proceed to maximize entropy, that is, to become more randomized and to convert energy into a less available form. A direct consequence of fundamental importance is the implication that at thermodynamic equilibrium the entropy of a system is at a relative maximum; that is, no further increase in disorder is possible without changing by some external means (such as adding heat) the thermodynamic state of the system. A basic corollary of the SLT is the statement that the sum of the entropy changes of a system and that of its surroundings must always be positive, that is, the universe (the sum of all systems and surroundings) is constrained to become forever more disordered and to proceed toward thermodynamic equilibrium with some absolute maximum value of entropy. From a biological standpoint this is certainly a reasonable concept, since unless gradients in concentration and temperature are forcibly maintained by the consumption of energy, organisms proceed spontaneously toward the biological equivalent of equilibrium-death.

The SLT is quite general. However, when intermolecular forces are long range, as in the case of particles interacting through gravitation, there are difficulties because our classification into extensive variables (proportional to volume) and intensive variables

(independent of volume) does not apply. The total energy is no longer proportional to the volume. Fortunately, gravitational forces are very weak as compared to the short-range intermolecular forces. It is only on the astrophysical scale that this problem becomes important. The generality of the SLT gives us a powerful means to understand the thermodynamic aspects of real systems through the usage of ideal systems. A classic example is Planck's analysis of radiation in thermodynamic equilibrium with matter (blackbody radiation) in which Planck considered idealized simple harmonic oscillators interacting with radiation. Planck considered simple harmonic oscillators not merely because they are good approximations of molecules but because the properties of radiation in thermal equilibrium with matter are universal, regardless of the particular nature of the matter with which the radiation interacts. The conclusions one arrives at using idealized oscillators and the laws of thermodynamics must also be valid for all other forms of matter, however complex.

What makes this new statement of the SLT valuable as a guide to energy policy is the relationship between entropy and the usefulness of energy. Energy is most useful to us when we can get it to flow from one substance to another, e.g., to warm a house and we can use it to do work. Useful energy thus must have low entropy so that the SLT will allow transfer or conversions to occur spontaneously.

SLT Statements

- The **Clausius statement**. It is impossible for a system to transfer heat from a lower temperature reservoir to a higher temperature reservoir. Simply, heat transfer can only occur spontaneously in the direction of temperature decrease. For example, we cannot construct a refrigerator that operates without any work input.
- The **Kelvin-Planck statement**. It is impossible for a system to receive a given amount of heat from a high-temperature reservoir and provide an equal amount of work output. While a system converting work to an equivalent energy transfer as heat is possible, a device converting heat to an equivalent energy transfer as work is impossible. For example, we cannot build a heat engine that has a thermal efficiency of 100%.

Exothermic Reactions:

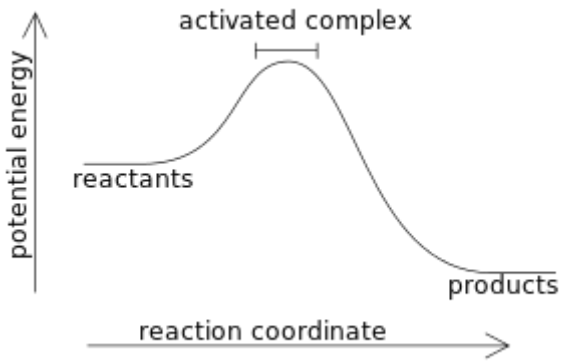
Exothermic reaction is a chemical reaction that releases energy through light or heat. It is opposite of an endothermic reaction.

$$\text{reactants} \rightarrow \text{products} + \text{energy}.$$

"Exo" means in Greek “out” and “thermic” means “heat”. So, the reaction in which there is release of heat with or without light is called exothermic reaction. The reaction in which there is release of heat with or without light is called exothermic reaction. It gives net energy to its surroundings. That is, the energy needed to initiate the reaction is less than the energy released. When the medium in which the reaction is taking place collects heat, the reaction is exothermic. When using a bomb calorimeter, the total amount of heat that flows into (or through) the calorimeter is the negative of the net change in energy of the system.

The absolute amount of energy in a chemical system is difficult to measure or calculate. The enthalpy change, Δ*H*, of a chemical reaction is much easier to work with. The enthalpy change equals the change in internal energy of the system plus the work needed to change the volume of the system against constant ambient pressure. A bomb calorimeter is very suitable for measuring the energy change, Δ*H*, of a combustion reaction. Measured and calculated ΔH values are related to bond energies by:

Δ*H* = (energy used in forming product bonds) − (energy released in breaking reactant bonds)



In an exothermic reaction, by definition, the enthalpy change has a negative value:

$$\Delta H < 0$$

Examples of exothermic reactions

- Combustion reactions of fuels or a substance

  Burning of natural gas:
- Neutralization
- The thermite reaction
- Reactions taking place in a self-heating can based on lime aluminium
- Many corrosion reactions such as oxidation of metals
- Most polymerization reactions

- The Haber process of ammonia production
- Respiration
- Decomposition of vegetable matter in to compost
- Solution of sulfuric acid in to water
- Dehydration of sugar upon contact with sulfuric acid
- Detonation of nitroglycerin
- Nuclear fission of Uranium-235

Endothermic reactions

An **endothermic process** is any process which requires or absorbs energy from its surroundings, usually in the form of heat. It may be a chemical process, such as dissolving ammonium nitrate in water, or a physical process, such as the melting of ice cubes. The term was coined by Marcellin Berthleot from the Greek roots *endo*-, derived from the word "endon" meaning "within", and the root "therm", meaning "hot" or "warm" in the sense that a reaction depends on absorbing heat if it is to proceed. The opposite of an endothermic process is an exothermic process, one that releases, "gives out" energy in the form of heat. Thus, in each term (endothermic & exothermic) the prefix refers to where heat goes as the reaction occurs, though in reality it only refers to where the energy goes, without necessarily being in the form of heat.

All chemical reactions involve both the breaking of existing and the making of new chemical bonds. A reaction to break a bond always requires the input of energy and so such a process is always endothermic. When atoms come together to form new chemical bonds, the electrostatic forces bringing them together leave the bond with a large excess of energy (usually in the form of vibrations and rotations). If that energy is not dissipated, the new bond would quickly break apart again. Instead, the new bond can shed its excess energy - by radiation, by transfer to other motions in the molecule, or to other molecules through collisions - and then become a stable new bond. Shedding this excess energy is the exothermicity that leaves the molecular system. Whether a given overall reaction is exothermic or endothermic is determined by the relative contribution of these bond breaking endothermic steps and new bond stabilizing exothermic steps.

The concept is frequently applied in physical sciences to, for example, chemical reactions where thermal energy (heat) is converted to chemical bond energy.

Endothermic (and exothermic) analysis only accounts for the enthalpy change (ΔH) of a reaction. The full energy analysis of a reaction is the Gibbs free energy (ΔG), which includes an entropy (ΔS) and temperature term in addition to the enthalpy. A

reaction will be a spontaneous process at a certain temperature if the products have a lower Gibbs free energy (an exergonic reaction) even if the enthalpy of the products is higher. Entropy and enthalpy are different terms, so the change in entropic energy can overcome an opposite change in enthalpic energy and make an endothermic reaction favourable.

Eg: Melting of Ice (Endothermic reaction)

ATP is an energy source

Adenosine triphosphate (ATP), the energy currency or coin of the cell pictured in Figfure.1, transfers energy from chemical bonds to endergonic (energy absorbing) reactions within the cell. Structurally, ATP consists of the adenine nucleotide (ribose sugar, adenine base, and phosphate group, PO_4^{-2}) plus two other phosphate groups.

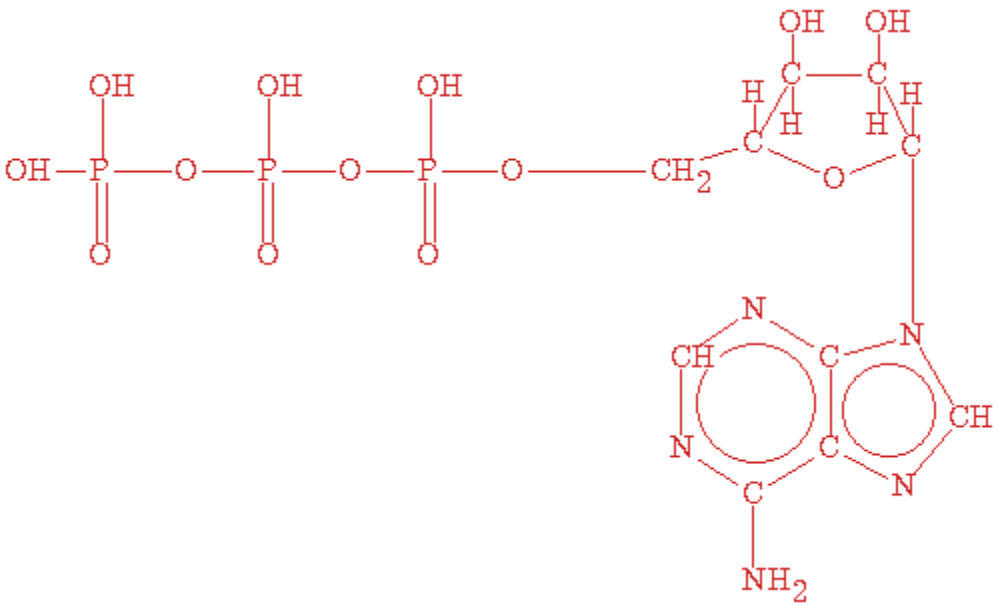


Figure.1: 2-D stick view of the structure of ATP

Energy is stored in the covalent bonds between phosphates, with the greatest amount of energy (approximately 7 kcal/mole) in the bond between the second and third phosphate groups. This covalent bond is known as a pyrophosphate bond.

We can write the chemical reaction for the formation of ATP as:

ADP + Pi + energy----> ATP

The chemical formula for the expenditure/release of ATP energy can be written as:

ATP ---> ADP + energy + Pi

An analogy between ATP and rechargeable batteries is appropriate. The batteries are used, giving up their potential energy until it has all been converted into kinetic energy and heat/unusable energy. Recharged batteries (into which energy has been put) can be used **only** after the input of additional energy. Thus, ATP is the higher energy form (the recharged battery) while ADP is the lower energy form (the used battery). When the terminal (third) phosphate is cut loose, ATP becomes ADP (Adenosine diphosphate; di= two), and the stored energy is released for some biological process to utilize. The input of additional energy (plus a phosphate group) "recharges" ADP into ATP (as in my analogy the spent batteries are recharged by the input of additional energy).

How to make ATP?

Two processes convert ADP into ATP

- Substrate level phosphorylation - Substrate-level phosphorylation occurs in the cytoplasm when an enzyme attaches a third phosphate to the ADP (both ADP and the phosphates are the substrates on which the enzyme acts).
- Chemiosmosis - Enzymes in chemiosmotic synthesis are arranged in an electron transport chain that is embedded in a membrane. In eukaryotes this membrane is in either the chloroplast or mitochondrion. According to the chemiosmosis hypothesis proposed by Peter Mitchell in 1961, a special ATP-synthesizing enzyme is also located in the membranes. Mitchell would later win the Nobel Prize for his work.

During chemiosmosis in eukaryotes, H^+ ions are pumped across an organelle membrane by membrane "pump proteins" into a confined space (bounded by membranes) that contains numerous hydrogen ions. The energy for the pumping comes from the coupled oxidation-reduction reactions in the electron transport chain. Electrons are passed from one membrane-bound enzyme to another, losing some energy with each transfer (as per the second law of thermodynamics). This "lost" energy allows for the pumping of hydrogen ions against the concentration gradient (there are fewer hydrogen ions outside the confined space than there are inside the confined space). The confined hydrogens cannot pass back through the membrane. Their only exit is through the ATP synthesizing enzyme that is located in the confining membrane. As the hydrogen passes through the ATP synthesizing enzyme, energy from the enzyme is used to attach a third phosphate to ADP, converting it to ATP.

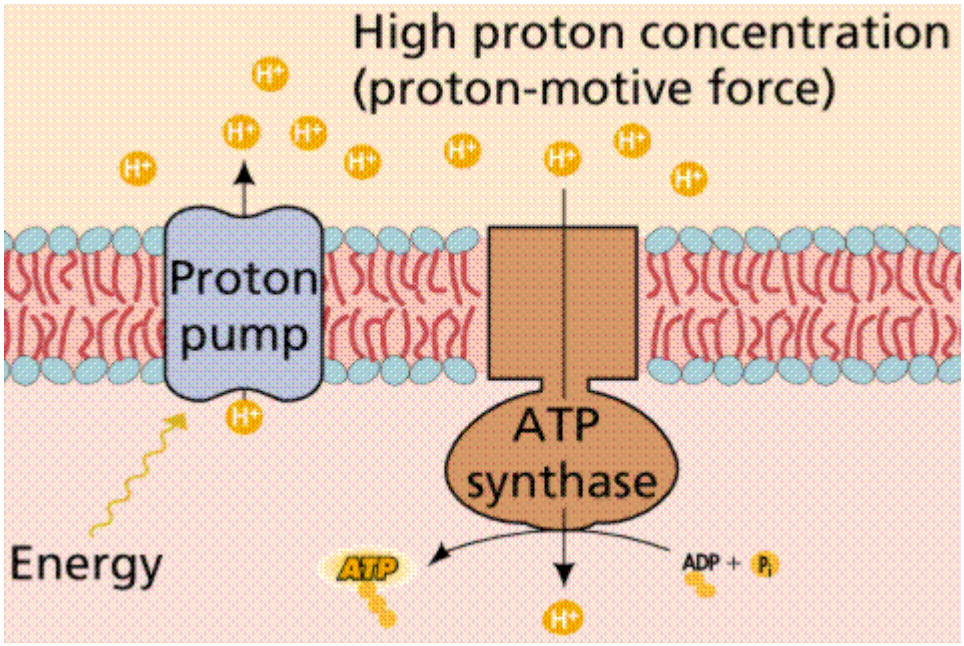


Figure: Generalized view of an electron transport system

Usually the terminal phosphate is not simply removed, but instead is attached to another molecule. This process is known as phosphorylation.

Glucose + ATP -----> Glucose~P + ADP

Glucose can be converted into Glucose-6-phosphate by the addition of the phosphate group from ATP.

ATP serves as the biological energy company, releasing energy for both anabolic and catabolic processes and being recharged by energy generated from other catabolic reactions.

Glycolysis

Introduction

Glycolysis is a metabolic pathway and an anaerobic source of energy that has evolved in nearly all types of organisms. The process entails the oxidation of glucose molecules, the single most important organic fuel in plants, microbes, and animals. Most cells prefer glucose (there are exceptions, such as acetic acid bacteria which prefer ethanol). In glycolysis, per molecule of glucose, 2 ATP molecules are utilized, while 4 ATP, 2 NADH, and 2pyruvates are produced. The pyruvate can be used in the citric acid cycle, or serve as a precursor for other reactions.

Molecule

Glucose is a hexose sugar, which means that it is a monosaccharide with 6 carbon atoms and 6 oxygen atoms. The first carbon consists of an aldehyde group, and the other 5 carbons have 1 hydroxyl group each. In glycolysis, glucose is broken down ultimately into pyruvate and energy, a total of 2 ATP, is derived in the process (Glucose + 2 NAD+ +2 ADP + 2 Pi $\rightarrow$ 2 Pyruvate + 2 NADH + 2 H+ + 2 ATP + 2 H_2O). The hydroxyl groups allow for phosphorylation. The specific form of glucose used in glycolysis is glucose 6phosphate.

Function

Glycolysis occurs in the cytosol of the cell. It is metabolic pathway which creates ATP without the use of oxygen but can occur in the presence of oxygen as well. In cells which use aerobic respiration as the primary source of energy, the pyruvate formed from the pathway can be used in the citric acid cycle and go through oxidative phosphorylation to be oxidized into carbon dioxide and water. Even if cells primarily use oxidative phosphorylation, glycolysis can serve as an emergency backup for energy or serve as the preparation step before oxidative phosphorylation. In highly oxidative tissue, such as the heart, the production of pyruvate is important for acetyl CoA synthesis and L-malate synthesis and serves as a precursor to many molecules, such as lactate, alanine, and oxaloacetate.

Glycolysis precedes lactic acid fermentation; the pyruvate made in the former process serves as the prerequisite for the lactate made in the latter process. Lactic acid fermentation is the main source of ATP in animal tissues with low metabolic requirements and with low mitochondrial levels. In erythrocytes, lactic acid fermentation is the sole source of ATP for these cells have no mitochondria, and once mature, the red blood cells have little demand for ATP. Another part of the body which relies entirely or almost entirely on anaerobic glycolysis is the lens of the eye, which is devoid of mitochondria to prevent light scattering.

Though skeletal muscles prefer to catalyse glucose into carbon dioxide and water during heavy exercise where the amount of oxygen is inadequate, the muscles simultaneously undergo anaerobic glycolysis along with oxidative phosphorylation.

Mechanism

Glycolysis Phases

There are two phases of glycolysis: the investment phase and the payoff phase. The investment phase is where energy as ATP is put in, and the payoff phase is where net ATP and NADH molecules are created. A total of 2 ATP is put in the investment

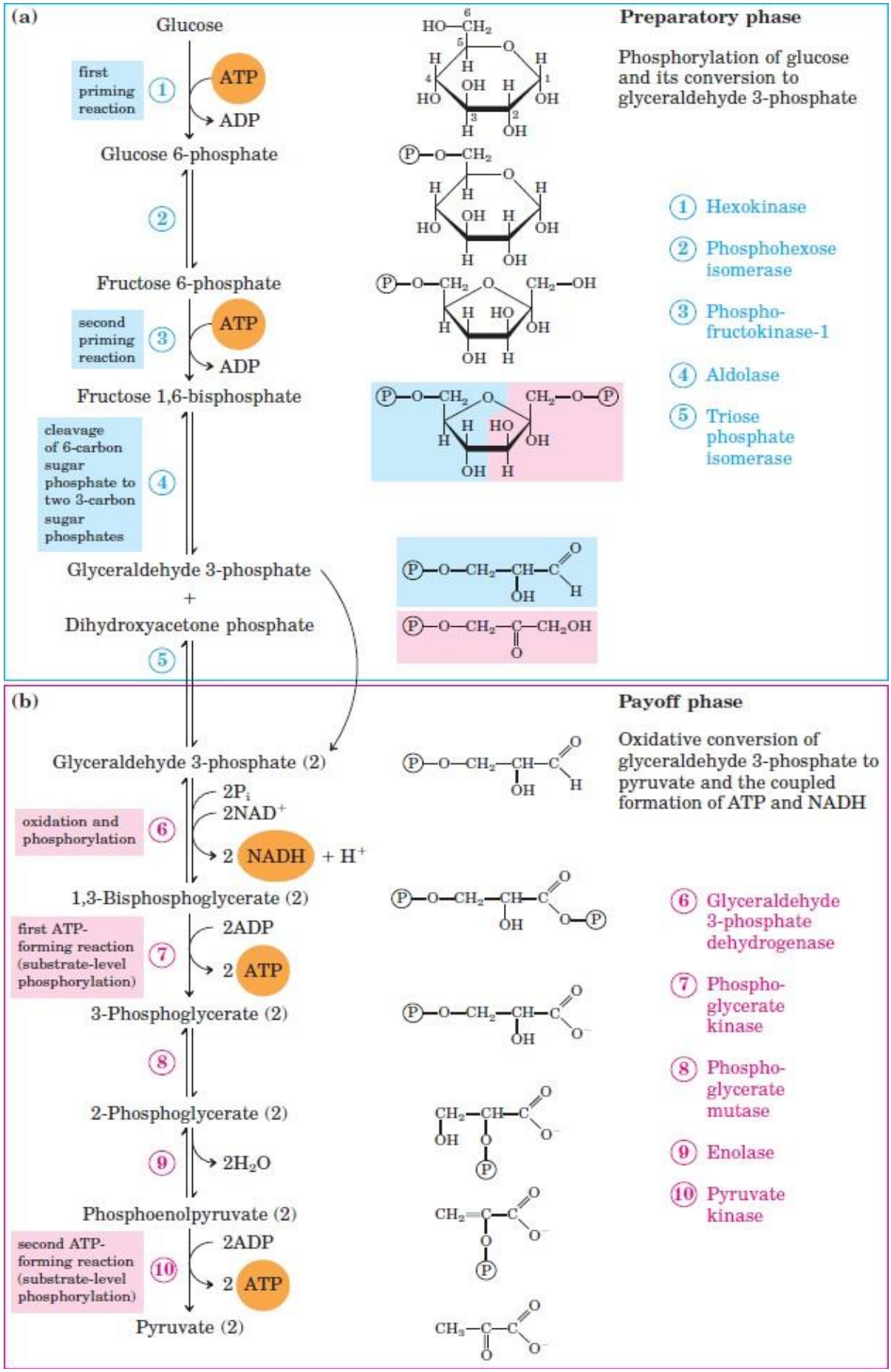
phase, and a total of 4 ATP is made in the payoff phase; thus, there is a net total of 2 ATP. The steps with which new ATP are created is called substrate level phosphorylation.

Investment Phase

In this phase, there are 2 phosphates added to glucose. Glycolysis begins with hexokinase phosphorylating glucose into glucose6 phosphate (G6P). This is the first transfer of a phosphate group and where the first ATP is used. Also, this step is an irreversible step. This phosphorylation traps the glucose molecule in the cell because it cannot readily pass the cell membrane. From there, phosphoglucose isomerase isomerizes G6P into fructose 6phosphate (F6P). Then, the second phosphate is added by phosphofructokinase (PFK1). PFK1 uses the second ATP and phosphorylates the F6P into fructose 1,6bisphosphate. This step is also irreversible and is the rate limiting step. In the following step, fructose 1,6bisphosphate is lysed into 2. Fructose - bisphosphate aldolase lyses it into dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3phosphate (G3P). DHAP is turned into G3P by triosephosphate isomerase. DHAP and G3p are in equilibrium with each other, meaning they transform back and forth.

payoff Phase

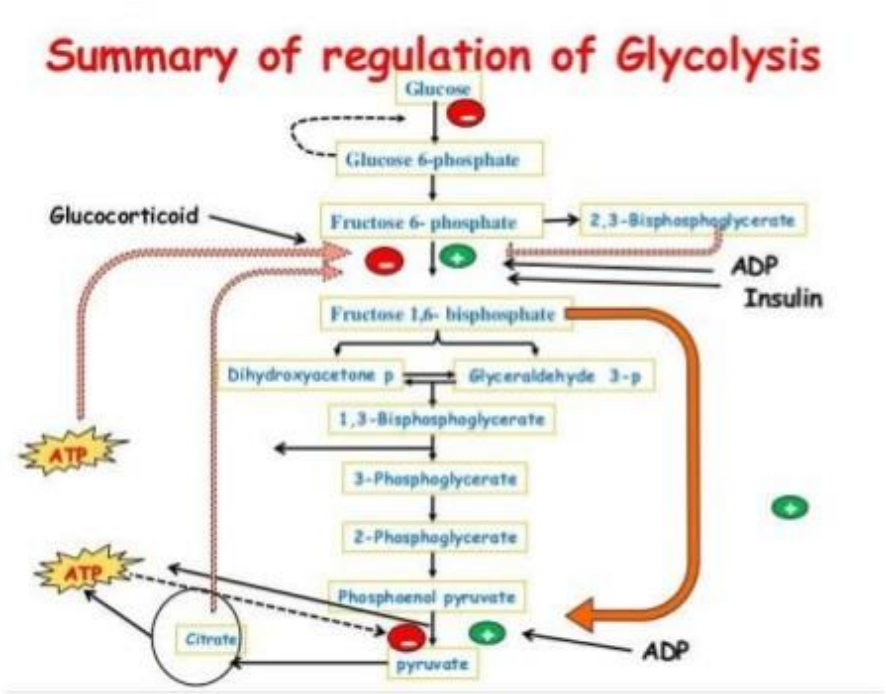
It is critical to remember that in this phase there are a total of 2, 3carbon sugars for every 1 glucose in the beginning. The enzyme, glyceraldehyde3phosphate dehydrogenase metabolizes the G3P into 1,3diphosphoglycerate by reducing NAD+ into NADH. Next, the 1,3diphosphoglycerate loses a phosphate group by way of phosphoglycerate kinase to make 3phosphoglycerate and creates an ATP through substrate level phosphorylation. At this point, there are 2 ATP created, one from each 3carbonmolecule. The 3phosphoglycerate turns into 2-phosphoglycerate by phosphoglycerate mutase, and then enolase turns the 2-phosphoglycerates into phosphoenolpyruvate (PEP). In the final step, pyruvate kinase turns PEP into pyruvate and phosphorylates ADP into ATP through substrate level phosphorylation, thus creating two more ATP. This step is also irreversible. Overall, the input for 1 glucose molecule is 2 ATP, and the output is 4 ATP and 2 NADH and 2 pyruvate molecules. In cells, it is critical that NADH is recycled back to NAD+ to keep glycolysis running. Without NAD+ the payoff phase will halt and cause a backup in glycolysis. In aerobic cells, NADH is recycled back into NAD+ by way of the oxidative phosphorylation. In aerobic cells, it is done through fermentation. There are 2 types of fermentation: lactic acid and alcohol fermentation.


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

Clinical Significance

Pyruvate kinase deficiency is an autosomal recessive mutation that causes hemolytic anemia. There is an inability to form ATP and causes cell damage. Cells become swollen and are taken up by the spleen, causing splenomegaly. Signs and symptoms include jaundice, icterus, elevated bilirubin, and splenomegaly. Arsenic poisoning also prevents ATP synthesis because arsenic takes the place of phosphate in the steps of glycolysis.

Regulation



Kreb's Cycle

Functions of the Citric Acid Cycle

- The final common oxidative pathway that oxidizes acetyl-CoA to CO_2.
- The source of reduced co enzymes that provide the substrate for the respiratory chain.
- The link between catabolic and anabolic pathways (amphibolic role).
- Provides precursors for synthesis of amino acids and nucleotides.
- Components of the cycle have a direct or indirect controlling effect on key enzymes of other pathways.

Reactions of the Cycle Preparatory Steps

Acetyl-CoA enters the cycle, and is completely oxidized. During this process, energy is trapped. Pyruvate derived from glycolysis is oxidatively decarboxylated to acetyl-coA by the pyruvate dehydrogenase. This is the link between the TCA cycle and

glycolysis. The acetyl-CoA is also derived from beta-oxidation of fatty acids. All the enzymes of citric acid cycle are located inside the mitochondria.

Formation of Citric Acid

The 4 carbon, oxaloacetate condenses with 2 carbon, acetyl-CoA to form 6 carbon compounds, the citrate Sources and utilization of acetyl-CoA Citric Acid Cycle (tricarboxylic acid). The enzyme is citrate synthase. The hydrolysis of the thioester bond in acetyl-CoA drives the reaction forward. This is an irreversible step. However, body can reverse this step by another enzyme, ATP-citrate lyase.

Formation of Isocitrate

Citrate is isomerized to isocitrate by aconitase. This reaction takes place in two steps, with cis-aconitate as the intermediary.

Formation of Alpha Ketoglutarate

This reaction is catalyzed by the enzyme, isocitrate dehydrogenase. First isocitrate is dehydrogenated to form oxalosuccinate. It undergoes spontaneous decarboxylation to form alpha ketoglutarate. The NADH generated in this step is later oxidized in electron transport chain (ETC) to generate ATPs. Isocitrate (6 carbons) undergoes oxidative decarboxylation to form alpha ketoglutarate (5 carbons). In this reaction, one molecule of CO2 is liberated.

Formation of Succinyl-CoA

Next, alpha ketoglutarate is oxidatively decarboxylated to form succinyl-CoA by the enzyme alpha ketoglutarate dehydrogenase (step 4, Fig. 20.2). The NADH thus generated enters into ETC to generate ATPs. Another molecule of CO_2 is removed in this step. This is the only irreversible step in the whole reaction cycle. The enzyme alpha ketoglutarate dehydrogenase is a multienzyme complex having 3 enzyme proteins and 5 coenzymes. This is similar to the pyruvate dehydrogenase reaction.

Generation of Succinate

The next reaction involves a substrate level phosphorylation whereby a high energy phosphate is generated from the energy trapped in the thioester bond of succinyl-CoA. The enzyme is succinate thiokinase. A molecule of GDP is phosphorylated to GTP and succinate is formed. The GTP can be converted to ATP by reacting with an ADP molecule: GTP + ADP → GDP + ATP

Formation of Fumarate

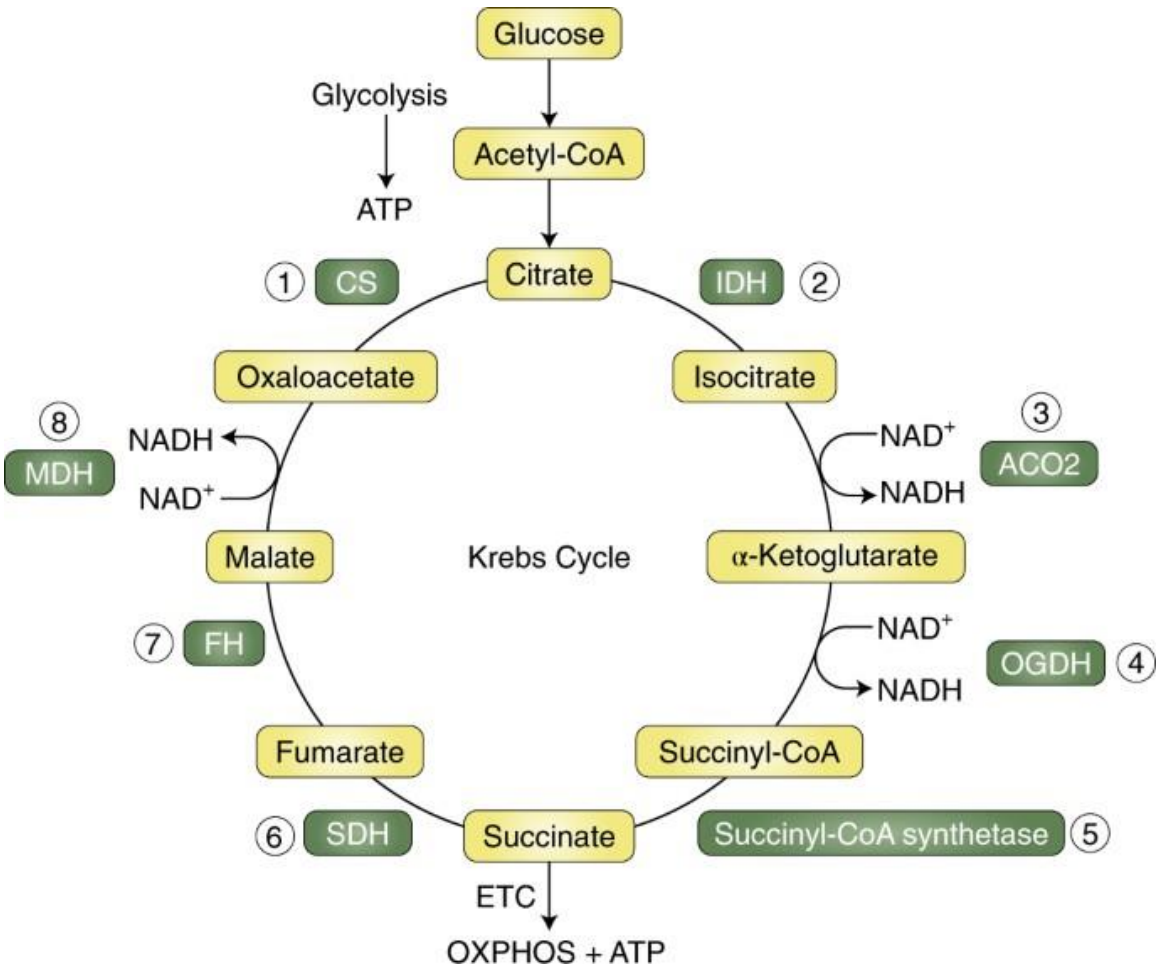
Succinate is dehydrogenated to fumarate, an unsaturated dicarboxylic acid, by succinate dehydrogenase.The hydrogen atoms are accepted by FAD. The FADH2 then enters into ETC to generate ATPs. The succinate dehydrogenase is competitively inhibited by malonate.

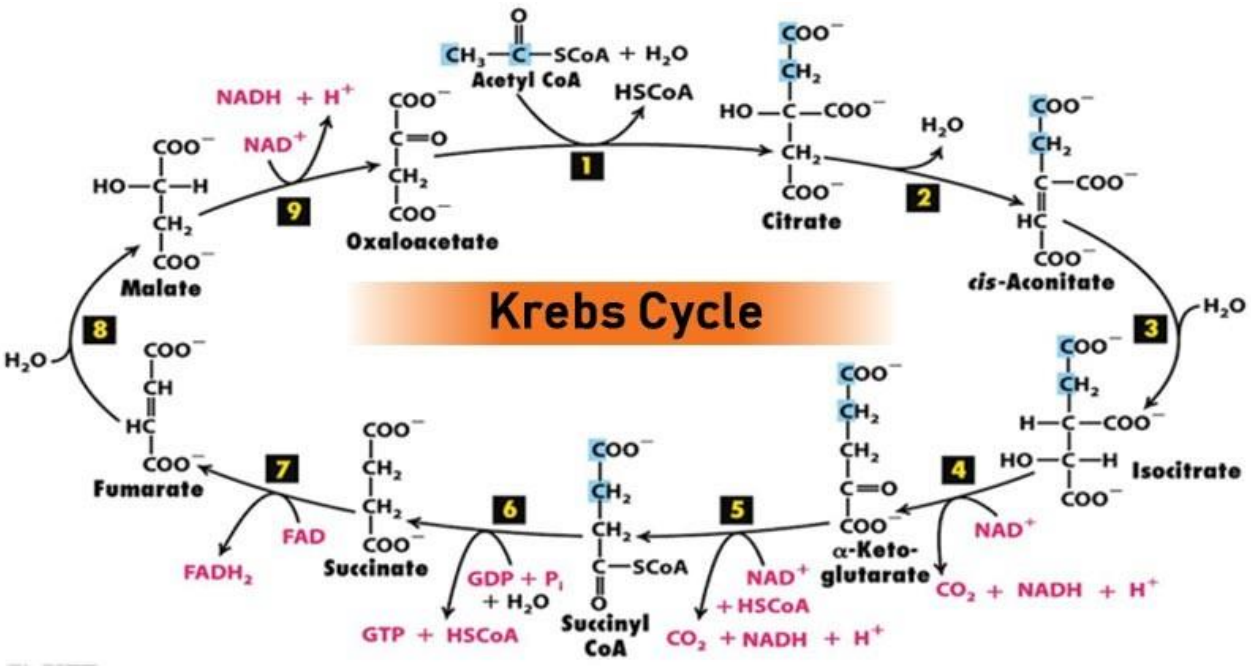
Formation of Malate

The formation of malate from fumarate is catalyzed by fumarase. The reaction involves the addition of a water molecule.

Regeneration of Oxaloacetate

Finally, malate is oxidized to oxaloacetate by malate dehydrogenase. The coenzyme is NAD^+. The NADH is generated in this step, which enters the electron transport chain, when ATPs are produced. The oxaloacetate can further condense with another acetyl-CoA molecule and the cycle continues.





1. Citrate synthase
2. Aconitase
3. Aconitase
4. Isocitrate dehydrogenase
5. α-ketoglutarate dehydrogenase
6. Succinyl CoA synthetase
7. Succinate dehydrogenase
8. Fumarase
9. Malate dehydrogenase

Significance of TCA cycle:

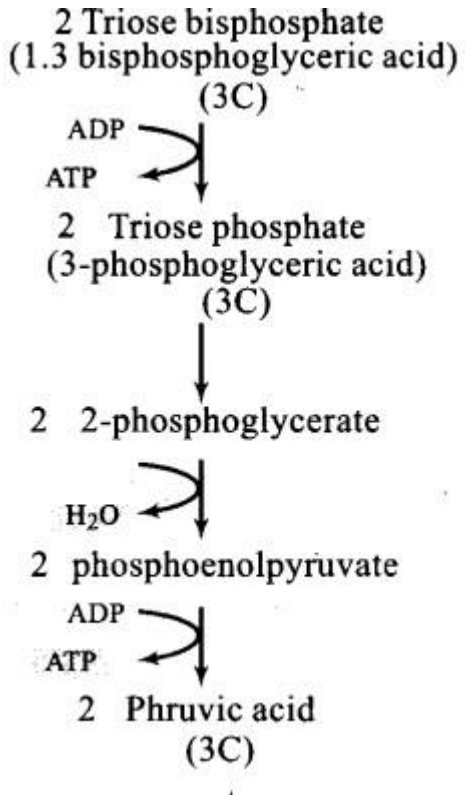
- Complete oxidation of acetyl CoA
- ATP generation
- Final common oxidative pathway
- Integration of major metabolic pathways
- Fat is burned on the wick of carbohydrates
- Excess carbohydrates as converted as neutral fat
- No net synthesis of carbohydrates from fat
- Amphibolic pathway
- Anaplerotic role

Photosynthesis

Energy yielding reactions

The energy-yielding reactions within the cell are therefore coupled to ATP synthesis, while the energy-requiring reactions are coupled to ATP hydrolysis. The high-energy bonds of ATP thus play a central role in cell metabolism by serving as a usable storage form of free energy.

Eg: The conversion of BPGA to 3-phosphoglyceric acid (PGA), is an energy yielding process; this energy is trapped by the formation of ATP. Another ATP is synthesised during the conversion of PEP to pyruvic acid.



Energy consuming reactions

Chemical energy, which is both produced and consumed in different chemical reactions, is an important way of storing energy in foods, fat reserves and fuels.....In energy consuming (endothermic) reactions the total energy of the products is more than that of the reactants - heat is taken from the surrounding substances.

Eg: The conversion of Glucose to Glucose-6-phosphate by hexokinase is energy utilizing reaction. The energy utilized in the form of ATP.

Enzymes

Enzymes are biocatalysts – the catalysis of life. A catalyst defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any

change in the overall process. They are protein in nature, colloidal and thermolabile in character and specific in their action.

- Enzymes, in Greek means *in living* (en=in, zyme=living)
- Biocatalysts are high molecular weight proteins
- Coined by Khune in 1878
- First enzyme extract from yeast cells by Buchner in 1897.
- First purified enzyme is urease by James B.Summer in 1926

Trivial names of enzymes

- The name of an enzyme
  - ✓ Usually ends with 'ase'
  - ✓ Identifies the reacting substance For eg: sucrase catalyses the reaction of sucrose.
  - ✓ Describes the function of an enzyme. For Eg: oxidase catalyses oxidation.
  - ✓ Could be a common name, particularly for the digestive enzymes, pepsin and trypsin.

Classification of Enzymes

Enzymes classifies according to the reaction they catalyse.

Class	**Reaction catalysed**
1.Oxidoreductases	Oxidation → Reduction
Alcohol dehydrogenases E.C.1.1.1.1	$AH_2 + B \rightarrow A + BH_2$
2. Transferases	Group transfer
Hexokinase E.C.2.7.1.1.	$A - X + B \rightarrow A + B - X$
3.Hydrolases	Hydrolysis
Lipase	$A - B + H_2O \rightarrow AH + BOH$
4.Lyases	Addition → Elimination
Aldolase	$A - B + X + Y \rightarrow AX - BY$
5.Isomerases	Interconversion of isomers
Triose phosphate isomerase	A → A'
6.Ligases	Condensation
Glutamine synthetase	$A + B \xrightarrow[ATP \quad ADP+P_i]{} A - B$

EC Number

Enzyme classified in to 6 groups according to the reaction being catalysed. Nomenclature was determined by the enzyme commission in 1961, hence all enzymes are assigned an EC number.

EC numbers are four digits, for example a.b.c.d where ‘a’ is the class, ‘b’ is the subclass, ‘c’ is the sub-sub class, ‘d’ is the sub-sub-class. ‘b’ and ‘c’ digit describe the reaction, while the ‘d’ digit is used to distinguish between different enzymes of the same function based on the actual substrate in the reaction.

3

GENETICS AND TRANSFORMATION TECHNOLOGY

3.1 MOLECULAR BASIS OF INHERITANCE

Inheritance is the process by which genetic information is passed on from parent to child. This is why members of the same family tend to have similar characteristics. We actually have two genomes. Each we get one copy of our genome from each of our parents. Inheritance describes how genetic material is passed on from parent to child Most of our cells contain two sets of 23 chromosomes (they are diploid). An exception to this rule are the sex cells (egg and sperm), also known as gametes, which only have one set of chromosomes each (they are haploid). However, in sexual reproduction the sperm cell combines with the egg cell to form the first cell of the new organism in a process called fertilization This cell (the fertilised egg) has two sets of 23 chromosomes (diploid) and the complete set of instructions needed to make more cells, and eventually a whole person. Each of the cells in the new person contains genetic material from the two parents. This passing down of genetic material is evident if you examine the characteristics of members of the same family, from average height to hair and eye colour to nose and ear shape, as they are usually similar.

Genetics mainly deals with the study of genes, heredity, and genetic variation. Genes exist on chromosomes and chromosomes are comprised of DNA and proteins. DNA is a molecule that carries genetic information in all living organisms and viruses where it is used in reproduction, functioning, growth, and development. It is a long polymer of deoxyribonucleotides.

a. DNA

DNA is a double-helical structure that carries all the genetic information. Its length is determined by the number of nucleotide pairs present in it. It is an acidic substance in the nucleus identified by Friedrich Meischer. Its double helical structure was given by Watson and Crick.DNA is made up of 6 molecular structures that comprise of one phosphate molecule and five carbon sugar termed deoxyribose. A nucleotide is a basic building block of DNA. A nucleotide is comprised of one of the 4 bases, one sugar molecule, and one phosphate molecule. A sugar-phosphate chain act as a backbone and bases are on the inside. Nucleotide subunits are linked together to form a DNA strand thus providing polar stability.

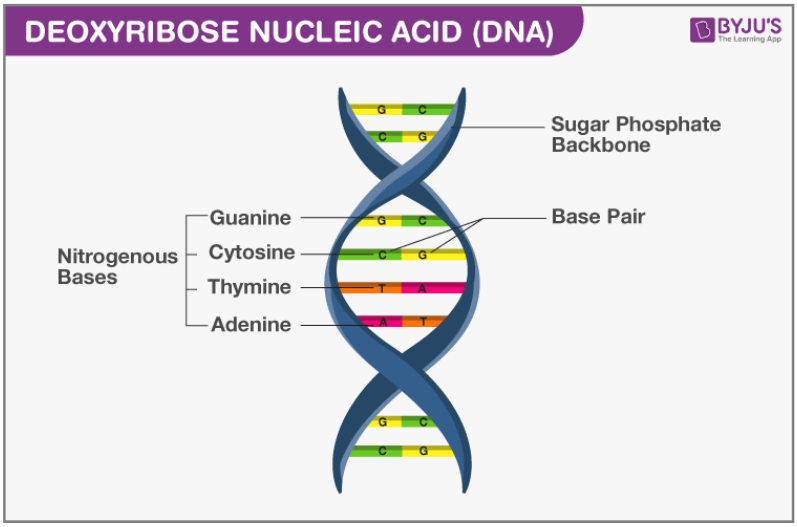


Fig. 3.1.DNA

The three-dimensional structure of DNA arises from chemical and structural features of 2 polynucleotide chain. A purine (A/G) base pairs up with pyrimidine (T/C) base. For instance guanine pairs with cytosine. So the two strands that are held together by a hydrogen bond are complementary to each other and they run in the antiparallel direction.

i. Process of information transfer (Central Dogma)

The central dogma was proposed by Crick. The central dogma states that the DNA is converted into RNA and the RNA is converted into proteins. In retroviruses, the flow of information is opposite, i.e., RNA to DNA to mRNA to Protein.

ii. DNA Packaging

The negatively charged DNA is packaged by surrounding the positively charged histone octamer. A structure called nucleosome is formed. The DNA is packed in chromatin of eukaryotes.

iii. DNA Replication

DNA is self-replicative. It occurs in the S-phase of the life cycle. It takes only a few minutes in prokaryotes but hours in eukaryotes. DNA undergoes semi-conservative replication, i.e., two strands of DNA are formed. One strand is the same as one of the strands while the other is complementary to the parent strand. The replication occurs in 5′-3′ direction.

b. RNA

Ribonucleic acid or RNA is a vital molecule with a long chain of nucleotides. It is the first genetic material. A nucleotide chain comprises a phosphate, a ribose sugar, and nitrogenous base. RNA acts as a catalyst and as genetic material. There are two types of RNA that is genetic and non-genetic.

c. Genetic Code

The genetic code can be defined as a set of rules wherein the information encoded in genetic materials are translated into proteins by living cells. The code defines how codons specify which amino acids will be added next during protein synthesis. The frequency of codon is termed as codon usage bias. It varies from species to species in terms of functional implications for the control of translation.

The genetic code can also be defined as a relationship between the sequence of amino acids in a nucleotide chain of mRNA or DNA and amino acid in a polypeptide chain. Nearly twenty types of amino acids participate in protein synthesis. Sixty-one codons out of sixty-four codons code only for amino acids. The characteristics of the genetic code are stated below:

- Degeneracy of genetic code.
- Non-overlapping.
- Universality.

- Triplet in nature.
- Comma-less.
- Non-ambiguous.

d. Human Genome Project

The human genome project was launched to sequence the entire human genome of 2.75 billion base pairs. The main goals of the human genome project are:

- To provide a complete sequence of 3 billion base pairs those make up the human genome.
- To sequence the genome of other organisms those are used in medical research. For eg, mouse, flies, etc.
- To develop new tools to obtain and analyse the data and to make this data widely available.
- It holds prospects for healthier living, a database of knowledge about designer drugs, genetically modified diets, and genetic identity.

3.2. DNA AS GENETIC MATERIALS

Our modern understanding of DNA's role in heredity has led to a variety of practical applications, including forensic analysis, paternity testing, and genetic screening. Today many people have at least a basic awareness of DNA. It may be surprising, then, to realize that less than a century ago, even the best-educated members of the scientific community did not know that DNA was the hereditary material! We have look at some of the classic experiments that led to the identification of DNA as the carrier of genetic information.

a. Protein vs. DNA

The work of Gregor Mendel showed that traits (such as flower colors in pea plants) were not inherited directly, but rather, were specified by genes passed on from parents to offspring. The work of additional scientists around the turn of the 20th

century, including Theodor Boveri, Walter Sutton, and Thomas Hunt Morgan, established that Mendel's heritable factors were most likely carried on chromosomes.

Scientists first thought that proteins, which are found in chromosomes along with DNA, would turn out to be the sought-after genetic material. Proteins were known to have diverse amino acid sequences, while DNA was thought to be a boring, repetitive polymer, due in part to an incorrect (but popular) model of its structure and composition. Today, we know that DNA is not actually repetitive and can carry large amounts of information. But how did scientists first come to realize that "boring" DNA might actually be the genetic material?

b. Frederick Griffith: Bacterial transformation

In 1928, British bacteriologist Frederick Griffith conducted a series of experiments using Streptococcus pneumoniae bacteria and mice. Griffith wasn't trying to identify the genetic material, but rather, trying to develop a vaccine against pneumonia. In his experiments, Griffith used two related strains of bacteria, known as R and S.

- **R strain.** When grown in a petri dish, the R bacteria formed colonies, or clumps of related bacteria, that had well-defined edges and a rough appearance (hence the abbreviation "R"). The R bacteria were nonvirulent, meaning that they did not cause sickness when injected into a mouse.

- **S strain.** S bacteria formed colonies that were rounded and smooth (hence the abbreviation "S"). The smooth appearance was due to a polysaccharide, orsugar-based, coat produced by the bacteria. This coat protected the S bacteria from the mouse immune system, making them virulent (capable of causing disease). Mice injected with live S bacteria developed pneumonia and died.

As part of his experiments, Griffith tried injecting mice with heat-killed S bacteria (that is, S bacteria that had been heated to high temperatures, causing the cells to die). Unsurprisingly, the heat-killed S bacteria did not cause disease in mice. The experiments took an unexpected turn, however, when harmless R bacteria were combined with heat-killed S bacteria and injected into a mouse. Not only did the mouse develop pnenumonia and die, but when Griffith took a blood sample from the dead mouse, he found that it contained living S bacteria!

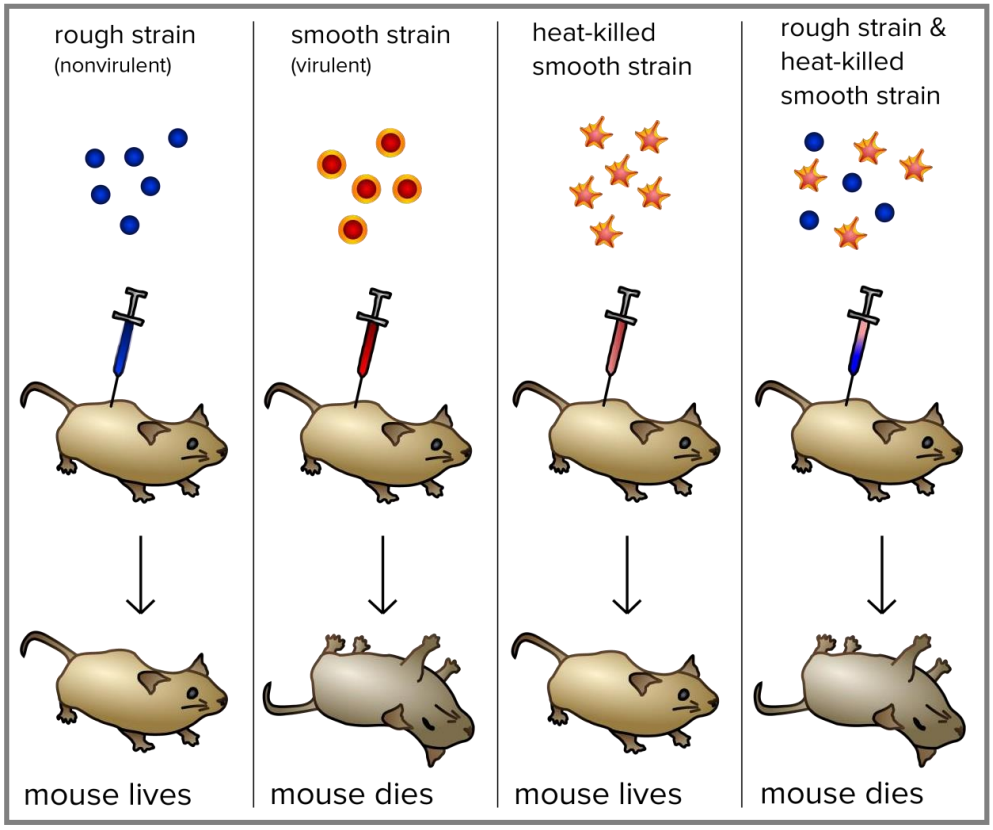


Fig.3.2 Diagram illustrating Frederick Griffith's experiment with S and R bacteria.

1. Rough strain (nonpathogenic). When this strain is injected into a mouse, the mouse lives.
2. Smooth strain (pathogenic). When this strain is injected into a mouse, the mouse gets pneumonia and dies.
3. Heat-killed smooth strain. When heat-killed smooth cells are injected into a mouse, the mouse lives.
4. Rough strain & heat-killed smooth strain. When these two types of cells are injected into a mouse as a mixture, the mouse gets pneumonia and dies.

Griffith concluded that the R-strain bacteria must have taken up what he called a "transforming principle" from the heat-killed S bacteria, which allowed them to "transform" into smooth-coated bacteria and become virulent.

c. Avery, McCarty, and MacLeod: Identifying the transforming principle

In 1944, three Canadian and American researchers, Oswald Avery, Maclyn McCarty, and Colin MacLeod, set out to identify Griffith's "transforming principle."To do so, they began with large cultures of heat-killed S cells and, through a long series of biochemical steps (determined by careful experimentation), progressively purified the transforming principle by washing away, separating out, or enzymatically destroying the other cellular components. The purified substance gave a negative result in chemical tests known to detect proteins, but a strongly positive result in a chemical test known to detect DNA.

- The elemental composition of the purified transforming principle closely resembled DNA in its ratio of nitrogen and phosphorous.
- Protein- and RNA-degrading enzymes had little effect on the transforming principle, but enzymes able to degrade DNA eliminated the transforming activity.

These results all pointed to DNA as the likely transforming principle. However, Avery was cautious in interpreting his results. He realized that it was still possible that some contaminating substance present in small amounts, not DNA, was the actual transforming principle. Because of this possibility, debate over DNA's role continued until 1952, when Alfred Hershey and Martha Chase used a different approach to conclusively identify DNA as the genetic material.

d. The Hershey-Chase experiments

In their now-legendary experiments, Hershey and Chase studied **bacteriophage**, or viruses that attack bacteria. The phages they used were simple particles composed of protein and DNA, with the outer structures made of protein and the inner core consisting of DNA. Hershey and Chase knew that the phages attached to the surface of a host bacterial cell and injected some substance (either DNA or protein) into the host. This substance gave "instructions" that caused the host bacterium to start making lots and lots of phages—in other words, it was the phage's genetic material..

To establish whether the phage injected DNA or protein into host bacteria, Hershey and Chase prepared two different batches of phage. In each batch, the phage

was produced in the presence of a specific radioactive element, which was incorporated into the macromolecules (DNA and protein) that made up the phage.

- One sample was produced in the presence of ^{35}S, a radioactive isotope of sulfur. Sulfur is found in many proteins and is absent from DNA, so only phage proteins were radioactively labeled by this treatment.
- The other sample was produced in the presence of ^{32}P, a radioactive isotope of phosphorous. Phosphorous is found in DNA and not in proteins, so only phage DNA sample was radioactively labeled by this treatment.

Each batch of phage was used to infect a different culture of bacteria. After infection had taken place, each culture was whirled in a blender, removing any remaining phage and phage parts from the outside of the bacterial cells. Finally, the cultures were centrifuged, or spun at high speeds, to separate the bacteria from the phage debris. Centrifugation causes heavier material, such as bacteria, to move to the bottom of the tube and form a lump called a pellet. Lighter material, such as the medium (broth) used to grow the cultures, along with phage and phage parts, remains near the top of the tube and forms a liquid layer called the supernatant.

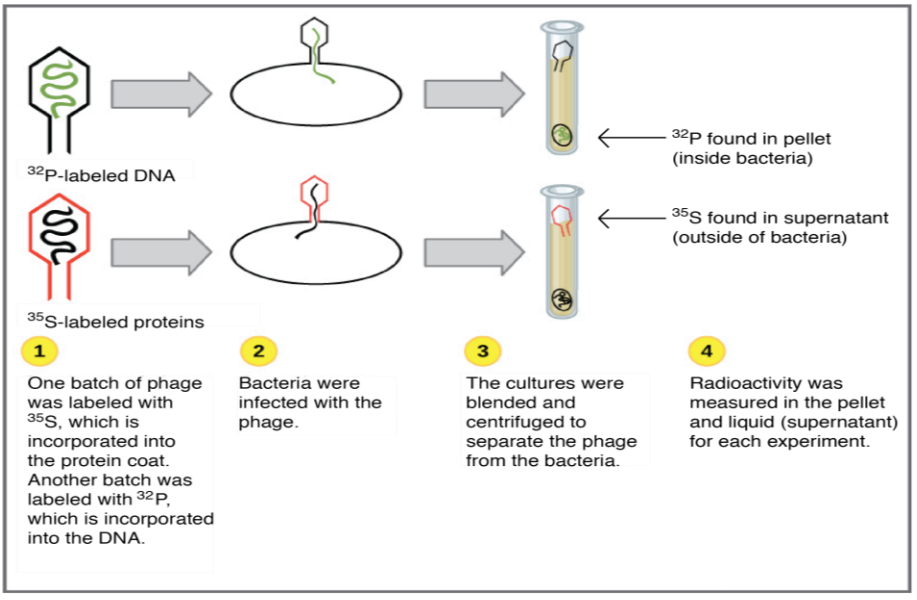


Fig.3.3The Hershey-Chase experiments

1. One batch of phage was labeled with ^{35}S, which is incorporated into the protein coat. Another batch was labeled with ^{32}P, which is incorporated into the DNA.

2. Bacteria were infected with the phage.
3. The cultures were blended and centrifuged to separate the phage from the bacteria.
4. Radioactivity was measured in the pellet and liquid (supernatant) for each experiment. ^{32}P was found in the pellet (inside the bacteria), while ^{35}S was found in the supernatant (outside of the bacteria)

When Hershey and Chase measured radioactivity in the pellet and supernatant from both of their experiments, they found that a large amount of ^{32}P in the pellet, whereas almost all of the ^{35}S appeared in the supernatant. Based on this and similar experiments, Hershey and Chase concluded that DNA, not protein, was injected into host cells and made up the genetic material of the phage.

3.3. GENETIC CODE

Have you ever written a secret message to one of your friends? If so, you may have used a code to keep the message is hidden. For instance, you may have replaced the letters of the word with numbers or symbols, following a particular set of rules. In order for your friend to understand the message, they would need to know the code and apply the same set of rules, in reverse, to decode it. Decoding messages is also a key step in gene expression, in which information from a gene is read out to build a protein. In this article, we'll take a closer look at the geneticcode, which allows DNA and RNA sequences to be "decoded" into the amino acids of a protein.

The instruction in a gene isthat tell the cell how to make a specific protein. A, C, G, and T are the "letters" of the DNA code; they stand for the chemicals adenine (A), cytosine (C), guanine (G), and thymine (T), respectively, that make up the nucleotide bases of DNA. Each gene's code combines the four chemicals in various ways to spell out three-letter "words" that specify which amino acid is needed at every step in making a protein.

Genetic code is the term we use for the way that the four bases of DNA. The A, C, G, and Tare strung together in a way that the cellular machinery, the ribosome, can read them and turn them into a protein. In the genetic code, each three nucleotides in a row count as a triplet and code for a single amino acid. So each sequence of three codes is responsible for an amino acid. And proteins are made up of sometimes hundreds of amino acids. So the code that would make one protein could have hundreds, sometimes even thousands of triplets contained in it.

a. Making a protein

Genes that provide instructions for proteins are expressed in a two-step process.

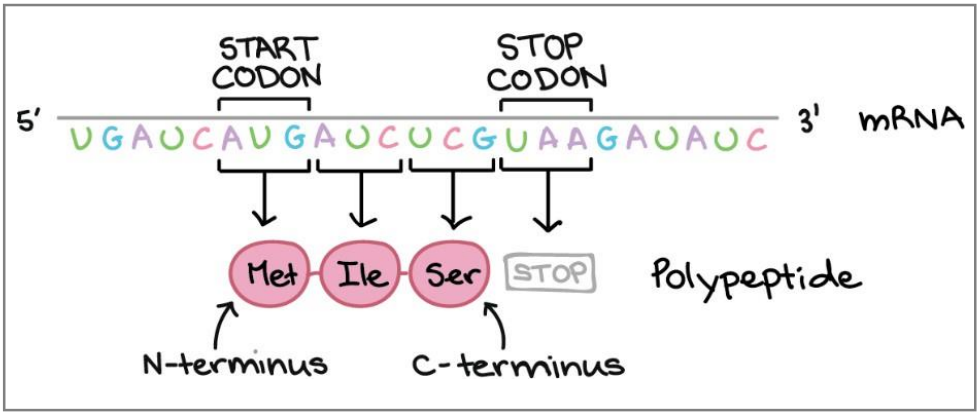
- In **transcription**, the DNA sequence of a gene is "rewritten" in RNA. In eukaryotes, the RNA must go through additional processing steps to become a messengerRNA, or mRNA.
- In **translation**, the sequence of nucleotides in the mRNA is "translated" into a sequence of amino acids in a polypeptide (protein chain).

b. Codons

Cells decode mRNAs by reading their nucleotides in groups of three, called codons. Here are some features of codons:

- Most codons specify an amino acid
- Three "stop" codons mark the end of a protein
- One "start" codon, AUG, marks the beginning of a protein and also encodes the amino acid methionine

Codons in an mRNA are read during translation, beginning with a start codon and continuing until a stop codon is reached. mRNA codons are read from 5' to 3' , and they specify the order of amino acids in a protein from N-terminus (methionine) to C-terminus.



What do 5' and 3' mean?

The two ends of a strand of DNA or RNA are different from each other. That is, a DNA or RNA molecule has **directionality**.

- At the **5' end** of the chain, the phosphate group of the first nucleotide in the chain sticks out. The phosphate group is attached to the 5' carbon of the sugar ring, which is why this is called the 5' end.

- At the other end, called the **3' end**, the hydroxyl of the last nucleotide added to the chain is exposed. The hydroxyl group is attached to the 3' carbon of the sugar ring, which is why this is called the 3' end.

Many processes, such as DNA replication and transcription, can only take place in one particular direction relative the directionality of a DNA or RNA strand.

What are the N- and C-terminus?

Polypeptides (chains of linked amino acids) have two distinct ends:

- An **N-terminus** with an amino group exposed

- A **C-terminus** with a carboxyl group exposed

c. The genetic code table

The full set of relationships between codons and amino acids (or stop signals) is called the geneticcode. The genetic code is often summarized in a table. The codon table may look kind of intimidating at first. Fortunately, it's organized in a logical way, and it's not too hard to use once you understand this organization. To see how the codon table works, let's walk through an example. Suppose that we are interested in the codon CAG and want to know which amino acid it specifies.

1. First, we look at the left side of the table. The axis on the left side refers to the first letter of the codon, so we find C along the left axis. This tells us the (broad) row of the table in which our codon will be found.

2. Next, we look at the top of the table. The upper axis refers to the second letter of the codon, so we find A along the upper axis. This tells us the column of the table in which our codon will be found.

The row and column from steps 1 and 2 intersect in a single box in the codon table, one containing four codons. It's often easiest to simply look at these four codons and see which one is the one you're looking for.If you want to use the structure of the table to the maximum, however, you can use the third axis (on the right side of the

table) corresponding to the intersect box. By finding the third nucleotide of the codon on this axis, you can identify the exact row within the box where your codon is found. For instance, if we look for G on this axis in our example above, we find that CAG encodes the amino acid glutamine (Gln).

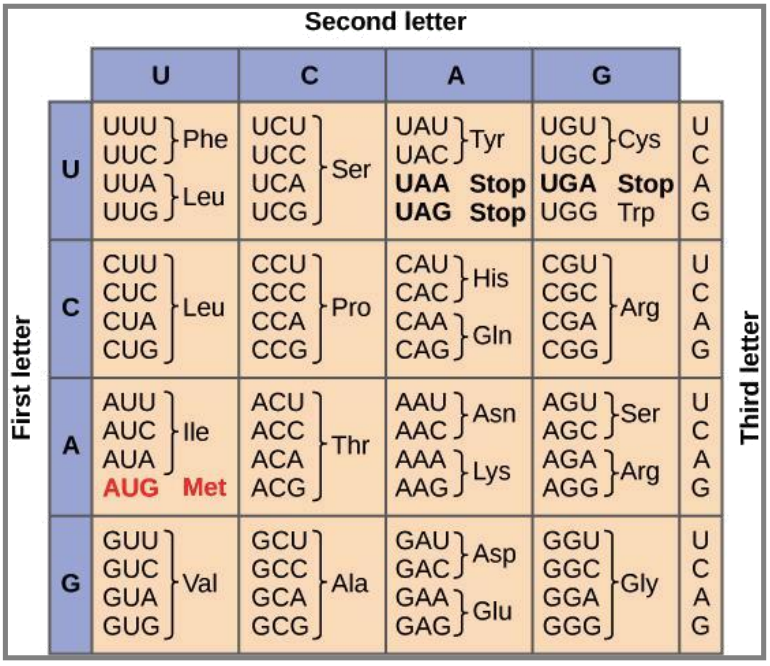


Fig.3.4The genetic code table

Each three-letter sequence of mRNA nucleotides corresponds to a specific amino acid, or to a stop codon. UGA, UAA, and UAG are stop codons. AUG is the codon for methionine, and is also the start codon. Notice that many amino acids are represented in the table by more than one codon. For instance, there are six different ways to "write" leucine in the language of mRNA.

An important point about the genetic code is that it's universal. That is, with minor exceptions, virtually all species (from bacteria to you!) use the genetic code shown above for protein synthesis.

d. Reading frame

To reliably get from an mRNA to a protein, we need one more concept: that of **reading frame**. Reading frame determines how the mRNA sequence is divided up into codons during translation.

That's a pretty abstract concept, so let's look at an example to understand it better. The mRNA below can encode three totally different proteins, depending on the frame in which it's read:

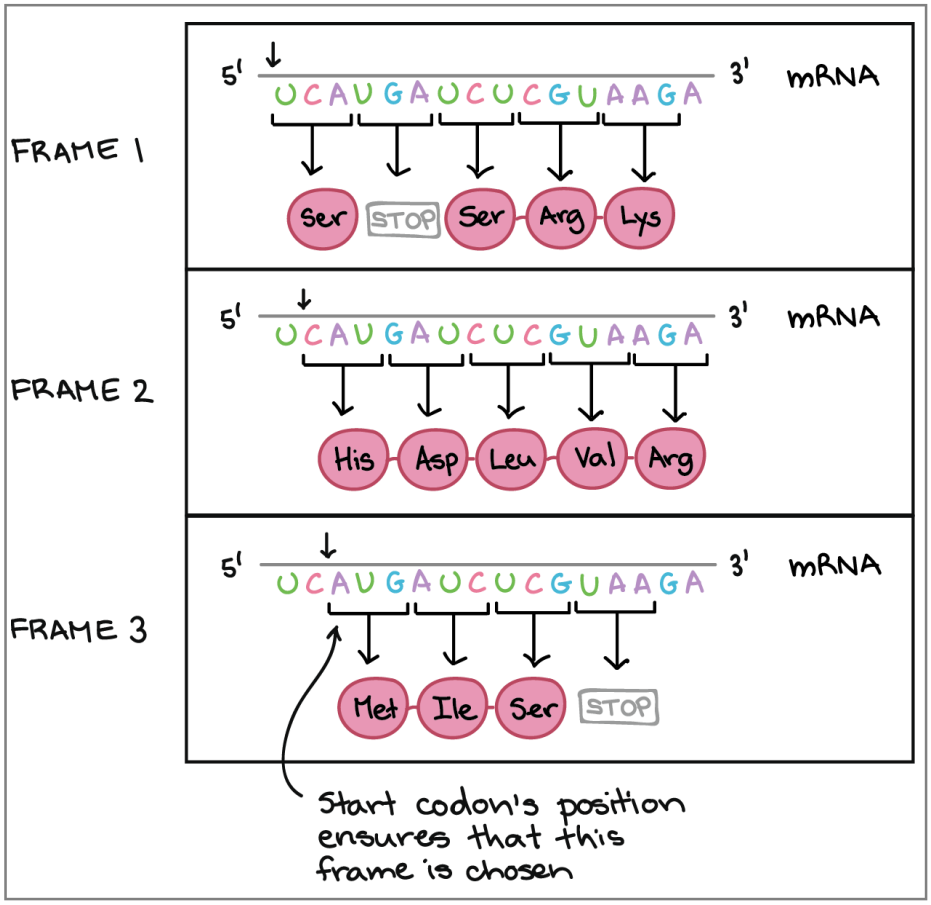


Fig.3.5 Reading frame

The start codon's position ensures that Frame 3 is chosen for translation of the mRNA. So, how does a cell know which of this protein to make? The start codon is the key signal. Because translation begins at the start codon and continues in successive groups of three, the position of the start codon ensures that the mRNA is read in the correct frame (in the example above, in Frame 3).

Mutations (changes in DNA) that insert or delete one or two nucleotides can change the reading frame, causing an incorrect protein to be produced "downstream" of the mutation site:

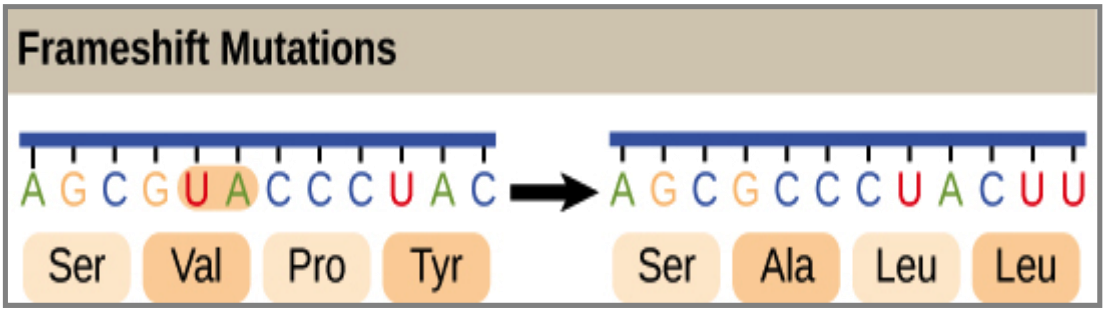


Illustration shows a frame shift mutation in which the reading frame is altered by the deletion of two amino acids.

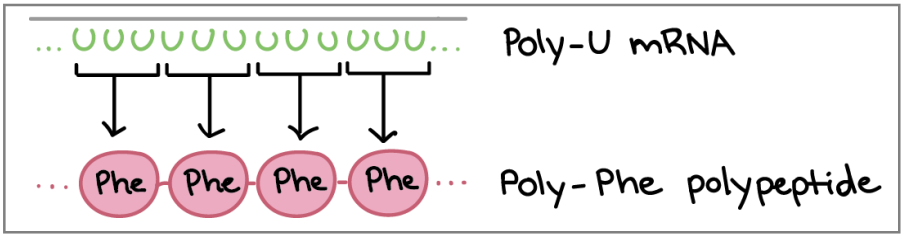
There are 16 unique groups of nucleotides if a doublet code is used and 64 unique groups if a triplet code is used. Why is this case? Let's take a closer look at the math behind these statements.

e. Matching codons to amino acids

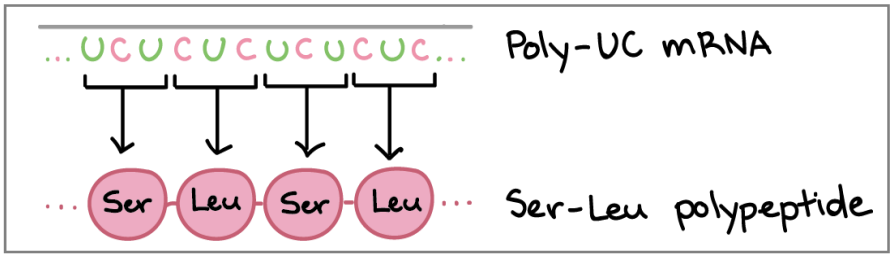
Gamow's triplet hypothesis seemed logical and was widely accepted. However, it had not been experimentally proven, and researchers still did not know which triplets of nucleotides corresponded to which amino acids. The cracking of the genetic code began in 1961, with work from the American biochemist Marshall Nirenberg. For the first time, Nirenberg and his colleagues were able to identify specific nucleotide triplets that corresponded to particular amino acids. Their success relied on two experimental innovations:

- A way to make artificial mRNA molecules with specific, known sequences.
- A system to translate mRNAs into polypeptides outside of a cell (a "cell-free" system). Nirenberg's system consisted of cytoplasm from burst E. coli cells, which contains all of the materials needed for translation.

First, Nirenberg synthesized an mRNA molecule consisting only of the nucleotide uracil (called poly-U). When he added poly-U mRNA to the cell-free system, he found that the polypeptides made consisted exclusively of the amino acid phenylalanine. Because the only triplet in poly-U mRNA is UUU, Nirenberg concluded that UUU might code for phenylalanine squared. Using the same approach, he was able to show that poly-C mRNA was translated into polypeptides made exclusively of the amino acid proline, suggesting that the triplet CCC might code for proline.



The poly-UC mRNA that it was translated into polypeptides with an alternating pattern of serine and leucine amino acids. These and other results confirmed that the genetic code was based on triplets, or **codons**. Today, we know that serine is encoded by the codon UCU, while leucine is encoded by CUC.



By 1965, using the cell-free system and other techniques, Nirenberg, Khorana, and their colleagues had deciphered the entire genetic code. That is, they had identified the amino acid or "stop" signal corresponding to each one of the 64646464 nucleotide codons. For their contributions, Nirenberg and Khorana (along with another genetic code researcher, Robert Holley) received the Nobel Prize in 1968.

3.4. MENDEL'S LAW

Mendelian inheritance (or Mendelian genetics or Mendelism) is a set of primary tenets relating to the transmission of hereditary characteristics from parent organisms to their children; it underlies much of genetics. The tenets were initially derived from the work of Gregor Mendel published in 1865 and 1866, which was ‑re-discovered‖ in 1900; they were initially very controversial, but they soon became the core of classical genetics.

The laws of inheritance were derived by Gregor Mendel, a 19th century monk conducting hybridization experiments in garden peas (Pisumsativum). Between 1856 and 1863, he cultivated and tested some 28,000 pea plants. From these experiments, he deduced two generalizations that later became known as Mendel's Laws of Heredity or Mendelian inheritance. He described these laws in a two part paper, ‑Experiments on Plant Hybridization‖, which was published in 1866.

Mendel discovered that by crossing true-breeding white flower and true-breeding purple flower plants, the result was a hybrid offspring. Rather than being a mix of the two colors, the offspring was purple flowered. He then conceived the idea of heredity units, which he called ―factors‖, one of which is a recessive characteristic and the other dominant. Mendel said that factors, later called genes, normally occur in pairs in ordinary body cells, yet segregate during the formation of sex cells. Each member of the pair becomes part of the separate sex cell. The dominant gene, such as the purple flower in Mendel's plants, will hide the recessive gene, the white flower. After Mendel self-fertilized the F1 generation and obtained an F2 generation with a 3:1 ratio, he correctly theorized that genes can be paired in three different ways for each trait: AA, aa, and Aa. The capital A represents the dominant factor while the lowercase a represents the recessive.

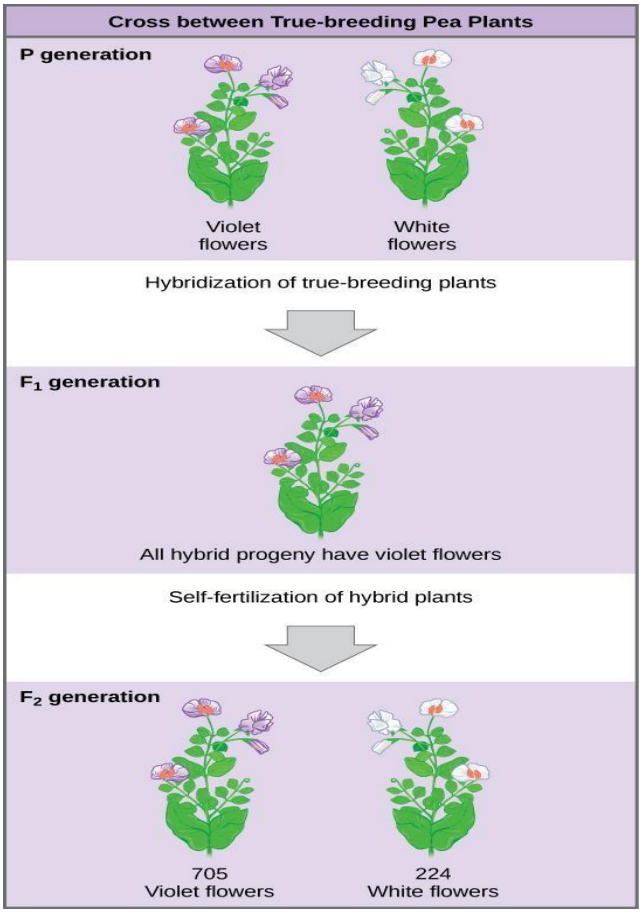


Fig.3.5Mendel's Pea Plants

Mendel's Pea Plants: In one of his experiments on inheritance patterns, Mendel crossed plants that were true-breeding for violet flower color with plants true-breeding for white flower color (the P generation). The resulting hybrids in the F1 generation all had violet flowers. In the F2 generation, approximately three-quarters of the plants had violet flowers, and one-quarter had white flowers.

Mendel stated that each individual has two alleles for each trait, one from each parent. Thus, he formed the ‒first rule‖, the Law of Segregation, which states individuals possess two alleles and a parent passes only one allele to his/her offspring. One allele is given by the female parent and the other is given by the male parent. The two factors may or may not contain the same information. If the two alleles are identical, the individual is called homozygous for the trait. If the two alleles are different, the individual is called heterozygous. The presence of an allele does not promise that the trait will be expressed in the individual that possesses it. In heterozygous individuals, the only allele that is expressed is the dominant. The recessive allele is present, but its expression is hidden. The genotype of an individual is made up of the many alleles it possesses. An individual's physical appearance, or phenotype, is determined by its alleles as well as by its environment.

Mendel also analyzed the pattern of inheritance of seven pairs of contrasting traits in the domestic pea plant. He did this by cross-breeding dihybrids; that is, plants that were heterozygous for the alleles controlling two different traits. Mendel then crossed these dihybrids. If it is inevitable that round seeds must always be yellow and wrinkled seeds must be green, then he would have expected that this would produce a typical monohybrid cross: 75 percent round-yellow; 25 percent wrinkled-green. But, in fact, his mating generated seeds that showed all possible combinations of the color and texture traits. He found 9/16 of the offspring were round-yellow, 3/16 were round-green, 3/16 were wrinkled-yellow, and 1/16 were wrinkled-green. Finding in every case that each of his seven traits was inherited independently of the others, he formed his ‒second rule‖, the Law of Independent Assortment, which states the inheritance of one pair of factors (genes) is independent of the inheritance of the other pair. Today we know that this rule holds only if the genes are on separate chromosomes

a. Mendel's Law of Dominance

In a heterozygote, the allele which masks the other is referred to as dominant, while the allele that is masked is referred to as recessive.

Alleles Can Be Dominant or Recessive

Most familiar animals and some plants have paired chromosomes and are described as diploid. They have two versions of each chromosome: one contributed by the female parent in her ovum and one by the male parent in his sperm. These are joined at fertilization. The ovum and sperm cells (the gametes) have only one copy of each chromosome and are described as haploid.

Mendel's law of dominance states that in a heterozygote, one trait will conceal the presence of another trait for the same characteristic. Rather than both alleles contributing to a phenotype, the dominant allele will be expressed exclusively. The recessive allele will remain ‑latent,‖ but will be transmitted to offspring by the same manner in which the dominant allele is transmitted. The recessive trait will only be expressed by offspring that have two copies of this allele; these offspring will breed true when self-crossed.

By definition, the terms dominant and recessive refer to the genotypic interaction of alleles in producing the phenotype of the heterozygote. The key concept is genetic: which of the two alleles present in the heterozygote is expressed, such that the organism is phenotypically identical to one of the two homozygotes. It is sometimes convenient to talk about the trait corresponding to the dominant allele as the dominant trait and the trait corresponding to the hidden allele as the recessive trait. However, this can easily lead to confusion in understanding the concept as phenotypic. For example, to say that ‑green peas‖ dominating ‑yellow peas‖ confuses inherited genotypes and expressed phenotypes. This will subsequently confuse discussion of the molecular basis of the phenotypic difference. Dominance is not inherent. One allele can be dominant to a second allele, recessive to a third allele, and codominant to a fourth. If a genetic trait is recessive, a person needs to inherit two copies of the gene for the trait to be expressed. Thus, both parents have to be carriers of a recessive trait in order for a child to express that trait.

b. Mendel's Law of Segregation

Mendel's Law of Segregation states that a diploid organism passes a randomly selected allele for a trait to its offspring, such that the offspring receives one allele from each parent. Observing that true-breeding pea plants with contrasting traits gave rise to F_1 generations that all expressed the dominant trait and F_2 generations that expressed the dominant and recessive traits in a 3:1 ratio, Mendel proposed the law of segregation.

The law of segregation states that each individual that is a diploid has a pair of alleles (copy) for a particular trait. Each parent passes an allele at random to their offspring resulting in a diploid organism. The allele that contains the dominant trait determines the phenotype of the offspring. In essence, the law states that copies of genes separate or segregate so that each gamete receives only one allele.

The Law of Segregation states that alleles segregate randomly into gametes:

When gametes are formed, each allele of one parent segregates randomly into the gametes, such that half of the parent's gametes carry each allele.

For the F_2 generation of a monohybrid cross, the following three possible combinations of genotypes could result: homozygous dominant, heterozygous, or homozygous recessive. Because heterozygotes could arise from two different pathways (receiving one dominant and one recessive allele from either parent), and because heterozygotes and homozygous dominant individuals are phenotypically identical, the law supports Mendel's observed 3:1 phenotypic ratio. The equal segregation of alleles is the reason we can apply the Punnett square to accurately predict the offspring of parents with known genotypes.

The physical basis of Mendel's law of segregation is the first division of meiosis in which the homologous chromosomes with their different versions of each gene are segregated into daughter nuclei. The behavior of homologous chromosomes during meiosis can account for the segregation of the alleles at each genetic locus to different gametes. As chromosomes separate into different gametes during meiosis, the two different alleles for a particular gene also segregate so that each gamete acquires one of the two alleles. In Mendel's experiments, the segregation and the independent assortment during meiosis in the F1 generation give rise to the F2 phenotypic ratios observed by Mendel. The role of the meiotic segregation of chromosomes in sexual reproduction was not understood by the scientific community during Mendel's lifetime.

a. Mendel's Law of Independent Assortment

Independent assortment allows the calculation of genotypic and phenotypic ratios based on the probability of individual gene combinations. Mendel's law of independent assortment states that genes do not influence each other with regard to the sorting of alleles into gametes: every possible combination of alleles for every gene is equally likely to occur. The independent assortment of genes can be illustrated by the

dihybrid cross: a cross between two true-breeding parents that express different traits for two characteristics. Consider the characteristics of seed color and seed texture for two pea plants: one that has green, wrinkled seeds (yyrr) and another that has yellow, round seeds (YYRR). Because each parent is homozygous, the law of segregation indicates that the gametes for the green/wrinkled plant all are yr, while the gametes for the yellow/round plant are all YR. Therefore, the F_1 generation of offspring all are YyRr.

For the F2 generation, the law of segregation requires that each gamete receive either an R allele or an r allele along with either a Y allele or a y allele. The law of independent assortment states that a gamete into which an r allele sorted would be equally likely to contain either a Y allele or a y allele. Thus, there are four equally likely gametes that can be formed when the YyRr heterozygote is self-crossed as follows: YR, Yr, yR, and yr. Arranging these gametes along the top and left of a 4 × 4 Punnett square gives us 16 equally likely genotypic combinations. From these genotypes, we infer a phenotypic ratio of 9 round/yellow:3 round/green:3 wrinkled/yellow:1 wrinkled/green. These are the offspring ratios we would expect, assuming we performed the crosses with a large enough sample size.

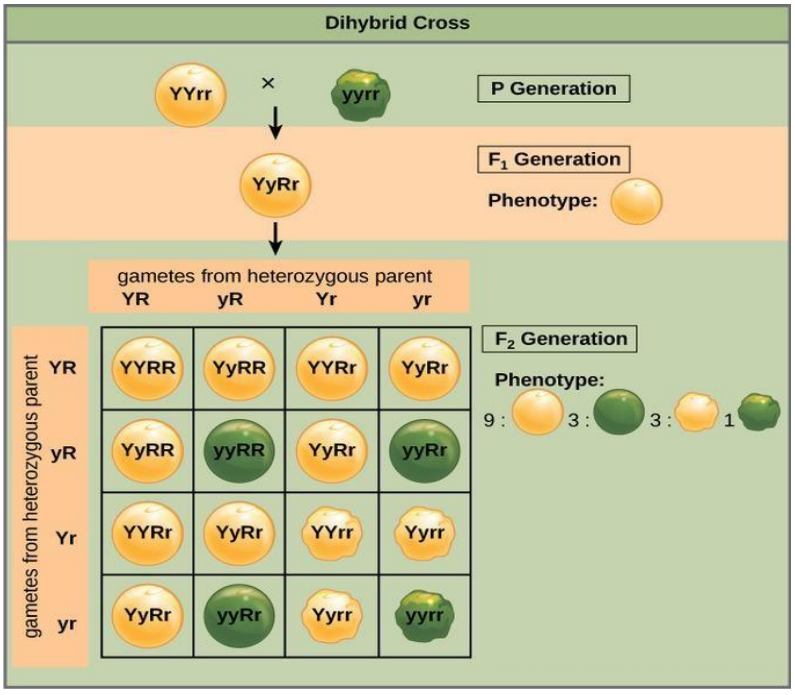


Fig.3.6 Dihybrid cross

i. Independent assortment of 2 genes:

This dihybrid cross of pea plants involves the genes for seed color and texture. Because of independent assortment and dominance, the 9:3:3:1 dihybrid phenotypic ratio can be collapsed into two 3:1 ratios, characteristic of any monohybrid cross that follows a dominant and recessive pattern. Ignoring seed color and considering only seed texture in the above dihybrid cross, we would expect that three-quarters of the F_2 generation offspring would be round and one-quarter would be wrinkled. Similarly, isolating only seed color, we would assume that three-quarters of the F_2 offspring would be yellow and one-quarter would be green. The sorting of alleles for texture and color are independent events, so we can apply the product rule. Therefore, the proportion of round and yellow F_2 offspring is expected to be $(3/4) \times (3/4) = 9/16$, and the proportion of wrinkled and green offspring is expected to be $(1/4) \times (1/4) = 1/16$. These proportions are identical to those obtained using a Punnett square. Round/green and wrinkled/yellow offspring can also be calculated using the product rule as each of these genotypes includes one dominant and one recessive phenotype. Therefore, the proportion of each is calculated as $(3/4) \times (1/4) = 3/16$.

ii. Probability Method

While the forked-line method is a diagrammatic approach to keeping track of probabilities in a cross, the probability method gives the proportions of offspring expected to exhibit each phenotype (or genotype) without the added visual assistance.

To fully demonstrate the power of the probability method, however, we can consider specific genetic calculations. For instance, for a tetrahybrid cross between individuals that are heterozygotes for all four genes, and in which all four genes are sorting independently in a dominant and recessive pattern, what proportion of the offspring will be expected to be homozygous recessive for all four alleles? Rather than writing out every possible genotype, we can use the probability method. We know that for each gene the fraction of homozygous recessive offspring will be 1/4. Therefore, multiplying this fraction for each of the four genes, $(1/4) \times (1/4) \times (1/4) \times (1/4)$, we determine that 1/256 of the offspring will be quadruply homozygous recessive.

iii. Genetic Linkage and Violation of the Law of Independent Assortment

Genes that are on the same chromosome, or ‑linked‖, do not assort independently, but can be separated by recombination. Although all of Mendel’s pea characteristics behaved according to the law of independent assortment, we now know

that some allele combinations are not inherited independently of each other. Genes that are located on separate non-homologous chromosomes will always sort independently. However, each chromosome contains hundreds or thousands of genes organized linearly on chromosomes like beads on a string. The segregation of alleles into gametes can be influenced by linkage, in which genes that are located physically close to each other on the same chromosome are more likely to be inherited as a pair. However, because of the process of recombination, or ‒crossover,‖ it is possible for two genes on the same chromosome to behave independently, or as if they are not linked. To understand this, let's consider the biological basis of gene linkage and recombination.

Homologous chromosomes possess the same genes in the same linear order. The alleles may differ on homologous chromosome pairs, but the genes to which they correspond do not. In preparation for the first division of meiosis, homologous chromosomes are replicate and synapse. Like genes on the homologs align with each other. At this stage, segments of homologous chromosomes exchange linear segments of genetic material. This process is called recombination, or crossover, and it is a common genetic process. Because the genes are aligned during recombination, the gene order is not altered. Instead, the result of recombination is that maternal and paternal alleles are combined onto the same chromosome. Across a given chromosome, several recombination events may occur, causing extensive shuffling of alleles.

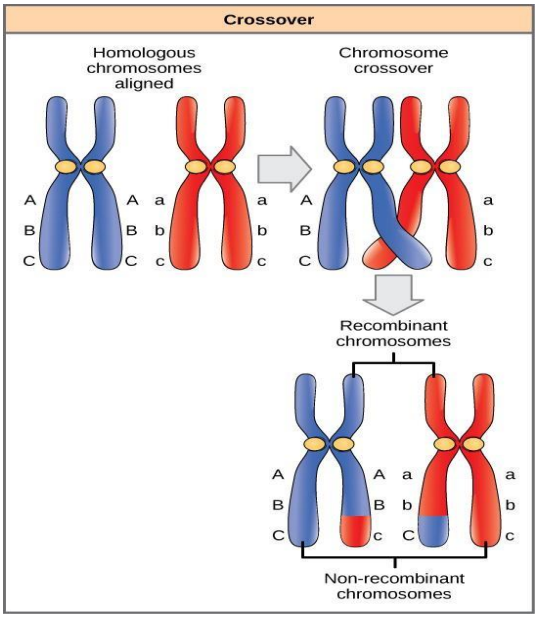


Fig.3.7 Crossover

iv. Linked genes can be separated by recombination:

The process of crossover, or recombination, occurs when two homologous chromosomes align during meiosis and exchange a segment of genetic material. Here, the alleles for gene C were exchanged. The result is two recombinant and two non-recombinant chromosomes.

When two genes are located in close proximity on the same chromosome, they are considered linked, and their alleles tend to be transmitted through meiosis together. To exemplify this, imagine a dihybrid cross involving flower color and plant height in which the genes are next to each other on the chromosome. If one homologous chromosome has alleles for tall plants and red flowers, and the other chromosome has genes for short plants and yellow flowers, then when the gametes are formed, the tall and red alleles will go together into a gamete and the short and yellow alleles will go into other gametes. These are called the parental genotypes because they have been inherited intact from the parents of the individual producing gametes. But unlike if the genes were on different chromosomes, there will be no gametes with tall and yellow alleles and no gametes with short and red alleles. If you create the Punnett square with these gametes, you will see that the classical Mendelian prediction of a 9:3:3:1 outcome of a dihybrid cross would not apply. As the distance between two genes increases, the probability of one or more crossovers between them increases, and the genes behave more like they are on separate chromosomes. Geneticists have used the proportion of recombinant gametes (the ones not like the parents) as a measure of how far apart genes are on a chromosome. Using this information, they have constructed elaborate maps of genes on chromosomes for well-studied organisms, including humans.

Mendel's seminal publication makes no mention of linkage, and many researchers have questioned whether he encountered linkage, but chose not to publish those crosses out of concern that they would invalidate his independent assortment postulate. The garden pea has seven chromosomes and some have suggested that his choice of seven characteristics was not a coincidence. However, even if the genes he examined were not located on separate chromosomes, it is possible that he simply did not observe linkage because of the extensive shuffling effects of recombination.

v. Epistasis

Epistasis occurs when one gene masks or interferes with the expression of another. Mendel's studies in pea plants implied that the sum of an individual's phenotype was controlled by genes (or as he called them, unit factors): every characteristic was distinctly and completely controlled by a single gene. In fact, single observable characteristics are almost always under the influence of multiple genes (each with two or more alleles) acting in unison. For example, at least eight genes contribute to eye color in humans.

In some cases, several genes can contribute to aspects of a common phenotype without their gene products ever directly interacting. In the case of organ development, for instance, genes may be expressed sequentially, with each gene adding to the complexity and specificity of the organ. Genes may function in complementary or synergistic fashions: two or more genes need to be expressed simultaneously to affect a phenotype. Genes may also oppose each other with one gene modifying the expression of another.

In epistasis, the interaction between genes is antagonistic: one gene masks or interferes with the expression of another. ―Epistasis‖ is a word composed of Greek roots that mean ―standing upon.‖ The alleles that are being masked or silenced are said to be hypostatic to the epistatic alleles that are doing the masking. Often the biochemical basis of epistasis is a gene pathway in which the expression of one gene is dependent on the function of a gene that precedes or follows it in the pathway.

An example of epistasis is pigmentation in mice. The wild-type coat color, agouti (AA), is dominant to solid-colored fur (aa). However, a separate gene (C) is necessary for pigment production. A mouse with a recessive c allele at this locus is unable to produce pigment and is albino regardless of the allele present at locus A. Therefore, the genotypes AAcc, Aacc, and aacc all produce the same albino phenotype. A cross between heterozygotes for both genes (AaCc x AaCc) would generate offspring with a phenotypic ratio of 9 agouti:3 solid color:4 albino. In this case, the C gene is epistatic to the A gene.

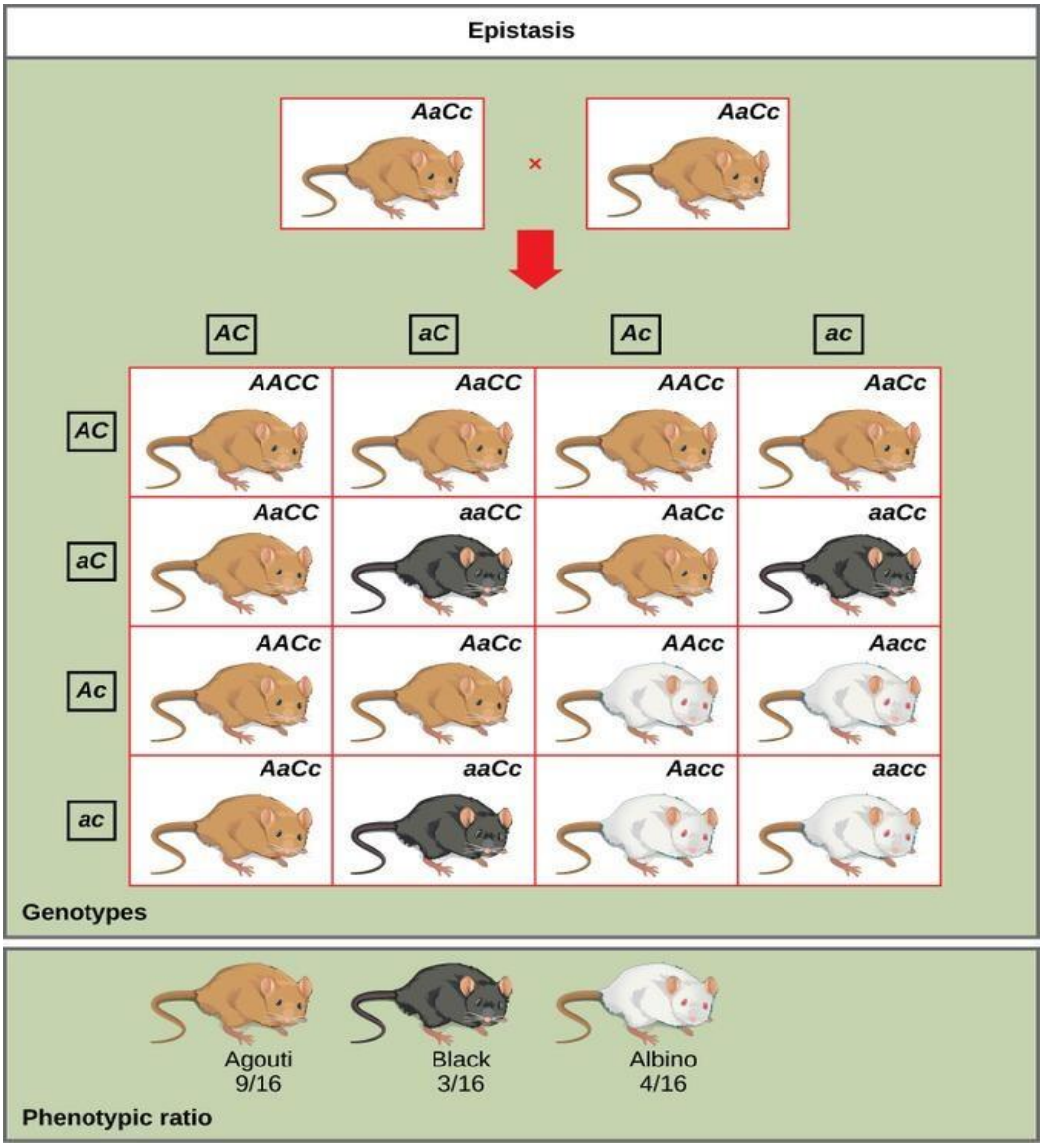


Fig.3.8 Epistasis

vi. Epistasis in mouse coat color:

In mice, the mottled agouti coat color (A) is dominant to a solid coloration, such as black or gray. A gene at a separate locus (C) is responsible for pigment production. The recessive c allele does not produce pigment and a mouse with the homozygous recessive cc genotype is albino regardless of the allele present at the A locus. Thus, the C gene is epistatic to the A gene.

Epistasis can also occur when a dominant allele masks expression at a separate gene. Fruit color in summer squash is expressed in this way. Homozygous recessive expression of the W gene (ww) coupled with homozygous dominant or heterozygous expression of the Y gene (YY or Yy) generates yellow fruit, while the wwyy genotype produces green fruit. However, if a dominant copy of the W gene is present in the homozygous or heterozygous form, the summer squash will produce white fruit regardless of the Y alleles. A cross between white heterozygotes for both genes (WwYy × WwYy) would produce offspring with a phenotypic ratio of 12 white:3 yellow:1 green.

Finally, epistasis can be reciprocal: either gene, when present in the dominant (or recessive) form, expresses the same phenotype. In the shepherd's purse plant (Capsella bursa-pastoris), the characteristic of seed shape is controlled by two genes in a dominant epistatic relationship. When the genes A and B are both homozygous recessive (aabb), the seeds are ovoid. If the dominant allele for either of these genes is present, the result is triangular seeds. That is, every possible genotype other than aabb results in triangular seeds; a cross between heterozygotes for both genes (AaBb x AaBb) would yield offspring with a phenotypic ratio of 15 triangular:1 ovoid.

Keep in mind that any single characteristic that results in a phenotypic ratio that totals 16 is typical of a two-gene interaction. Recall the phenotypic inheritance pattern for Mendel's dihybrid cross, which considered two non-interacting genes: 9:3:3:1. Similarly, we would expect interacting gene pairs to also exhibit ratios expressed as 16 parts. Note that we are assuming the interacting genes are not linked; they are still assorting independently into gametes.

vii. Allele Definition

An allele is specific variation of a gene. Bacteria, because they have a single ring of DNA, have one allele per gene per organism. In sexually reproducing organisms, each parent gives an allele for each gene, giving the offspring two alleles per gene. Because alleles are just variants of specific genes, different alleles are found on the same locations on the chromosomes of different individuals. This is important because it gives organisms to be incredibly varied in the functions of their various alleles, while at the same time being able to reproduce. This creates variety caused by mutations in specific genes gives rise to a wide number of alleles for any trait in a given population.

Some areas of the genome are more protected against mutation than other areas. For instance, the ends of chromosomes are often broken and changed chemically because of the interactions with the surrounding cytosol and membranes it may come into contact with. This breakage or damage necessitates DNA repair. While the enzymes that repair DNA are extremely efficient, they sometimes make mistakes.

The repair of DNA molecules is carried out by a variety of enzymes, one of the most important of which is DNA polymerase. DNA polymerase uses free floating nucleic acid bases to ‑rebuild‖ the DNA, one nucleic acid at a time. After the DNA is unwound by another enzyme, helicase, DNA polymerase goes to work on each strand of the two-stranded DNA molecule. By ‑reading‖ one strand and adding nucleic acid bases together, it creates a brand new strand that can couple to the first strand. DNA bases have counterparts that always go together. Guanine (G) is the basepair of cytosine (C). Thymine (T) is always the base pair of adenine (A).

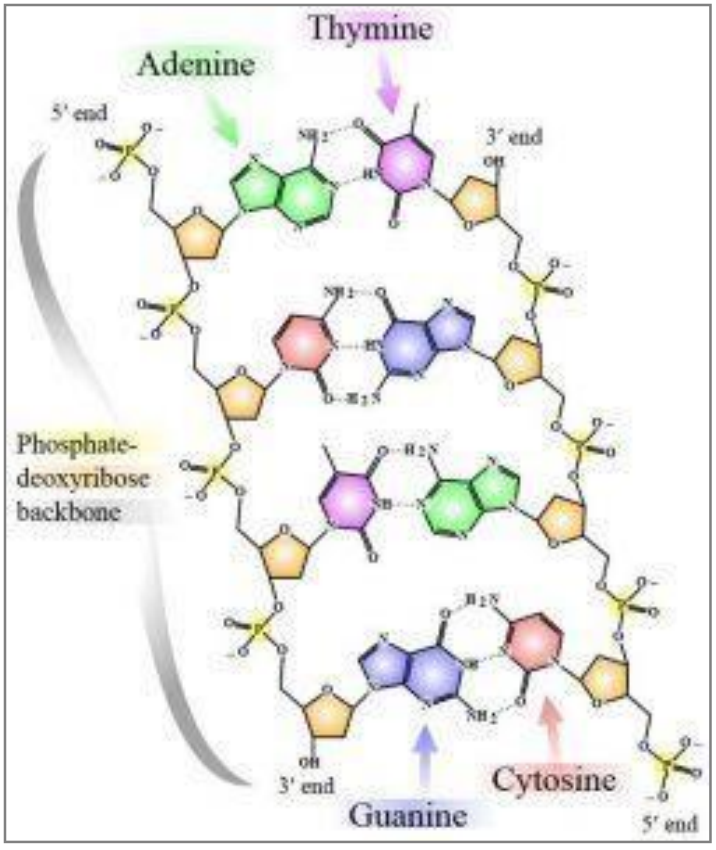


Fig.3.9 DNA chemical structure

Sometimes, the polymerase makes a mistake, and the wrong base pairs are put together. Other enzymes are designed to ‒check‖ the DNA after it has been synthesized to find these errors. The enzyme runs along the DNA, checking for bumps that signify two base pairs are not properly bonded. If all these mechanisms fail to catch the mutation, it will be replicated the next time the cell divides. In bacteria this can give rise to whole colonies that have novel mutations and can be easily studied. In sexually reproducing organisms, a beneficial mutation is only valuable if it happens early in development or in the production of gametes. A mutation in a single skin cell, for instance, will not be able to help the organism in a large way. The cell may give rise to a few thousand ‒good‖ skin cells, but compared to the trillions on your body, they wouldn't matter. In early development or in the production of gametes however, the mutations of a gene into different alleles can be passed onto entire organisms, which can then reproduce the allele to its full benefit.

Other mutations, known as deleterious mutations, cause a disruption of cellular function. These mutations cause non-functional alleles to arise, as is the case in cancers. Some cancers are caused by mutations in tumor-suppressing genes, which regulate the size, shape, and growth of individual cells. A non-functional allele at this gene means the cell will continue to grow and divide, regardless of the signals it receives. As part of a functioning body of trillions of cells, this can cause a terrible amount of damage if the cancerous cells are in a sensitive or vital area.

viii. Examples of Allele: Flower Color in Peas

The founder of the field of genetics, Gregor Mendel, was a friar who studied peas. One of the traits that he studied was flower color. Mendel's peas produced two different colors of flower, purple and white. Although he did not know it at the time, these two colors represented the interactions of different alleles in the genomes of the plants. Plants are sexually reproducing, meaning they receive two alleles for each trait. The trait for flower color is determined by a gene that creates an enzyme responsible for creating the pigment we see as purple. Plants that received even one functioning allele produce purple flowers, while plants that receive two non-functioning alleles produce white flowers. Because one functioning allele can completely mask the effects of the non-functioning allele, the former is said to be the dominant allele, while the non-functioning allele is the recessive.

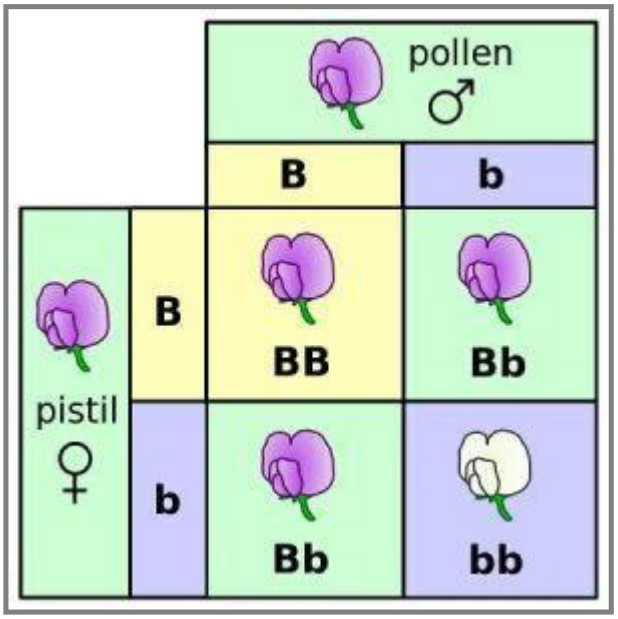


Fig.3.10Punnett squaremendel flowers

The interactions between these alleles are produces important variability in the flowers. While the recessive allele can be masked by the dominant allele, it does not mean that the dominant allele is better for the plant. It could be true that white flowers attract more pollinators, and are therefore more successful at reproducing. If this were true, the allele frequency of the non-functioning allele would increase in the population, even though it is not functioning. Sometimes the most adaptable function of an enzyme is to not have the enzyme functioning at all.

ix. Multiple Genes in Peas

One of the things that most interested Mendel was the enormous variety he could obtain by crossing two seemingly identical plants. Below is a table of the various traits Mendel observed. He observed that while each of these traits only had two forms, the different alleles could be mix-matched in an enormous variety of patterns and shapes. What Mendel was beginning to describe were the laws of segregation and independent assortment.

Related Biology Terms

- **Polymerase** – An enzyme used to bind monomers into polymers, or smaller molecules into large ones.

- **Mutation** – When DNA polymerase makes a mistake, and places the wrong nucleic acid in a DNA chain.

- **Genotype** – The alleles present at a specific locus in the DNA, which give rise to phenotypes through the production (or lack of production) of protein.

- **Phenotype** – The physical manifestation of the DNA, expressed in terms of protein.

3.5. GENETIC MAPPING

3.5.1 Genes and loci

In the prerecombinant DNA era, all genes were defined by the existence of alternative alleles that produced alternative phenotypes that segregated in genetic crosses. Today, with the use of molecular technologies, the ability to recognize genes has expanded tremendously. Monomorphic genes (those with only a single allele) can now be recognized through their transcriptional activity alone. Recognition of putative genes within larger genomic sequences can also be accomplished through the identification of open reading frames, flanking tissue-specific enhancers and other regulatory elements, internal splicing signals, and sequence conservation across evolutionary lines. Sequence-specific epigenetic phenomena such as imprinting, methylation, and DNase sensitivity can also be used to elucidate the existence of functional genomic elements.

Mouse geneticists use the term locus to describe any DNA segment that is distinguishable in some way by some form of genetic analysis. In the prerecombinant DNA era, only genes distinguished by phenotype could be recognized as loci. But today, with the use of molecular tools, it is possible to distinguish "loci" in the genome that have no discernible function at all. In fact, any change in the DNA sequence, no matter how small or large, whether in a gene or elsewhere, can be followed potentially as an alternative allele in genetic crosses. When alternative alleles exist in a genomic sequence that has no known function, the polymorphic site is called an anonymous locus. With an average rate of polymorphism of one base difference in a thousand between individual chromosome homologs within a species, the pool of potential anonymous loci is enormous.

3.5.2. Maps

A genetic map is simply a representation of the distribution of a set of loci within the genome. The loci included by an investigator in any one mapping project may bear no relation to each other at all, or they may be related according to any of a number of parameters including functional or structural homologies or a pre-determined chromosomal assignment. Mapping of these loci can be accomplished at many different levels of resolution. At the lowest level, a locus is simply assigned to a particular chromosome without any further localization. At a step above, an assignment may be made to a particular subchromosomal region. At a still higher level of resolution, the relative order and approximate distances that separate individual loci within a linked set can be determined. With ever-increasing levels of resolution, the order and interlocus distances can be determined with greater and greater precision. Finally, the ultimate resolution is attained when loci are mapped onto the DNA sequence itself.

The simplest genetic maps can contain information on as few as two linked loci. At the opposite extreme will be complete physical maps that depict the precise physical location of all of the thousands of genes that exist along an entire chromosome. The first step toward the generation of these complete physical maps has recently been achieved with the establishment of single set of DNA sequence of overlapping clones across the length of two complete human chromosome arms. By the time this book is actually read, it is likely that complete overlapping DNA sequence across other human as well as mouse chromosomes will also be attained. However, it is still a long journey from simply having a set of clones to deciphering the genetic information within them.

a. Linkage maps

The linkage map, also referred to as a recombination map, was the first to be developed soon after the re-discovery of Mendel's work at the beginning of the 20th century. Linkage maps can only be constructed for loci that occur in two or more heritable forms, or alleles. Thus, monomorphic loci - those with only a single allele - cannot be mapped in this fashion. Linkage maps are generated by counting the number of offspring that receive either parental or recombinant allele combinations from a parent that carries two different alleles at two or more loci. Analyses of this type of data allow one to determine whether loci are "linked" to each other and, if they are, their relative order and the relative distances that separate them.

A chromosomal assignment is accomplished whenever a new locus is found to be in linkage with a previously assigned locus. Distances are measured in centimorgans, with one centimorgan equivalent to a crossover rate of 1%. The linkage map is the only type based on classical breeding analysis. The term "genetic map" is sometimes used as a false synonym for "linkage map"; a genetic map is actually more broadly defined to include both chromosomal and physical maps as well.

b. Chromosome maps

The chromosomemap (or cytogenetic map) is based on the karyotype of the mouse genome. All mouse chromosomes are defined at the cytogenetic level according to their size and banding pattern and ultimately, all chromosomal assignments are made by direct cytogenetic analysis or by linkage to a locus that has previously been mapped in this way. Chromosomal map positions are indicated with the use of band names. Inherent in this naming scheme is a means for ordering loci along the chromosome.

Today, several different approaches, with different levels of resolution, can be used to generate chromosome maps. First, in some cases, indirect mapping can be accomplished with the use of one or more somatic cell hybrid lines that contain only portions of the mouse karyotype within the milieu of another species' genome. By correlating the presence or expression of a particular mouse gene with the presence of a mouse chromosome or subchromosomal region in these cells, one can obtain a chromosomal, or subchromosomal, assignment.

The second approach can be used in those special cases where karyotypic abnormalities appear in conjunction with particular mutant phenotypes. When the chromosomal lesion and the phenotype assort together, from one generation to the next, it is likely that the former causes the latter. When the lesion is a deletion, translocation, inversion, or duplication, one can assign the mutant locus to the chromosomal band that has been disrupted.

Finally, with the availability of a locus-specific DNA probe, it becomes possible to use the method of in situ hybridization to directly visualize the location of the corresponding sequence within a particular chromosomal band. This approach is not dependent on correlations or assumptions of any kind and, as such, it is the most direct mapping approach that exists. However, it is technically demanding and its resolution is not nearly as high as that obtained with linkage or physical approaches.

d. Physical maps

The third type of map is a physical map. All physical maps are based on the direct analysis of DNA. Physical distances between and within loci are measured in basepairs (bp), kilobasepairs (kb) or megabasepairs (mb). Physical maps are arbitrarily divided into short range and long range. Short range mapping is commonly pursued over distances ranging up to 30 kb. In very approximate terms, this is the average size of a gene and it is also the average size of cloned inserts obtained from cosmid-based genomic libraries. Cloned regions of this size can be easily mapped to high resolution with restriction enzymes and, with advances in sequencing technology, it is becoming more common to sequence interesting regions of this length in their entirety.

Direct long-range physical mapping can be accomplished over megabase-sized regions with the use of rare-cutting restriction enzymes together with various methods of gel electrophoresis referred to generically as pulsed field gel electrophoresis or PFGE, which allow the separation and sizing of DNA fragments of 6 mb or more in length. PFGE mapping studies can be performed directly on genomic DNA followed by Southern blot analysis with probes for particular loci. It becomes possible to demonstrate physical linkage whenever probes for two loci detect the same set of large restriction fragments upon sequential hybridizations to the same blot.

Long-range mapping can also be performed with clones obtained from large insert genomic libraries such as those based on the yeast artificial chromosome (YAC) cloning vectors, since regions within these clones can be readily isolated for further analysis. In the future, long-range physical maps consisting of overlapping clones will cover each chromosome in the mouse genome. Short-range restriction maps of high resolution will be merged together along each chromosomal length, and ultimately, perhaps, the highest level of mapping resolution will be achieved with whole chromosome DNA sequences.

3.5.3. Connections between maps

In theory, linkage, chromosomal, and physical maps should all provide the same information on chromosomal assignment and the order of loci. However, the relative distances that are measured within each map can be quite different. Only the physical map can provide an accurate description of the actual length of DNA that separates loci from each other. This is not to say that the other two types of maps are inaccurate. Rather, each represents a version of the physical map that has been modulated according to a different parameter. Cytogenetic distances are modulated by the relative

packing of the DNA molecule into different chromosomal regions. Linkage distances are modulated by the variable propensity of different DNA regions to take part in recombination events.

In practice, genetic maps of the mouse are often an amalgamation of chromosomal, linkage, and physical maps, but at the time of this writing, it is still the case that classical recombination studies provide the great bulk of data incorporated into such integrated maps. Thus, the primary metric used to chart interlocus distances has been the centimorgan. However, it seems reasonable to predict that, within the next five years, the megabase will overtake the centimorgan as the unit for measurement along the chromosome.

3.6. CELL MULTIPLICATION

a. Cell division and growth

In unicellular organisms, cell division is the means of reproduction; in multicellular organisms, it is the means of tissuegrowth and maintenance. Survival of the eukaryotes depends upon interactions between many cell types, and it is essential that a balanced distribution of types be maintained. This is achieved by the highly regulated process of cell proliferation. The growth and division of different cell populations are regulated in different ways, but the basic mechanisms are similar throughout multicellular organisms.

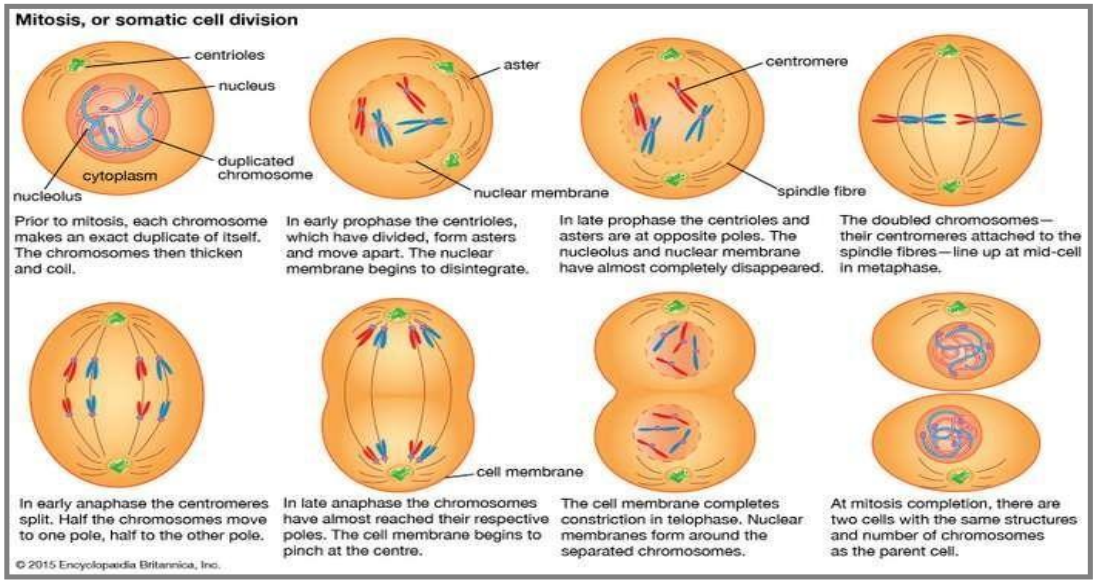


Fig.3.11 Mitosis

Mitosis One cell gives rise to two genetically identical daughter cells during the process of mitosis.

Most tissues of the body grow by increasing their cell number, but this growth is highly regulated to maintain a balance between different tissues. In adults most cell division is involved in tissue renewal rather than growth, many types of cells undergoing continuous replacement. Skin cells, for example, are constantly being sloughed off and replaced; in this case, the mature differentiated cells do not divide, but their population is renewed by division of immature stem cells. In certain other cells, such as those of the liver, mature cells remain capable of division to allow growth or regeneration after injury.

In contrast to these patterns, other types of cells either cannot divide or are prevented from dividing by certain molecules produced by nearby cells. As a result, in the adult organism, some tissues have a greatly reduced capacity to renew damaged or diseased cells. Examples of such tissues include heart muscle, nerve cells of the central nervous system, and lens cells in mammals. Maintenance and repair of these cells is limited to replacing intracellular components rather than replacing entire cells.

b. Duplication of the genetic material

Before a cell can divide, it must accurately and completely duplicate the genetic information encoded in its DNA in order for its progeny cells to function and survive. This is a complex problem because of the great length of DNA molecules. Each human chromosome consists of a long double spiral, or helix, each strand of which consists of more than 100 million nucleotides.

The duplication of DNA is called DNA replication, and it is initiated by complex enzymes called DNA polymerases. These progress along the molecule, reading the sequences of nucleotides that are linked together to make DNA chains. Each strand of the DNA double helix, therefore, acts as a template specifying the nucleotide structure of a new growing chain. After replication, each of the two daughter DNA double helices consists of one parental DNA strand wound around one newly synthesized DNA strand.

In order for DNA to replicate, the two strands must be unwound from each other. Enzymes called helicases unwind the two DNA strands, and additional proteins bind to the separated strands to stabilize them and prevent them from pairing again. In addition, a remarkable class of enzyme called DNA topoisomerase removes the helical

twists by cutting either one or both strands and then resealing the cut. These enzymes can also untangle and unknot DNA when it is tightly coiled into a chromatin fibre.

In the circular DNA of prokaryotes, replication starts at a unique site called the origin of replication and then proceeds in both directions around the molecule until the two processes meet, producing two daughter molecules. In rapidly growing prokaryotes, a second round of replication can start before the first has finished. The situation in eukaryotes is more complicated, as replication moves more slowly than in prokaryotes. At 500 to 5,000 nucleotides per minute (versus 100,000 nucleotides per minute in prokaryotes), it would take a human chromosome about a month to replicate if started at a single site. Actually, replication begins at many sites on the long chromosomes of animals, plants, and fungi. Distances between adjacent initiation sites are not always the same; for example, they are closer in the rapidly dividing embryonic cells of frogs or flies than in adult cells of the same species.

Accurate DNA replication is crucial to ensure that daughter cells have exact copies of the genetic information for synthesizing proteins. Accuracy is achieved by a ‑proofreading‖ ability of the DNA polymerase itself. It can erase its own errors and then synthesize anew. There are also repair systems that correct genetic damage to DNA. For example, the incorporation of an incorrect nucleotide, or damage caused by mutagenic agents, can be corrected by cutting out a section of the daughter strand and recopying the parental strand.

c. Cell division

In eukaryotes the processes of DNA replication and cell division occur at different times of the cell division cycle. During cell division, DNA condenses to form short, tightly coiled, rodlike chromosomes. Each chromosome then splits longitudinally, forming two identical chromatids. Each pair of chromatids is divided between the two daughter cells during mitosis, or division of the nucleus, a process in which the chromosomes are propelled by attachment to a bundle of microtubules called the mitotic spindle.

Mitosis can be divided into five phases. In prophase the mitotic spindle forms and the chromosomes condense. In prometaphase the nuclear envelope breaks down (in many but not all eukaryotes) and the chromosomes attach to the mitotic spindle. Both chromatids of each chromosome attach to the spindle at a specialized chromosomal region called the kinetochore. In metaphase the condensed chromosomes align in a

plane across the equator of the mitotic spindle. Anaphase follows as the separated chromatids move abruptly toward opposite spindle poles. Finally, in telophase a new nuclear envelope forms around each set of unraveling chromatids.

An essential feature of mitosis is the attachment of the chromatids to opposite poles of the mitotic spindle. This ensures that each of the daughter cells will receive a complete set of chromosomes. The mitotic spindle is composed of microtubules, each of which is a tubular assembly of molecules of the protein tubulin. Some microtubules extend from one spindle pole to the other, while a second class extends from one spindle pole to a chromatid. Microtubules can grow or shrink by the addition or removal of tubulin molecules. The shortening of spindle microtubules at anaphase propels attached chromatids to the spindle poles, where they unravel to form new nuclei.

The two poles of the mitotic spindle are occupied by centrosomes, which organize the microtubule arrays. In animal cells each centrosome contains a pair of cylindrical centrioles, which are themselves composed of complex arrays of microtubules. Centrioles duplicate at a precise time in the cell division cycle, usually close to the start of DNA replication.

After mitosis comes cytokinesis, the division of the cytoplasm. This is another process in which animal and plant cells differ. In animal cells cytokinesis is achieved through the constriction of the cell by a ring of contractile microfilaments consisting of actin and myosin, the proteins involved in muscle contraction and other forms of cell movement. In plant cells the cytoplasm is divided by the formation of a new cell wall, called the cell plate, between the two daughter cells. The cell plate arises from small Golgi-derived vesicles that coalesce in a plane across the equator of the late telophase spindle to form a disk-shaped structure. In this process, each vesicle contributes its membrane to the forming cell membranes and its matrix contents to the forming cell wall. A second set of vesicles extends the edge of the cell plate until it reaches and fuses with the sides of the parent cell, thereby completely separating the two new daughter cells. At this point, cellulose synthesis commences, and the cell plate becomes a primary cell wall.

d. Meiosis

A specialized division of chromosomes called meiosis occurs during the formation of the reproductive cells, or gametes, of sexually reproducing organisms. Gametes such as ova, sperm, and pollen begin as germ cells, which, like other types of

cells, have two copies of each gene in their nuclei. The chromosomes composed of these matching genes are called homologs. During DNA replication, each chromosome duplicates into two attached chromatids. The homologous chromosomes are then separated to opposite poles of the meiotic spindle by microtubules similar to those of the mitotic spindle. At this stage in the meiosis of germ cells, there is a crucial difference from the mitosis of other cells. In meiosis the two chromatids making up each chromosome remain together, so that whole chromosomes are separated from their homologous partners. Cell division then occurs, followed by a second division that resembles mitosis more closely in that it separates the two chromatids of each remaining chromosome. In this way, when meiosis is complete, each mature gamete receives only one copy of each gene instead of the two copies present in other cells.

e. The cell division cycle

In prokaryotes, DNA synthesis can take place uninterrupted between cell divisions, and new cycles of DNA synthesis can begin before previous cycles have finished. In contrast, eukaryotes duplicate their DNA exactly once during a discrete period between cell divisions. This period is called the S (for synthetic) phase. It is preceded by a period called G_1 (meaning ‑first gap‖) and followed by a period called G_2, during which nuclear DNA synthesis does not occur.

The four periods G_1, S, G_2, and M (for mitosis) make up the cell division cycle. The cell cycle characteristically lasts between 10 and 20 hours in rapidly proliferating adult cells, but it can be arrested for weeks or months in quiescent cells or for a lifetime in neurons of the brain. Prolonged arrest of this type usually occurs during the G_1 phase and is sometimes referred to as G_0. In contrast, some embryonic cells, such as those of fruit flies (vinegar flies), can complete entire cycles and divide in only 11 minutes. In these exceptional cases, G_1 and G_2 are undetectable, and mitosis alternates with DNA synthesis. In addition, the duration of the S phase varies dramatically. The fruit flyembryo takes only four minutes to replicate its DNA, compared with several hours in adult cells of the same species.

f. Controlled proliferation

Several studies have identified the transition from the G_1 to the S phase as a crucial control point of the cell cycle. Stimuli are known to cause resting cells to proliferate by inducing them to leave G_1 and begin DNA synthesis. These stimuli, called growth factors, are naturally occurring proteins specific to certain groups of cells

in the body. They include nerve growth factor, epidermal growth factor, and platelet-derived growth factor. Such factors may have important roles in the healing of wounds as well as in the maintenance and growth of normal tissues. Many growth factors are known to act on the external membrane of the cell, by interacting with specialized proteinreceptor molecules. These respond by triggering further cellular changes, including an increase in calcium levels that makes the cell interior more alkaline and the addition of phosphate groups to the amino acidtyrosine in proteins. The complex response of cells to growth factors is of fundamental importance to the control of cell proliferation.

g. Failure of proliferation control

Cancer can arise when the controlling factors over cell growth fail and allow a cell and its descendants to keep dividing at the expense of the organism. Studies of viruses that transform cultured cells and thus lead to the loss of control of cell growth have provided insight into the mechanisms that drive the formation of tumours. Transformed cells may differ from their normal progenitors by continuing to proliferate at very high densities, in the absence of growth factors, or in the absence of a solid substrate for support.

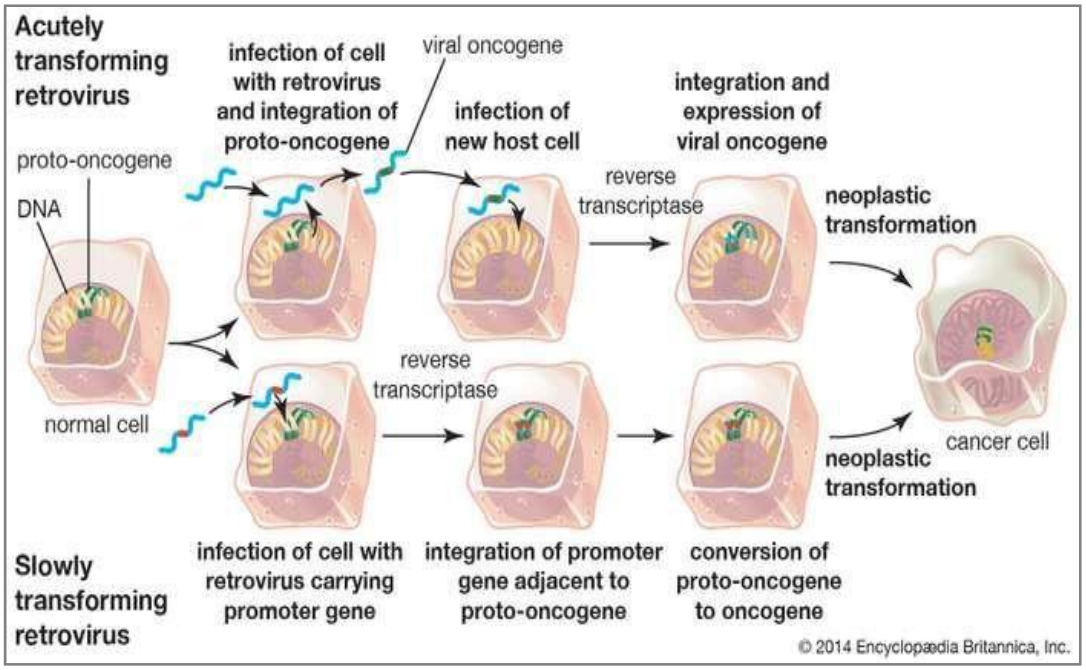


Fig.3.12Cell division

h. Cancer-causing retroviruses:

Retroviral insertion can convert a proto-oncogene, integral to the control of cell division, into an oncogene, the agent responsible for transforming a healthy cell into a cancer cell. An acutely transforming retrovirus, which produces tumours within weeks of infection, incorporates genetic material from a host cell into its own genome upon infection, forming a viral oncogene. When the viral oncogene infects another cell, an enzyme called reverse transcriptase copies the single-stranded genetic material into double-stranded DNA, which is then integrated into the cellular genome. A slowly transforming retrovirus (shown at bottom), which requires months to elicit tumour growth, does not disrupt cellular function through the insertion of a viral oncogene. Rather, it carries a promoter gene that is integrated into the cellular genome of the host cell next to or within a proto-oncogene, allowing conversion of the proto-oncogene to an oncogene.

Major advances in the understanding of growth control have come from studies of the viral genes that cause transformation. These viral oncogenes have led to the identification of related cellular genes called protooncogenes. Protooncogenes can be altered by mutationor epigenetic modification, which converts them into oncogenes and leads to cell transformation. Specific oncogenes are activated in particular human cancers. For example, an oncogene called RAS is associated with many epithelial cancers, while another, called MYC, is associated with leukemias.

An interesting feature of oncogenes is that they may act at different levels corresponding to the multiple steps seen in the development of cancer. Some oncogenes immortalize cells so that they divide indefinitely, whereas normal cells die after a limited number of generations. Other oncogenes transform cells so that they grow in the absence of growth factors. A combination of these two functions leads to loss of proliferation control, whereas each of these functions on its own cannot. The mode of action of oncogenes also provides important clues to the nature of growth control and cancer. For example, some oncogenes are known to encode receptors for growth factors that may cause continuous proliferation in the absence of appropriate growth factors.

Loss of growth control has the added consequence that cells no longer repair their DNA effectively, and thus aberrant mitoses occur. As a result, additional mutations arise that subvert a cell's normal constraints to remain in its tissue of origin. Epithelial tumour cells, for example, acquire the ability to cross the basal lamina and

enter the bloodstream or lymphatic system, where they migrate to other parts of the body, a process called metastasis. When cells metastasize to distant tissues, the tumour is described as malignant, whereas prior to metastasis a tumour is described as benign.

3.7. SINGLE GENE DISORDERS

When a certain gene is known to cause a disease, we refer to it as a single gene disorder or a Mendelian disorder. For example, you may have heard of cystic fibrosis, sickle cell disease, Fragile X syndrome, muscular dystrophy, or Huntington disease. These are all examples of single gene disorders. As a rule, single gene disorders are not very common. For example, only one in 2,500 people are born with cystic fibrosis. There are a number of inheritance patterns of single gene disorders that are predictable when you know what they are.

Autosomal dominant means that a person only needs one copy of the changed gene (genetic difference) in order to have the disorder. Usually, the changed gene is inherited from a parent who also has the disorder and every generation in the family may have members with the disorder. There are some instances in which a person has the gene that causes the disorder and does not show symptoms of the disorder, but can still pass the gene to his or her children. A person who carriers a gene for an autosomal dominant disorder has a 50% chance of passing the gene to each child.

Autosomal recessive means that it is necessary to have two copies of the changed gene to have the disorder. Each parent contributes one changed copy of the gene to the child who has the disorder. The parents are called carriers of the disorder because they have one normal copy of the gene and one changed copy of the gene, but they do not show symptoms of the disorder. When both parents are carriers of the changed gene, each of their children has a 25% chance of having the disorder, a 50% chance of being a carrier of the disorder (like their parents), and a 25% chance of neither being a carrier nor having the disorder. These risks are the same for each pregnancy. When there is more than one person in a family who has the disease, these people are often in the same generation.

X-linked dominant inheritance follows a pattern similar to autosomal dominant inheritance except that more females are affected than males. However, X-linked dominant disorders are very rare.

X-linked recessive disorders are usually only seen in males and they are much more common than X-linked dominant disorders. People with an X-linked recessive disorder do not have any normal copies of the gene. Males only have one X chromosome, so if a male inherits a changed gene on his X chromosome (which is always inherited from his mother), then he does not have another copy of the working gene to compensate. Females with one copy of a changed gene on one X chromosome are called carriers of X-linked recessive disorder. It is rare for a female to have the changed gene on both her X chromosomes. In most cases, females who are carriers do not show symptoms because the working copy of the gene compensates for the non-working copy of the gene. Carrier females have a 25% of having a son with the disorder, a 25% chance of having a son without the disorder, a 25% chance of having a carrier daughter and a 25% chance of having a daughter who is not a carrier. Males with an X-linked recessive disorder cannot pass the disorder to their sons, but 100% of their daughters will be carriers.

4

CLASSIFICATION AND SYSTEM ENGINEERING

4.1 PROKARYOTES - STRUCTURE AND FUNCTIONS

Prokaryotes are microscopic organisms, which include both bacteria and archaea, are found almost everywhere, in every ecosystem, on every surface of our homes, and inside of our bodies! Some live in environments too extreme for other organisms, such as hot vents on the ocean floor. Although they are found all around us, prokaryotes can be hard to detect, count, and classify. The prokaryotic species we know of today are a tiny fraction of all prokaryotic species thought to exist. Then, we'll explore why it‟s often tricky to identify and classify them. Finally, we'll see how DNA sequencing methods are helping us get a better picture of the prokaryotes around us and the structure of Prokaryotes shown in Fig. 4.1.

i) The capsule

Many prokaryotes have a sticky outermost layer called the **capsule**, which is usually made of polysaccharides (sugar polymers).The capsule helps prokaryotes cling to each other and to various surfaces in their environment, and also helps prevent the cell from drying out. In the case of disease-causing prokaryotes that have colonized the body of a host organism, the capsule or slime layer may also protect against the host‟s immune system.

ii) The cell wall

All prokaryotic cells have a stiff **cell wall**, located underneath the capsule (if there is one). This structure maintains the cell‟s shape, protects the cell interior, and prevents the cell from bursting when it takes up water.The cell wall of most bacteria

contains **peptidoglycan**, a polymer of linked sugars and polypeptides. Peptidoglycan is unusual in that it contains not only L-amino acids, the type normally used to make proteins, but also D-amino acids ("mirror images" of the L-amino acids). Archaeal cell walls don't contain peptidoglycan, but some include a similar molecule called pseudopeptidoglycan, while others are composed of proteins or other types of polymers.

Some eukaryotes, such as plants and fungi, also have cell walls, but they are made of different materials than those of prokaryotes. Plant cell walls are made primarily of cellulose, and fungal cell walls are made of a modified polysaccharide called chitin.

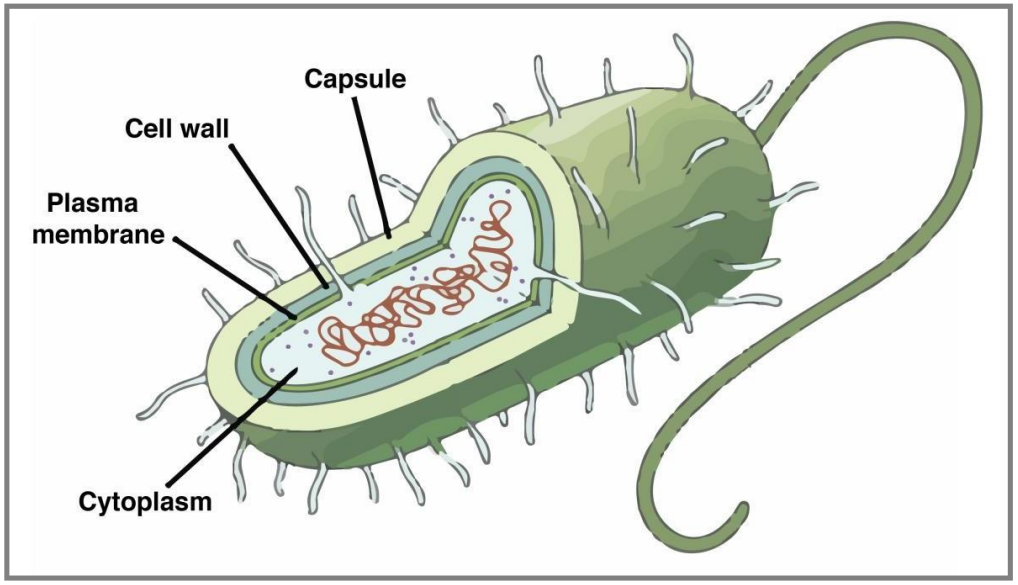


Fig. 4.1.The external structures of the prokaryotic cell

Some of the antibiotics used to treat bacterial infections in humans and other animals act by targeting the bacterial cell wall. For instance, some antibiotics contain D-amino acids similar to those used in peptidoglycan synthesis, "faking out" the enzymes that build the bacterial cell wall (but not affecting human cells, which don't have a cell wall or utilize D-amino acids to make polypeptides).

ii) The plasma membrane

Underneath the cell wall lies the **plasmamembrane**. The basic building block of the plasmamembrane is the **phospholipid**, a lipid composed of a glycerol molecule attached a hydrophilic (water-attracting) phosphate head and to two hydrophobic

(water-repelling) fatty acid tails. The phospholipids of a eukaryotic or bacterial membrane are organized into two layers, forming a structure called a **phospholipidbilayer**.

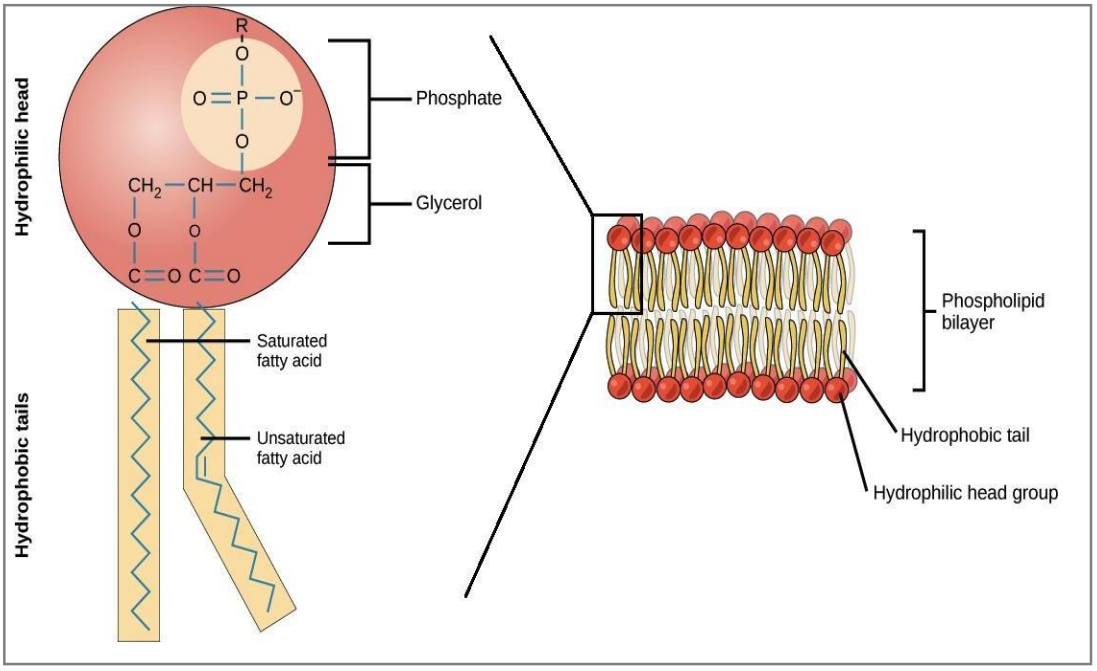


Fig. 4.2. Structure of a phospholipid

Fig4.2. Structure of a phospholipid, showing hydrophobic fatty acid tails and hydrophilic head (including ester linkages, glycerol backbone, phosphate group, and attached R group on phosphate group). A bilayered membrane consisting of phospholipids arranged in two layers, with their heads pointing out and their tails sandwiched in the middle, is also shown.

The plasma membranes of archaea have some unique properties, different from those of both bacteria and eukaryotes. For instance, in some species, the opposing phospholipid tails are joined into a single tail, forming a monolayer instead of a bilayer. This modification may stabilize the membrane at high temperatures, allowing the archaea to live happily in boiling hot springs.

In addition to consisting of monolayers in some cases, archaeal plasma membranes differ from those of bacteria and eukaryotes in other ways shows in Fig 4.3. Below are three other main differences:

- First, archaea have phospholipids with **isoprenoidtails** instead of fatty acid tails. The isoprenoid tails are branched, and while the fatty acid tails are not.
- Second, the bond that joins the glycerol molecule to isoprenoid tails in archaea is an **etherlinkage**. In bacteria (and eukaryotes), by contrast, the glycerol is joined to fatty acid tails by an **esterlinkage**, as shown below.
- Third, the glycerol molecules used to make archaeal phospholipids are mirror images of those used to make bacterial and eukaryotic phospholipids (when considered in three-dimensional space). These forms of glycerol are enantiomers of each other and are designated **L-glycerol** (found in archaea) and **D-glycerol** (found in bacteria and eukaryotes).

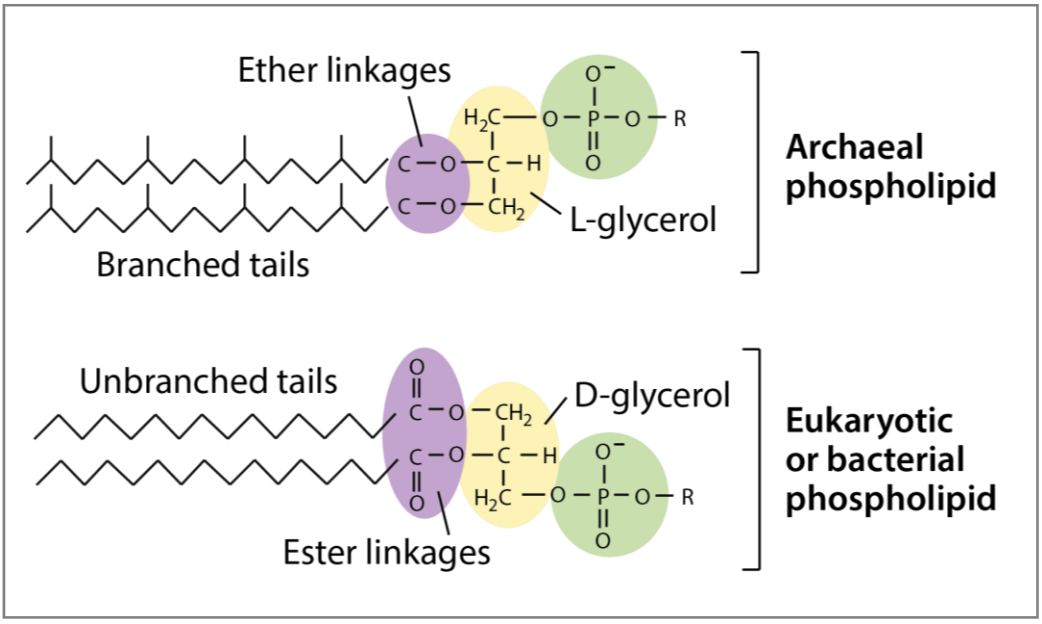


Fig. 4.3. Diffetrent types of phospholipid

The plasma membranes of archaea have several differences from bacteria (and eukaryotes). First, whereas bacteria and eukaryotes have fatty acid tails, archaea have isoprenoid tails. Second, the glycerol molecule of bacteria and eukaryotes is bound to fatty acid tails through an ether linkage. In archaea, it is bound to isoprenoid tails through an ester linkage. Third, the glycerol molecule of bacteria and archaea are mirror images of each other (enantiomers). Archaea have L-glycerol and bacteria and eukaryotes have D-glycerol.

The plasma membrane of bacterial and eukaryotic (and some archael) cells is composed of a phospholipid bilayer. The tails of opposite-facing phospholipids remain separated, forming two separate layers. The plasma membrane of some archaeal cells is composed of a phospholipid monolayer. The tails of opposite-facing phospholipids become united, forming a single layer.

iv) Appendages

Prokaryotic cells often have appendages (protrusions from the cell surface) that allow the cell to stick to surfaces, move around, or transfer DNA to other cells as shown in Fig 4.4.Thin filaments called **fimbriae** (singular: **fimbria**), like those shown in Fig 4.5., are used for adhesion and help cells stick to objects and surfaces in their environment.

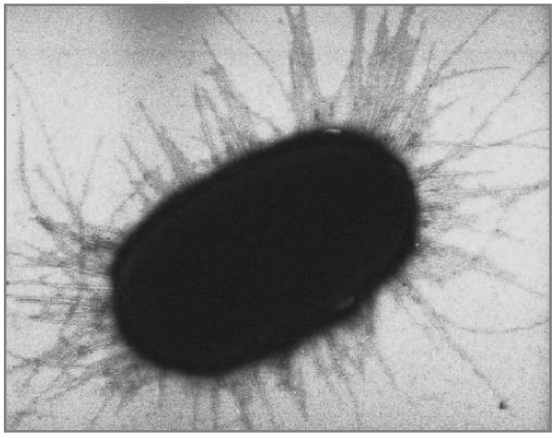


Fig. 4.4. Structure of appendages

A fimbria (plural: fimbriae) is a type of appendage of prokaryotic cells. Longer appendages, called **pili** (singular: **pilus**), come in several types that have different roles. For instance, a **sex pilus** holds two bacterial cells together and allows DNA to be transferred between them in a process called conjugation. Another class of bacterial pili, called **type IV pili**, help the bacterium move around its environment. The most common appendages used for getting around, however, are **flagella** (singular: **flagellum**). These tail-like structures whip around like propellers to move cells through watery environments. Flagella are present not only in bacteria and archaea, but on some eukaryotic cells as well. However, the structure and chemical makeup of the flagella is different in each group, indicating that the three types of flagella evolved independently of one another.

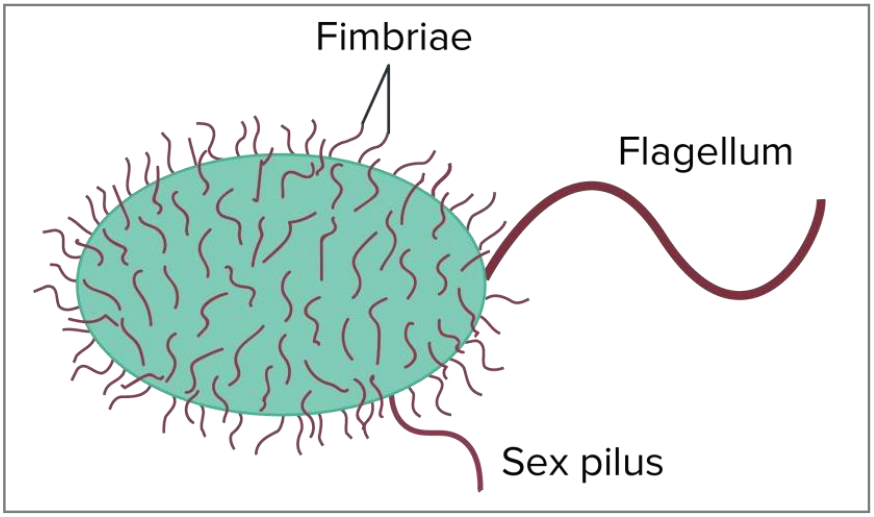


Fig. 4.5. Structure of fimbria in prokaryotic cells

Bacteria may have various types of surface structures. These include fimbriae, short protrusions found all over the surface of the bacterium; a flagellum, found at the back of the bacterium and used for propulsion; and a sex pilus, used to grab on to other bacteria for exchange of genetic material.Bacterial flagella are helical propellers turned by rotary motors in the cell membrane. The fuel for rotation is the membrane gradient of ions, H^+ in most neutrophiles and Na^+ in alkalophiles and marine Vibrio species. Bacteria control their flagella so that swimming is directed toward environments that promote survival. In many species, including Escherichia coliand Salmonella, the motors can rotate either clockwise (CW) or counterclockwise (CCW), and cells direct their movement by regulating switching between the two directions. Some flagellated species exhibit other modes of swimming, such as unidirectional rotation punctuated by occasional stops (Rhodobactersphaeroides) or regulated variations in motor speed (Sinorhizobiummeliloti). In E. coli or Salmonella, CCW rotation allows several filaments on a cell to join in a bundle and drive the cell smoothly forward (a “run”), whereas CW rotation disrupts the filament bundle and causes rapid somersaulting (a “tumble”). When a cell swims in an isotropic environment, the flagellar motors reverse direction at random intervals, and the trajectory is a random walk consisting of runs of about 1 s alternating with short tumbles. In a spatial gradient of a chemical attractant such as serine or maltose, cells increase the duration of runs that happen to be carrying them up the gradient, while not altering (or only slightly shortening) runs down the gradient, thus biasing their movement toward regions of higher attractant concentration.

v) Chromosome and plasmids

Most prokaryotes have a single circular chromosome, and thus a single copy of their genetic material. Eukaryotes like humans, in contrast, tend to have multiple rod-shaped chromosomes and two copies of their genetic material (on homologous chromosomes). Some types of prokaryotes differ from the normal. Species with linear chromosomes, multiple chromosomes, or multiple copies of the same chromosome have been described.

Also, prokaryotic genomes are generally much smaller than eukaryotic genomes. For instance, the E. coli genome is less than half the size of the genome of yeast (a simple, single-celled eukaryote), and almost 700 times smaller than the human genome. By definition, prokaryotes lack a membrane-bound nucleus to hold their chromosomes. Instead, the chromosome of a prokaryote is found in a part of the cytoplasm called a **nucleoid**.

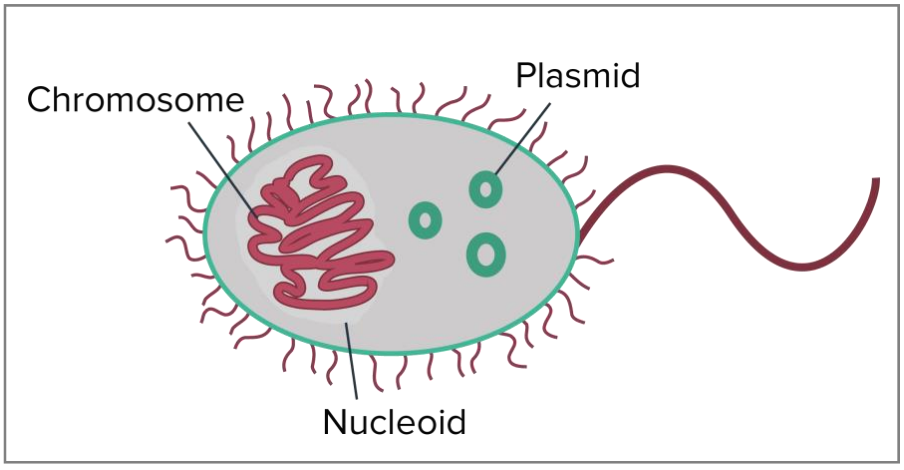


Fig. 4.6. Chromosome and Plasmids in prokaryotic cells

Prokaryotes generally have a single circular chromosome that occupies a region of the cytoplasm called a nucleoid. They also may contain small rings of double-stranded extra-chromosomal DNA called plasmids. In addition to the chromosome, many prokaryotes have **plasmids**, which are small rings of double-stranded extra-chromosomal ("outside the chromosome") DNA. Plasmids carry a small number of non-essential genes and are copied independently of the chromosome inside the cell as shown in Fig 4.6. They can be transferred to other prokaryotes in a population, sometimes spreading genes that are beneficial to survival.

For instance, some plasmids carry genes that make bacteria resistant to antibiotics. (These genes are called **R genes**.) When the plasmids carrying R genes are exchanged in a population, they can quickly make the population resistant to antibiotic drugs. While beneficial to the bacteria, this process can make it difficult for doctors to treat harmful bacterial infections.

Antibiotics are vital for treating harmful bacterial infections, such as pneumonia and tuberculosis. However, the extensive use of antibiotics has led to some strains of pathogenic bacteria becoming resistant. How does this happen?

Some bacteria carry genes (or specific versions of genes) that confer antibiotic resistance. These genes may allow the bacteria to combat natural antibiotics released by other organisms for competition or defense. If resistant bacteria happen to be in an environment that is exposed to antibiotics, then they will survive and reproduce more than non-resistant bacteria (that is, they will be favored by natural selection). If the resistant individuals carry their antibiotic-resistance genes on plasmids, they can also transmit the resistance to other individuals in the population. Through these mechanisms, colonies of resistant bacteria can quickly form and may be very difficult to kill. For this reason, many scientists urge caution regarding the widespread use of antibiotics.

vi) Internal compartments

Prokaryotic cells sometimes need to increase membrane surface area for reactions or concentrate a substrate around its enzyme, just like eukaryotic cells. Because of this, some prokaryotes have membrane folds or compartments functionally similar to those of eukaryotes. For example, photosynthetic bacteria often have extensive membrane folds to increase surface area for the light-dependent reactions, similar to the thylakoid membranes of a plant cell. These bacteria may also have carboxysomes, protein-enclosed cellular compartments where carbon dioxide is concentrated for fixation in the Calvin cycle.

4.2 EUKARYOTES – STRUCTURE AND FUNCTIONS

Eukaryotic cells are defined as cells containing organized nucleus and organelles which are enveloped by membrane-bound organelles. Examples of eukaryotic cells are plants, animals, protists, fungi. Their genetic material is organized in chromosomes. Golgi apparatus, Mitochondria, Ribosomes, Nucleus are parts of Eukaryotic Cells. Let‟s learn about the parts of eukaryotic cells in detail.

4.2.1. Structure of Eukaryotic Cell

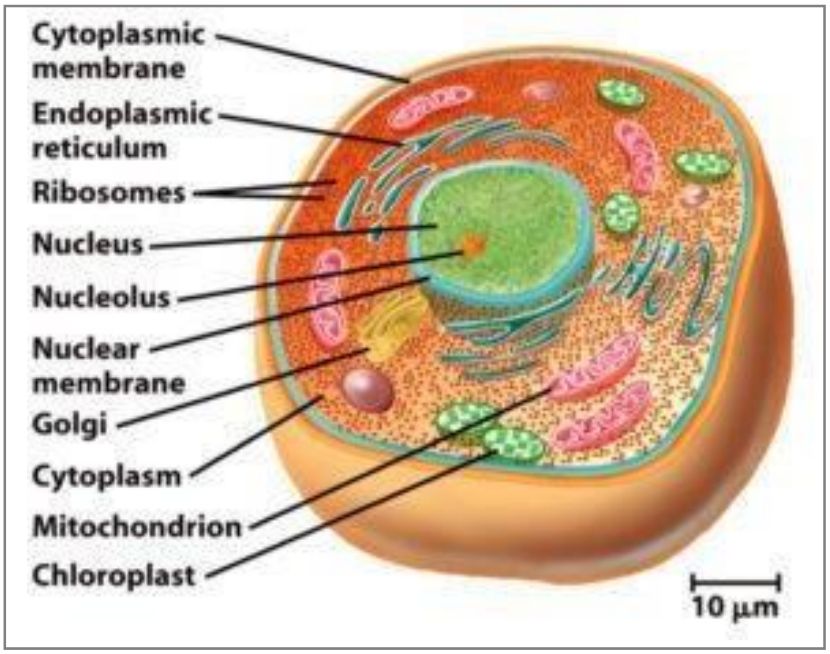


Fig. 4.7. Structure of Eukaryotic Cell

i) Cytoplasmic Membrane

It is also called plasma membrane orcell membrane. The plasma membrane is a semi-permeable membrane that separates the inside of a cell from the outside.

In eukaryotic cells, the plasma membrane consists of proteins, carbohydrates and two layers of phospholipids (i.e. lipid with a phosphate group). These phospholipids are arranged as follows:

- The polar, hydrophilic (water-loving) heads face the outside and inside of the cell. These heads interact with the aqueous environment outside and within a cell.
- The non-polar, hydrophobic (water-repelling) tails are sandwiched between the heads and are protected from the aqueous environments.

Scientists Singer and Nicolson described the structure of the phospholipid bilayer as the „Fluid Mosaic Model". The reason is that the bi-layer looks like a mosaic and has a semi-fluid nature that allows lateral movement of proteins within the bilayer.

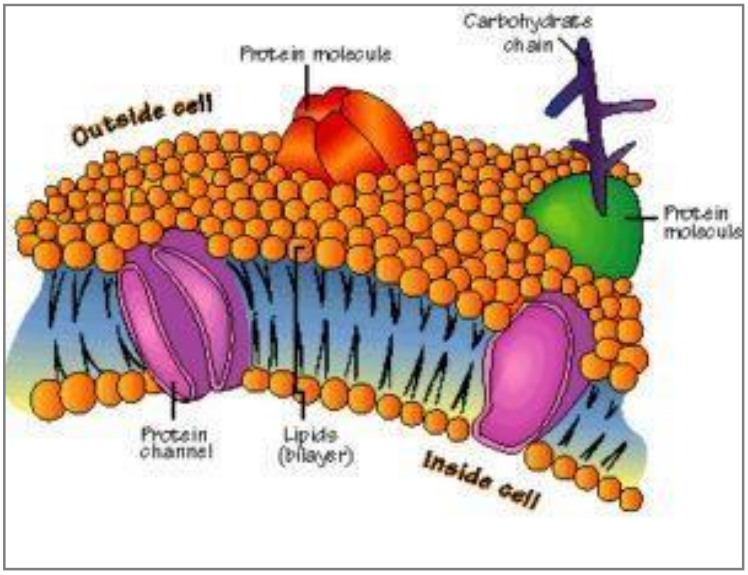


Fig. 4.7. Structure of Cytoplasmic Membrane

Image: Fluid mosaic model. Orange circles – Hydrophilic heads; Lines below – Hydrophobic tails.

Functions of plasma membrane are as follows,

- The plasma membrane is selectively permeable i.e. it allows only selected substances to pass through.
- It protects the cells from shock and injuries.
- The fluid nature of the membrane allows the interaction of molecules within the membrane. It is also important for secretion, cell growth, and division etc.
- It allows transport of molecules across the membrane. This transport can be of two types:
    - ✓ Active transport – This transport occurs against the concentration gradient and therefore, requires energy. It also needs carrier proteins and is a highly selective process.
    - ✓ Passive transport – This transport occurs along the concentration gradient and therefore, does not require energy. Thus, it does not need carrier proteins and is not selective.

ii) Cell Wall

The cell wall is a non-living, rigid structure outside the plasma membrane in plant cells and fungi. It is absent in Eukaryotic cells of animals. It is made of different components in different Eukaryotes:

- Cellulose, hemicellulose, proteins, and pectin – in plants.
- Cellulose, galactans, mannans and calcium carbonate – in fungi.

The cell wall is divided into the following three layers:

- Middle lamella – It is the outermost layer and is made of calcium pectates. It holds adjoining cells together.
- Primary wall – It is the middle layer and is made of cellulose and hemicellulose. It is present in young, growing cells and is capable of growth.
- Secondary wall – It is the innermost layer and similar in composition to the primary wall.

Functions of cell wall are as follows,

- Provides shape to the cell.
- Helps in cell-cell interaction.
- Protects the cell from injury, undesirable molecules and pathogens.

iii) Endoplasmic reticulum (ER)

It is a network of small, tubular structures. It divides the space inside of Eukaryotic cells into two parts – luminal (inside ER) and extra-luminal (cytoplasm).

Endoplasmic reticulum can be divided in two types –

Smooth Endoplasmic Reticulum (SER)	**Rough Endoplasmic Reticulum (RER)**
Smooth due to lack of ribosomes	Rough due to the presence of ribosomes
The main site of lipid synthesis	Site of protein synthesis.

SER is involved in lipid synthesis and RER is involved in protein synthesis.

RER helps in folding proteins and transports it to the Golgi apparatus in vesicles.

iv) Golgi Apparatus

It is named after the scientist who discovered it, Camillo Golgi. Golgi is made of many flat, disc-shaped structures called cisternae. It is present in all eukaryotic cells except human red blood cells and sieve cells of plants. The cisternae are arranged in parallel and concentrically near the nucleus as follows:

- Cis face (forming face) – It faces the plasma membrane and receives secreted material in vesicles.
- Trans face (maturing face) – It faces the nucleus and releases the received material into the cell.

Functions of Golgi Apparatus as follows,

- An important site for packaging material within the cell.
- Proteins are modified in the Golgi.
- An important site for the formation of glycolipids (i.e. lipids with carbohydrate) and glycoproteins (i.e. proteins with carbohydrates).

v) Ribosomes

These structures are not bound by a membrane. Ribosomes are also called „Protein factories" since they are the main site of protein synthesis. They are made of ribonucleic acids and proteins. Eukaryotic ribosomes are of the 80S type, with 60S (large subunit) and 40S (small subunit).

vi) Mitochondria

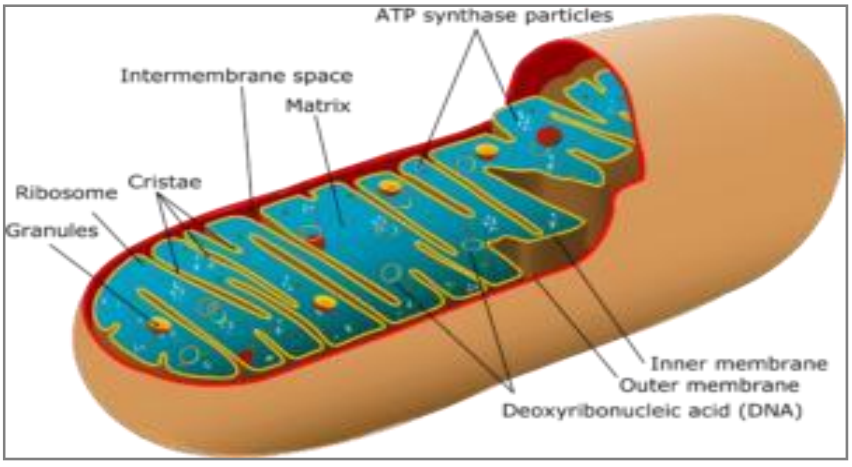


Fig. 4.8. Structure of Mitochondria

They are membrane-bound organelles, also known as „powerhouses of the cell". It has two membranes – outer and inner. The outer membrane forms a continuous boundary around the mitochondria. The inner membrane is semi-permeable and divided into folds called „cristae". The membranes divide the lumen of the mitochondria into an inner and outer compartment. The inner compartment is called matrix and outer compartment forms the inter membrane space as shown in Fig. 4.8.

Functions of mitochondria as follows,

- They produce energy (ATP) and therefore are called the „powerhouse of the cell".
- Helps in regulating cell metabolism.
- Mitochondria possess their own DNA, RNA and components required for protein synthesis.

vii) Lysosomes

They are membrane-bound vesicles formed in the Golgi apparatus. Lysosomes are also called „suicidal bags" since they are rich in hydrolytic enzymes such as lipases, proteases, carbohydrates etc. These enzymes are optimally active at acidic pH (less than 7).The main function of lysosomes is to digest lipids, proteins, carbohydrates and nucleic acids.

viii) Nucleus

Nucleus is the main organelle of a cell. It is a double membrane structure with all the genetic information. Therefore, it is also called the „brain" of a cell. The nucleus is found in all eukaryotic cells except human RBCs and sieve cells of plants.

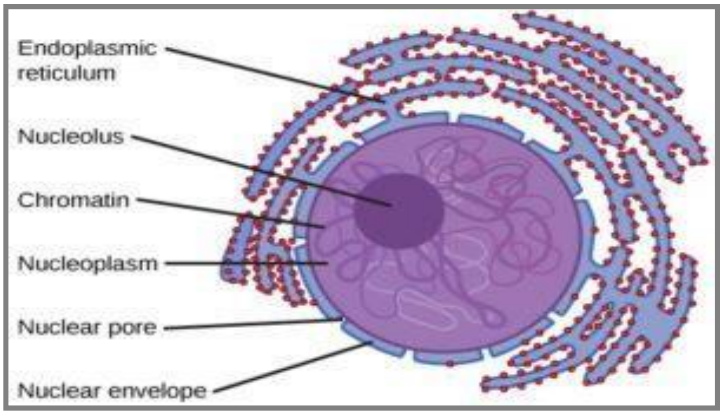


Fig. 4.9. Structure of Nucleus

A nucleus has the following parts:

Nuclear envelope – It is a double membrane structure that surrounds the nucleus. The outer membrane is continuous with the endoplasmic reticulum. The inner membrane has small pores called „nuclear pores".

Nucleoplasm – It is the fluid material in the nucleus that contains the nucleolus and chromatin.

Nucleolus – Nucleoli are not membrane-bound and are active sites for ribosomal RNA synthesis.

Chromatin – It consists of DNA and proteins called „histones". The DNA is organised into chromosomes. Chromosomes have certain constriction sites called „centromeres". Based on the position of the centromere, they can be divided as follows:

- Metacentric – With centromere in the centre and having equal chromosome arms.
- Sub-metacentric – Centromere is slightly off-centre creating one short and one long arm.
- Acrocentric – Centromere is extremely off-centre with one very long and one very short chromosome arm.
- Telocentric – Centromere is placed at one end of the chromosome. Humans do not possess telocentric chromosomes.

Functions of nucleus are as follows,

- It stores genetic information (in the form of DNA) necessary for development and reproduction.
- It contains all information necessary for protein synthesis and cellular functions.

ix) Cytoskeleton

It is the filamentous network present in the cytoplasm of a cell. It provides mechanical support, maintains the shape of the cell and helps in motility.

x) Cilia and Flagella

They are both responsible for the movement of a cell.

Table.4.1 Difference between Cilia and Flagelle

Cilia	Flagella
Short, hair-like structures	Long structures
There are many cilia per cell	There are fewer flagella per cell
Cover the entire surface of a cell	Are present at one end of a cell
Rowing movement	Up and down movement

xi) Plastids

They are double membrane organelles found in plant cells. They contain pigments and are of three types:

- Chloroplasts – They contain chlorophyll and are involved in photosynthesis, where light energy is converted to chemical energy. Chloroplasts contain compartments called stroma and grana. Grana contains structures called thylakoids that contain chlorophyll. Stroma contains enzymes needed for carbohydrate and protein synthesis.
- Chromoplasts – These give plants yellow, red or orange colours because they contain pigments like carotene.
- Leucoplasts – These are colourless plastids that store either carbohydrates (Amyloplasts), oils and fats (Elaioplasts) or proteins (Aleuroplasts).

4.2.2. Prokaryotes vs. Eukaryotes

Prokaryotes and eukaryotes are similar in some fundamental ways, reflecting their shared evolutionary ancestry. For instance, both you and the bacteria in your gut decode genes into proteins through transcription and translation. Similarly, you and your prokaryotic inhabitants both pass genetic information on to your offspring in the form of DNA. In other ways, prokaryotes and eukaryotes are quite different. That may be obvious when we are comparing humans to bacteria. It is less obvious when we are

comparing a bacterium to a yeast (which is tiny and unicellular, but eukaryotic). What actually separates these categories of organisms? The most fundamental differences between prokaryotes and eukaryotes relate to how their cells are set up. Specifically:

- Eukaryotic cells have a **nucleus**, a membrane-bound chamber where DNA is stored, while prokaryotic cells don't. This is the feature that formally separates the two groups.
- Eukaryotes usually have other membrane-bound organelles in addition to the nucleus, while prokaryotes don't.
- Cells in general are small, but prokaryotic cells are really small. Typical prokaryotic cells range from 0.20 to 2 μm in diameter, while typical eukaryotic cells range from 10 – 100 μm.

Many prokaryotic cells have sphere (called cocci), rod (called bacilli), or spiral shapes (as shown below). In the following sections, we shall walk through the structure of a prokaryotic cell, starting on the outside and moving towards the inside of the cell.

4.3 ANIMAL HABITATS

4.3.1. Aquatic Habitats:

The animals which live in water are called aquatic animals. According to the nature of the water aquatic animals may be marine or fresh-water.

i) Marine Animals:

About three fourths of the earth‟s surface is covered by the oceans. The salt water serves as the home for the marine animals who can survive neither in fresh water nor on land. The sea beach is occupied by the littoral animals, some of which live on sand, others in mud, and still others remain attached to submerged rocks or to sea weeds. The depth of the ocean is inhabited by the abyssal or deep-sea animals which are collectively known as the benthos.

The open ocean is thickly populated by the surface-dwelling pelagic animals which either swim freely or float passively and are drifted from place to place by the high waves. The pelagic fauna is thus subdivided into nekton, which includes the active swimmers, and plankton, which includes the smaller passive forms that are at the mercy of the currents. Marine animals are more or less restricted to their own respective zone either the sea beach, or the abyssal depth of the bottom, or the surface of the open sea.

ii) Fresh-Water Animals:

Fresh-water animals are found in ponds, pools, rivers, lakes and swamps. Some prefer to live in stagnant water but others choose the running stream

4.3.2. Terrestrial Habitats:

Terrestrial animals are those who live on the land. They may be simple surface dwellers or they may burrow beneath the soil and thus become sub terrestrial. The surface communities may choose to live on rocks, or plains, or desert or damp forest. The aerial animals spend a part of their time in the air, but they depend on the surface for rest. A number of animals are arboreal, homing amongst the branches of the tree.

4.3.3. Animal Associations

Some animals habitually live upon or within other animals.

There are four main types of such animal associations:

(1) Commensalism,

(2) Mutualism,

(3) Symbiosis, and

(4) Parasitism.

These four categories are absolutely man-made, which are not discontinuous in nature but are diverse aspects of the same general laws. Commensalism indicates an association between two individuals which live together with no metabolic interdependence and the relationship is not necessarily continuous for the life of the partners. Commensals may be external as well as internal. The best example of commensalism is the suckerfish Remora which develops a sucker for attaching to sharks. The Remora is thus carried from place to place and protected from enemies. They never injure their host and feed upon scraps that are rejected by the shark.

Mutualism involves an association between two animals in which there is a metabolic dependence, but it is not obligatory for existence. Association of hermit crab and a sea-anemone may be taken as example of mutualism.

Symbiosis indicates an association between two individuals in which there is metabolic dependence beneficial to both and both the partners are mutually benefited. In extreme cases, the two partners cannot live without each other. One of the best

example of symbiosis is the association between termites and a kind of small flagellates which five within their digestive canal. The termites feed on wood but are unable to digest the same; the flagellates help them by digesting the wood and thus save them from actual starvation. In return, the flagellates are safely housed, protected from enemies and supplied with food by their host.

Parasitism is an association between two individuals in which one is metabolically dependent on other and is benefited at its expense; the one benefited is the parasite. The host is always the losing partner. The protozoa causing malaria is a parasite; the host in this case is man. The parasites may live externally (ectoparasite) or internally (endoparasite).

4.4 BIOLOGICAL KINGTOM

In our daily life, we come across several animals, plants, and microbes, which have been named in order to understand their importance and to communicate with them. However, this communication about organisms becomes difficult in an area or a region where they do not occur or if they occur but are recognized by some other name. Further, organic evolution has caused a great number of biodiversity adding another problem to biologists to remember, and to identify new ones. All these factors contribute to a need for developing a system, called taxonomy. Taxonomy is the branch of science dealing with naming, Taxonomy is the branch of science dealing with naming, the grouping of organisms on the basis of the degree of similarity and arranging them in an order on the basis of their evolutionary relationship. Therefore, in other words, taxonomy is related to nomenclature, classification and phylogeny of organisms. Taxonomy unlike natural sciences such as Botany, Zoology, Physics, Chemistry, etc. is considered as a synthetic (man-made) and multidisciplinary science. It owes its progress on the advancement made in other branches of sciences like morphology, histology, physiology, cell biology, biochemistry, genetics, molecular biology, computational biology etc.

4.4.1. Nomenclature and Taxonomic Hierarchy

Carolus Linnaeus (1707-78) a Swedish botanist known as the father of taxonomy is credited for the establishment of taxonomy as a separate science. He was instrumental in framing the rules for naming the organisms, which he applied uniformly while giving his classification. It was he who popularized the binomial nomenclature that is the modern scientific way of naming organisms.

- In binomial nomenclature name of every organism is composed of two parts: first is called generic name representing the taxon – Genus to which it belongs and second is called specific epithet- Species.
- The generic name always starts with the capital letter and specific name always with the small letter.
- These scientific names are used uniformly regardless of regions/countries or languages and two different organisms cannot possess the same scientific name.
- The names of different organisms used in binomial nomenclature system must be derived from Latin or if names to be used are from different languages they must be treated as Latin.

The nomenclature of organisms is governed by a set of rules framed by International Codes of Nomenclature. There are different codes of nomenclature for different groups of organisms for example, naming of bacteria, animals and plants are governed by International Code for Nomenclature for Bacteria (ICNB), International Code of Zoological Nomenclature (ICZN) and International Code of Botanical Nomenclature (ICBN) respectively. The scientific name of an organism, when cited in any text, is always mentioned as in italics or underlined font style. The name of the author who first gave the correct name as per rules is written in abbreviated form after the specific name and is written in Roman.

4.4.2. The Current System

As scientists learn more about organisms, classification systems change. Genetic sequencing has given researchers a whole new way of analyzing relationships between organisms. The current Three Domain System groups organisms primarily based on differences in ribosomal RNA (rRNA) structure. Ribosomal RNA is a molecular building block for ribosomes.

Under this system, organisms are classified into three domains and six kingdoms. The domains are

- Archaea
- Bacteria
- Eukarya

The kingdoms are

- Archaebacteria (ancient bacteria)
- Eubacteria (true bacteria)
- Protista
- Fungi
- Plantae
- Animalia

i) Archaea Domain

This Archaea domain contains single-celled organisms. Archaea have genes that are similar to both bacteria and eukaryotes. Because they are very similar to bacteria in appearance, they were originally mistaken for bacteria. Like bacteria, archaea are prokaryotic organisms and do not have a membrane-bound nucleus. They also lack internal cell organelles and many are about the same size as and similar in shape to bacteria. Archaea reproduce by binary fission, have one circular chromosome, and use flagella to move around in their environment as do bacteria.

Archaea differ from bacteria in cell wall composition and differ from both bacteria and eukaryotes in membrane composition and rRNA type. These differences are substantial enough to warrant that archaea have a separate domain. Archaea are extreme organisms that live under some of the most extreme environmental conditions. This includes within hydrothermal vents, acidic springs, and under Arctic ice. Archaea are divided into three main phyla: Crenarchaeota, Euryarchaeota, and Korarchaeota.

- Crenarchaeota include many organisms that are hyperthermophiles and thermoacidophiles. These archaea thrive in environments with great temperature extremes (hyperthermophiles) and in extremely hot and acidic environments (thermoacidophiles.)
- Archaea known as methanogens are of the Euryarchaeota phylum. They produce methane as a byproduct of metabolism and require an oxygen-free environment.
- Little is known about Korarchaeota archaea as few species have been found living in places such as hot springs, hydrothermal vents, and obsidian pools.

ii) Bacteria Domain

Bacteria are classified under the Bacteria Domain. These organisms are generally feared because some are pathogenic and capable of causing disease. However, bacteria are essential to life as some are part of the human microbiota. These bacteria preform vital functions, such as enabling us to properly digest and absorb nutrients from the foods we eat. Bacteria that live on the skin prevent pathogenic microbes from colonizing the area and also aid in the activation of the immune system.

Bacteria are also important for the recycling of nutrients in the global ecosystem as they are primary decomposers. Bacteria have a unique cell wall composition and rRNA type. They are grouped into five main categories:

- **Proteobacteria:** This phylum contains the largest group of bacteria and includes E.coli, Salmonella, Heliobacter pylori, and Vibrio. bacteria.
- **Cyanobacteria:** These bacteria are capable of photosynthesis. They are also known as blue-green algae because of their color.
- **Firmicutes:** These gram-positive bacteria include Clostridium, Bacillus, and mycoplasmas (bacteria without cell walls.)
- **Chlamydiae:** These parasitic bacteria reproduce inside their host's cells. Organisms include Chlamydia trachomatis (causes chlamydia STD) and Chlamydophila pneumoniae (causes pneumonia.)
- **Spirochetes:** These corkscrew-shaped bacteria exhibit a unique twisting motion. Examples include Borrelia burgdorferi (cause Lyme disease) and Treponema pallidum (cause syphilis.)

Domain Bacteria contains 5 major groups: proteobacteria, chlamydias, spirochetes, cyanobacteria, and gram-positive bacteria. The proteobacteria are subdivided into five groups, alpha through epsilon. Species in these groups have a wide range of lifestyles. Some are symbiotic with plants, others live in hot vents deep under the sea, and others yet cause human diseases, such as stomach ulcers (Helicobacter pylori) and food poisoning (Salmonella).

The other four major groups of bacteria are similarly diverse. Chlamydias are pathogens that live inside host cells, while cyanobacteria are photosynthesizers that make much of Earth's oxygen. Spirochetes include both harmless bacteria and harmful

ones, like the Borrelia burgdorferi that cause Lyme disease. The same is true of Gram-positive bacteria, which range from probiotic bacteria in yogurt to the Bacillus anthracis that cause anthrax.

Characteristics of the five phyla of bacteria are described. The first phylum described is proteobacteria, which includes five classes, alpha, beta, gamma, delta and epsilon. Most species of Alpha Proteobacteria are photoautotrophic but some are symbionts of plants and animals, and others are pathogens.

Eukaryotic mitochondria are thought be derived from bacteria in this group. Representative species include Rhizobium, a nitrogen-fixing endosymbiont associated with the roots of legumes, and Rickettsia, obligate intracellular parasite that causes typhus and Rocky Mountain Spotted Fever (but not rickets, which is caused by Vitamin D deficiency).

Beta Proteobacteria is a diverse group of bacteria. Some species play an important role in the nitrogen cycle. Representative species include Nitrosomonas, which oxidize ammonia into nitrate, and Spirillumminus, which causes rat bite fever.

Gamma Proteobacteria include many are beneficial symbionts that populate the human gut, as well as familiar human pathogens. Some species from this subgroup oxidize sulfur compounds. Representative species include Escherichiacoli, normally beneficial microbe of the human gut, but some strains cause disease; Salmonella, certain strains of which cause food poisoning, and typhoid fever; Yersiniapestis–the causative agent of Bubonic plague; Psuedomonasaeruganosa– causes lung infections; Vibriocholera, the causative agent of cholera, and Chromatium–sulfur producing bacteria that oxidize sulfur, producing H_2S.

Some species of delta Proteobacteria generate a spore-forming fruiting body in adverse conditions. Others reduce sulfate and sulfur. Representative species include Myxobacteria, which generate spore-forming fruiting bodies in adverse conditions and Desulfovibriovulgaris, an anaerobic, sulfur-reducing bacterium.

Epsilon Proteobacteria includes many species that inhabit the digestive tract of animals as symbionts or pathogens. Bacteria from this group have been found in deep-sea hydrothermal vents and cold seep habitats.

The next phylum described is chlamydias. All members of this group are obligate intracellular parasites of animal cells. Cells walls lack peptidoglycan. Chlamydia infection is the most common sexually transmitted disease and can lead to blindness.

All members of the phylum Spirochetes have spiral-shaped cells. Most are free-living anaerobes, but some are pathogenic. Flagella run lengthwise in the periplasmic space between the inner and outer membrane. Representative species include Treponemapallidum, the causative agent of syphilis and Borreliaburgdorferi, the causative agent of Lyme disease.

Bacteria in the phylum Cyanobacteria, also known as blue-green algae, obtain their energy through photosynthesis. They are ubiquitous, found in terrestrial, marine, and freshwater environments. Eukaryotic chloroplasts are thought be derived from bacteria in this group. The Cyanobacteriumprochlorococcus is believed to be the most abundant photosynthetic organism on earth, responsible for generating half the world‟s oxygen.

Gram-positive Bacteria have a thick cell wall and lack an outer membrane. Soil-dwelling members of this subgroup decompose organic matter. Some species cause disease. Representative species include Bacillusanthracis, which causes anthrax; Clostridiumbotulinum, which causes botulism; Clostridiumdifficile, which causes diarrhea during antibiotic therapy; Streptomyces, from which many antibiotics, including streptomyocin, are derived; and Mycoplasmas, the smallest known bacteria, which lack a cell wall. Some are free-living, and some are pathogenic.

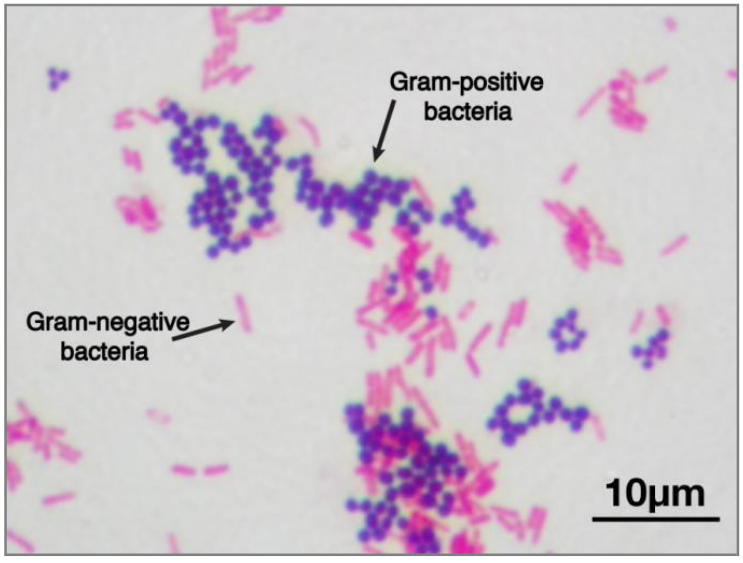


Fig. 4.9. A gram stain depicting both, gram-positive bacteria (violet) and gram-negative bacteria (pink).

Although most bacterial cell walls contain peptidoglycan, they can differ in other ways. In fact, these differences are used to classify bacteria using a technique called the **Gram stain** shown as in Fig. 4.2. Developed by the 19th-century Danish physician Christian Gram, this technique categorizes strains of bacteria into one of two groups: **gram-positive** or **gram-negative**.

The staining process involves applying two dyes to a sample of bacteria. First, a violet dye and iodine are applied, which bind together and form a bulky molecule in the cell walls of the bacteria. Alcohol is then used to wash the sample, removing leftover or loosely bound violet dye-iodine complexes, and a red dye is applied to the sample.

The structure of the cell wall of the bacteria determines which dye is visible at the end of the procedure. Gram-positive bacteria have a thick cell wall of peptidoglycan, which traps the bulky violet dye-iodine molecules. (The red dye also binds, but the violet dye covers it up.) Gram-negative bacteria, on the other hand, have a thin layer of peptidoglycan and an additional outer membrane (see image below). The thin peptidoglycan layer only retains the red dye, giving gram-negative bacteria a pinkish hue.

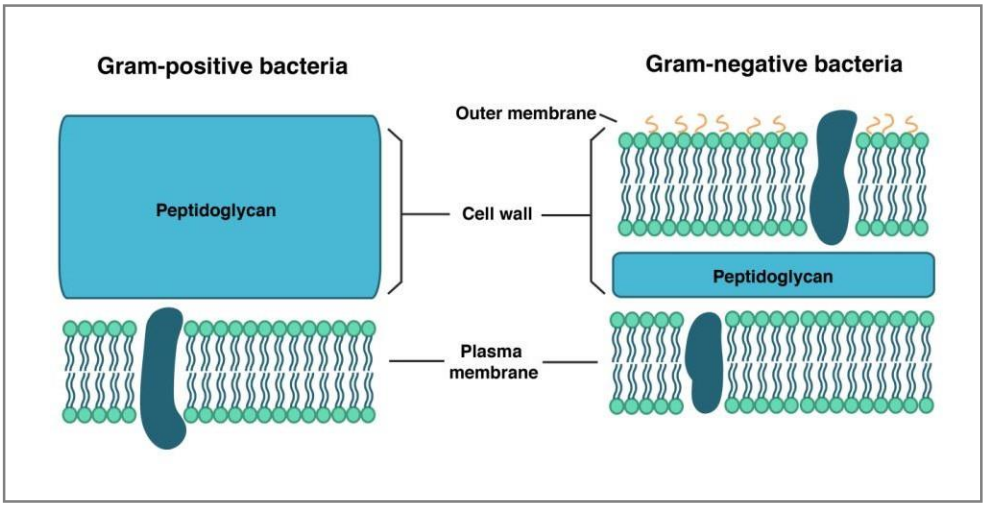
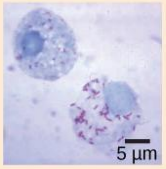
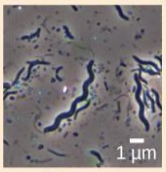
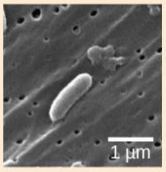
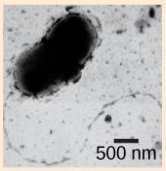
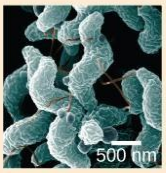


Fig.4.10. Gram-positive bacteria and Gram-negative bacteria

Fig.4.10. shows the Gram-positive bacteria have an inner plasma membrane and a thick cell wall composed of peptidoglycan. Gram-negative bacteria have an inner plasma membrane and a thin cell wall composed of peptidoglycan and an outer membrane.

Table 4.2. Bacteria of Phylum Proteobacteria

Bacteria of Phylum Proteobacteria		
Class	**Representative organisms**	**Representative micrograph**
Alpha Proteobacteria Some species are photoautotrophic but some are symbionts of plants and animals and others are pathogens. Eukaryotic mitochondria are thought be derived from bacteria in this group.	*Rhizobium* Nitrogen-fixing endosymbiont associated with the roots of legumes *Rickettsia* Obligate intracellular parasite that causes typhus and Rocky Mountain Spotted Fever (but not rickets, which is caused by Vitamin D deficiency)	 *Rickettsia rickettsia,* stained red, grow inside a host cell.
Beta Proteobacteria This group of bacteria is diverse. Some species play an important role in the nitrogen cycle.	*Nitrosomas* Species from this group oxidize ammonia into nitrite. *Spirillum minus* Causes rat-bite fever	 *Spirillum minus*
Gamma Proteobacteria Many are beneficial symbionts that populate the human gut, but others are familiar human pathogens. Some species from this subgroup oxidize sulfur compounds.	*Escherichia coli* Normally beneficial microbe of the human gut, but some strains cause disease *Salmonella* Certain strains cause food poisoning or typhoid fever *Yersinia pestis* Causative agent of Bubonic plague *Psuedomonas aeruginosa* Causes lung infections *Vibrio cholera* Causative agent of cholera *Chromatium* Sulfur-producing bacteria that oxidize sulfur, producing H_2S	 *Vibrio cholera*
Delta Proteobacteria Some species generate a spore-forming fruiting body in adverse conditions. Others reduce sulfate and sulfur.	*Myxobacteria* Generate spore-forming fruiting bodies in adverse conditions *Desulfovibrio vulgaris* Aneorobic, sulfate-reducing bacterium	 *Desulfovibrio vulgaris*
Epsilon Proteobacteria Many species inhabit the digestive tract of animals as symbionts or pathogens. Bacteria from this group have been found in deep-sea hydrothermal vents and cold seep habitats.	*Campylobacter* Causes blood poisoning and intestinal inflammation *Heliobacter pylori* Causes stomach ulcers	 *Campylobacter*

iii) Eukarya Domain

The Eukarya domain includes eukaryotes or organisms that have a membrane-bound nucleus.This domain is further subdivided into the kingdoms

- Protista
- Fungi
- Plantae
- Animalia

Eukaryotes have rRNA that is distinct from bacteria and archaeans. Plant and fungiorganisms contain cell walls that are different in composition than bacteria. Eukaryotic cells are typically resistant to antibacterial antibiotics. Organisms in this domain include protists, fungi, plants, and animals. Examples include algae, amoeba, fungi, molds, yeast, ferns, mosses, flowering plants, sponges, insects, and mammals.

Table.4.3. Difference between Bacteria, Archaea and Eukarya

Character	Bacteria	Archaea	Eukarya
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
Cell wall	Present; contain peptidoglycan	Present; peptidoglycan absent	Present/absent; peptidoglycan absent
Membrane lipids	Diacyl glycerol diesters	isoprenoid glycerol diethers or diglycerol tetraethers	Glycerol fattyacyl diesters
Genetic material	Small circular DNA not associated with histones	Small circular DNA associated with histones like proteins	Large linear DNA associated with histones
Translation (first amino acid)	Formylmethionine	Methionine	Methionine

RNA polymerase	One; simple	One; complex	One; complex
tRNA (TψC arm)	Thymine present	Thymine absent	Thymine present
Intron	Absent	Present rarely	Present
Antibiotic sensitivity	Yes	No	No
Diphtheria toxin sensitivity	No	Yes	Yes
Reproduction	Spore formation present	Spore formation absent	Spore formation present or absent
Habit	Variable	Extremophile	Variable

4.5. Microorganism classification

4.5.1 Introduction

Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists. The most important level of this type of classification is the species level.A species name should mean the same thing to everyone. Within one species, strains and subgroups can differ by the disease they produce, their environmental habitat, and many other characteristics. Formerly, species were created on the basis of such criteria, which may be extremely important for clinical microbiologists and physicians but which are not a sufficient basis for establishing a species. Verification of existing species and creation of new species should involve biochemical and other phenotypic criteria as well as DNA relatedness. In numerical or phenetic approaches to classification, strains are grouped on the basis of a large number of phenotypic characteristics. DNA relatedness is used to group strains on the basis of overall genetic similarity.

Species are identified in the clinical laboratory by morphological traits and biochemical tests, some of which are supplemented by serologic assessments (e.g., identification of Salmonella and Shigella species). Because of differences in pathogenicity (Escherichia coli) or the necessity to characterize a disease outbreak (Vibrio cholerae, methicillin-resistant Staphylococcus aureus), strains of medical interest are often classified below the species level by serology or identification of toxins. Pathogenic or epidemic strains also can be classified by the presence of a

specific plasmid, by their plasmid profile (the number and sizes of plasmids), or by bacteriophage susceptibility patterns (phage typing). Newer molecular biologic techniques have enabled scientists to identify some species and strains (without the use of biochemical tests) by identifying a specific gene or genetic sequence, sometimes directly from the clinical specimen.

Laboratories have no difficulty in identifying typical strains of common bacteria using commonly available test systems. Problems do arise, however, when atypical strains or rare or newly described species are not in the data base. Such difficulties are compounded when the strains are misidentified rather than unidentified, and so laboratory personnel and physicians (at least infectious diseases specialists) should be familiar with taxonomic reference texts and journals that publish papers on new species. Bacterial nomenclature at the genus and species level changes is based primarily on the use of newer genetic techniques. A species may acquire more than one name. In some cases the recognition of a new species results in a unique correlation with specific clinical problems. For example, recognition of Porphyromonasgingivalis as a unique species, separate from its previous inclusion within Bacteroides melaninogenicus (now known to be composed of several taxonomic groups of black-pigmenting anaerobic gram-negative bacilli), elucidated its role as a key pathogen in adult periodontitis. It is important to understand why these changes and synonyms exist in taxonomy.

The clinical laboratory is concerned with the rapid, sensitive, and accurate identification of microbes involved in producing disease. The number and types of tests done in such a laboratory depend on its size and the population it serves. Highly specialized or rarely performed tests should be done only by reference laboratories. Physicians, clinical laboratory personnel, and reference laboratory personnel must have a good working relationship if patients are to receive first-rate care.In addition, the physician and the clinical laboratory personnel must know which diseases and isolates are reportable to public health laboratories and how to report them.

4.5.2 Taxonomy

Taxonomy is the science of classification, identification, and nomenclature. For classification purposes, organisms are usually organized into subspecies, species, genera, families, and higher orders. For eukaryotes, the definition of the species usually stresses the ability of similar organisms to reproduce sexually with the formation of a zygote and to produce fertile offspring. However, bacteria do not undergo sexual reproduction in the eukaryotic sense. Other criteria are used for their classification.

Classification

Classification is the orderly arrangement of bacteria into groups. There is nothing inherently scientific about classification, and different groups of scientists may classify the same organisms differently. For example, clinical microbiologists are interested in the serotype, antimicrobial resistance pattern, and toxin and invasiveness factors in Escherichia coli, whereas geneticists are concerned with specific mutations and plasmids.

Identification

Identification is the practical use of classification criteria to distinguish certain organisms from others, to verify the authenticity or utility of a strain or a particular reaction, or to isolate and identify the organism that causes a disease.

Nomenclature

Nomenclature (naming) is the means by which the characteristics of a species are defined and communicated among microbiologists. A species name should mean the same thing to all microbiologists, yet some definitions vary in different countries or microbiologic specialty groups. For example, the organism known as Clostridium perfringens in the United States is called Clostridium welchii in England.

Species

A bacterial species is a distinct organism with certain characteristic features, or a group of organisms that resemble one another closely in the most important features of their organization. In the past, unfortunately, there was little agreement about these criteria or about the number of features necessary to distinguish a species. Species were often defined solely by such criteria as host range, pathogenicity, or ability to produce gas during the fermentation of a given sugar. Without a universal consensus, criteria reflected the interests of the investigators who described a particular species. For example, bacteria that caused plant diseases were often defined by the plant from which they were isolated; also, each new Salmonella serotype that was discovered was given species status. These practices have been replaced by generally accepted genetic criteria that can be used to define species in all groups of bacteria.

4.5.3. Approaches to Taxonomy

i) Numerical Approach

Classification and identification of an organism should be based on its overall morphologic and biochemical pattern. A single characteristic (pathogenicity, host range,

or biochemical reaction), regardless of its importance, is not a sufficient basis for classifying or identifying an organism.A large and diverse strain sample must be tested to determine accurately the biochemical characteristics used to distinguish a given species.Atypical strains often are perfectly typical members of a given biogroup within an existing species, but sometimes they are typical members of an unrecognized new species.

In numerical taxonomy (also called computer or phenetic taxonomy) many (50 to 200) biochemical, morphological, and cultural characteristics, as well as susceptibilities to antibiotics and inorganic compounds, are used to determine the degree of similarity between organisms. In numerical studies, investigators often calculate the coefficient of similarity or percentage of similarity between strains (where strain indicates a single isolate from a specimen). A dendrogram or a similarity matrix is constructed that joins individual strains into groups and places one group with other groups on the basis of their percentage of similarity.

In some cases, certain characteristics may be weighted more heavily; for example, the presence of spores in Clostridium might be weighted more heavily than the organism's ability to use a specific carbon source. A given level of similarity can be equated with relatedness at the genus, species, and, sometimes, subspecies levels. For instance, strains of a given species may cluster at a 90% similarity level, species within a given genus may cluster at the 70 percent level, and different genera in the same family may cluster at the 50 percent or lower level. When this approach is the only basis for defining a species, it is difficult to know how many and which tests should be chosen; whether and how the tests should be weighted; and what level of similarity should be chosen to reflect relatedness at the genus and species levels.

Most bacteria have enough DNA to specify some 1,500 to 6,000 average-sized genes. Therefore, even a battery of 300 tests would assay only 5 to 20 percent of the genetic potential of a bacterium. Tests that are comparatively simple to conduct (such as those for carbohydrate utilization and for enzymes, presence of which can be assayed colorimetrically) are performed more often than tests for structural, reproductive, and regulatory genes, presence of which is difficult to assay. Thus, major differences may go undetected.

Other types of errors may occur when species are classified solely on the basis of phenotype. For example, different enzymes (specified by different genes) may catalyze the same reaction. Also, even if a metabolic gene is functional, negative reactions can occur because of the inability of the substrate to enter the cell, because of

a mutation in a regulatory gene, or by production of an inactive protein. There is not necessarily a one-to-one correlation between a reaction and the number of genes needed to carry out that reaction. For instance, six enzymatic steps may be involved in a given pathway. If an assay for the end product is performed, a positive reaction indicates the presence of all six enzymes, whereas a negative reaction can mean the absence or nonfunction of one to six enzymes. Several other strain characteristics can affect phenotypic characterization; these include growth rate, incubation temperature, salt requirement, and pH. Plasmids that carry metabolic genes can enable strains to carry out reactions atypical for strains of that species.

The same set of "definitive" reactions cannot be used to classify all groups of organisms, and there is no standard number of specific reactions that allows identification of a species. Organisms are identified on the basis of phenotype, but, from the taxonomic standpoint, definition of species solely on this basis is subject to error.

ii) Phylogenetic Approach

The ideal means of identifying and classifying bacteria would be to compare each gene sequence in a given strain with the gene sequences for every known species. This cannot be done, but the total DNA of one organism can be compared with that of any other organism by a method called nucleic acid hybridization or DNA hybridization. This method can be used to measure the number of DNA sequences that any two organisms have in common and to estimate the percentage of divergence within DNA sequences that are related but not identical. DNA relatedness studies have been done for yeasts, viruses, bacteriophages, and many groups of bacteria.

Five factors can be used to determine DNA relatedness: genome size, guanine-plus-cytosine (G+C) content, DNA relatedness under conditions optimal for DNA reassociation, thermal stability of related DNA sequences, and DNA relatedness under conditions supraoptimal for DNA reassociation. Because it is not practical to conduct these genotypic or phylogenetic evaluations in clinical laboratories, the results of simpler tests usually must be correlated with known phylogenetic data. For example, yellow strains of Enterobacter cloacae were shown, by DNA relatedness, to form a separate species, Enterobacter sakazakii, but were not designated as such until results of practical tests were correlated with the DNA data to allow routine laboratories to identify the new species.

a) Genome Size

True bacterial DNAs have genome sizes (measured as molecular weight) between 1×10^9 and 8×10^9. Genome size determinations sometimes can distinguish between groups. They were used to distinguish Legionella pneumophila (the legionnaire's disease bacterium) from Bartonella (Rickettsia) quintana, the agent of trench fever. L. pneumophila has a genome size of about 3×10^9; that of B. quintana is about 1×10^9.

b) Guanine-plus-Cytosine Content

The G+C content in bacterial DNA ranges from about 25 to 75 percent. This percentage is specific, but not exclusive, for a species; two strains with a similar G+C content may or may not belong to the same species. If the G+C contents are very different, however, the strains cannot be members of the same species.

c) DNA Relatedness under Conditions Optimal for DNA Reassociation

DNA relatedness is determined by allowing single-stranded DNA from one strain to reassociate with single-stranded DNA from a second strain, to form a double-stranded DNA molecule (Figure 3-2). This is a specific, temperature-dependent reaction. The optimal temperature for DNA reassociation is 25 to 30°C below the temperature at which native double-stranded DNA denatures into single strands. Many studies indicate that a bacterial species is composed of strains that are 70 to 100 percent related. In contrast, relatedness between different species is 0 to about 65 percent. It is important to emphasize that the term “related” does not mean “identical” or “homologous.” Similar but nonidentical nucleic acid sequences can reassociate.

d)Thermal Stability of Related DNA Sequences

Each 1 percent of unpaired nucleotide bases in a double-stranded DNA sequence causes a 1 percent decrease in the thermal stability of that DNA duplex. Therefore, a comparison between the thermal stability of a control double-stranded molecule (in which both strands of DNA are from the same organism) and that of a heteroduplex (DNA strands from two different organisms) allows assessment of divergence between related nucleotide sequences.

e) DNA Relatedness under Supraoptimal Conditions for DNA Reassociation

When the incubation temperature used for DNA reassociation is raised from 25-30° C below the denaturation temperature to only 10-15° C below the denaturation

temperature, only very closely related (and therefore highly thermally stable) DNA sequences can reassociate. Strains from the same species are 60 percent or more related at these supraoptimal incubation temperatures.

f) Defining Species on the Basis of DNA Relatedness

Use of these five factors allows a species definition based on DNA. Thus, E. coli can be defined as a series of strains with a G+C content of 49 to 52 moles percent, a genome molecular weight of 2.3×10^9 to 3.0×10^9, relatedness of 70 percent or more at an optimal reassociation temperature with 0 to 4 percent divergence in related sequences, and relatedness of 60 percent or more at a supraoptimal reassociation temperature. Experience with more than 300 species has produced an arbitrary phylogenetic definition of a species to which most taxonomists subscribe: "strains with approximately 70% or greater DNA-DNA relatedness and with 5° C or less divergence in related sequences." When these two criteria are met, genome size and G+C content are always similar, and relatedness is almost always 60 percent or more at supraoptimal incubation temperatures. The 70 percent species relatedness rule has been ignored occasionally when the existing nomenclature is deeply ingrained, as is that for E. coli and the four Shigella species. Because these organisms are all 70 percent or more related, DNA studies indicate that they should be grouped into a single species, instead of the present five species in two genera. This change has not been made because of the presumed confusion that would result.

DNA relatedness provides one species definition that can be applied equally to all organisms. Moreover, it cannot be affected by phenotypic variation, mutations, or the presence or absence of metabolic or other plasmids. It measures overall relatedness, and these factors affect only a very small percentage of the total DNA.

iii) Polyphasic Approach

In practice, the approach to bacterial taxonomy should be polyphasic. The first step is phenotypic grouping of strains by morphological, biochemical and any other characteristics of interest. The phenotypic groups are then tested for DNA relatedness to determine whether the observed phenotypic homogeneity (or heterogeneity) is reflected by phylogenetic homogeneity or heterogeneity. The third and most important step is reexamination of the biochemical characteristics of the DNA relatedness groups. This allows determination of the biochemical borders of each group and determination of reactions of diagnostic value for the group. For identification of a given organism, the

importance of specific tests is weighted on the basis of correlation with DNA results. Occasionally, the reactions commonly used will not distinguish completely between two distinct DNA relatedness groups. In these cases, other biochemical tests of diagnostic value must be sought.

Morphologic Characteristics

Both wet-mounted and properly stained bacterial cell suspensions can yield a great deal of information. These simple tests can indicate the Gram reaction of the organism; whether it is acid-fast; its motility; the arrangement of its flagella; the presence of spores, capsules, and inclusion bodies; and, of course, its shape. This information often can allow identification of an organism to the genus level, or can minimize the possibility that it belongs to one or another group. Colony characteristics and pigmentation are also quite helpful. For example, colonies of several Porphyromonas species autofluoresce under long-wavelength ultraviolet light, and Proteus species swarm on appropriate media.

Growth Characteristics

A primary distinguishing characteristic is whether an organism grows aerobically, anaerobically, facultatively (i.e., in either the presence or absence of oxygen), or microaerobically (i.e., in the presence of a less than atmospheric partial pressure of oxygen). The proper atmospheric conditions are essential for isolating and identifying bacteria. Other important growth assessments include the incubation temperature, pH, nutrients required, and resistance to antibiotics. For example, one diarrheal disease agent, Campylobacter jejuni, grows well at 42° C in the presence of several antibiotics; another, Y. enterocolitica, grows better than most other bacteria at 4° C. Legionella, Haemophilus, and some other pathogens require specific growth factors, whereas E. coli and most other Enterobacteriaceae can grow on minimal media.

Antigens and Phage Susceptibility

Cell wall (O), flagellar (H), and capsular (K) antigens are used to aid in classifying certain organisms at the species level, to serotype strains of medically important species for epidemiologic purposes, or to identify serotypes of public health importance. Serotyping is also sometimes used to distinguish strains of exceptional virulence or public health importance, for example with V. cholerae (pandemic strain) and E. coli (enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteropathogenic serotypes).

Phage typing (determining the susceptibility pattern of an isolate to a set of specific bacteriophages) has been used primarily as an aid in epidemiologic surveillance of diseases caused by Staphylococcus aureus, mycobacteria, P, aeruginosa, V. cholerae, and S. typhi. Susceptibility to bacteriocins has also been used as an epidemiologic strain marker. In most cases recently, phage and bacteriocin typing have been supplanted by molecular methods.

Biochemical Characteristics

Most bacteria are identified and classified largely on the basis of their reactions in a series of biochemical tests. Some tests are used routinely for many groups of bacteria (oxidase, nitrate reduction, amino acid degrading enzymes, fermentation or utilization of carbohydrates); others are restricted to a single family, genus, or species (coagulase test for staphylococci, pyrrolidonylarylamidase test for Gram-positive cocci).

Both the number of tests needed and the actual tests used for identification vary from one group of organisms to another. Therefore, the lengths to which a laboratory should go in detecting and identifying organisms must be decided in each laboratory on the basis of its function, the type of population it serves, and its resources. Clinical laboratories today base the extent of their work on the clinical relevance of an isolate to the particular patient from which it originated, the public health significance of complete identification, and the overall cost-benefit analysis of their procedures. For example, the Centers for Disease Control and Prevention (CDC) reference laboratory uses at least 46 tests to identify members of the Enterobacteriaceae, whereas most clinical laboratories, using commercial identification kits or simple rapid tests, identify isolates with far fewer criteria.

4.5.4. Hazards of Clinical Laboratory Work

Clinical laboratory personnel, including support and clerical employees, are subject to the risk of infection, chemical hazards, and, in some laboratories, radioactive contamination. Such risks can be prevented or minimized by a laboratory safety program.

i) Radiation Hazards

Personnel who work with radioactive materials should have taken a radioactivity safety course; they should wear radiation monitor badges and be aware of the methods for decontaminating hands, clothing, work surfaces, and equipment. They should wear

gloves when working with radioactive compounds. When they work with high-level radiation, they should use a hood and stand behind a radiation shield. Preparative radioactive work should be done in a separate room with access only by personnel who are involved directly in the work.

ii) Chemical Hazards

Chemicals can harm laboratory personnel through inhalation or skin absorption of volatile compounds; bodily contact with carcinogens, acids, bases, and other harmful chemicals; or introduction of poisonous or skin-damaging liquids into the mouth. Good laboratory practices require that volatile compounds be handled only under a hood, that hazardous chemicals never be pipetted by mouth, and that anyone working with skin-damaging chemicals wear gloves, eye guards, and other personal protective equipment as necessary. Workers should be familiar with the materials safety data sheets (MSDS) posted in an accessible place in every laboratory. These forms contain information about chemical hazards and procedures for decontamination should an accident occur.

iii) Biologic Hazards

Microbiologic contamination is the greatest hazard in clinical microbiology laboratories. Laboratory infections are a danger not only to the clinical laboratory personnel but also to anyone else who enters the laboratory, including janitors, clerical and maintenance personnel, and visitors. The risk of infection is governed by the frequency and length of contact with the infectious agent, its virulence, the dose and route of administration, and the susceptibility of the host. The inherent hazard of any infectious agent is affected by factors such as the volume of infectious material used, handling of the material, effectiveness of safety containment equipment, and soundness of laboratory methods. Body fluids from patients, particularly those containing blood, are considered potentially infectious for blood-borne pathogens, and must be handled appropriately.

If possible, agents that are treated differently, such as viruses as opposed to bacteria, or M. tuberculosis in contrast to E. coli, should be handled in different laboratories or in different parts of the same laboratory. When the risk category of an agent is known, it should be handled in an area with appropriate containment. All specimens sent for microbiological studies and all organisms sent to the laboratory for identification should be assumed to be potentially infectious. A separate area should be set aside for the receipt of specimens. Personnel should be aware of the potential

hazards of improperly packed, broken, or leaking packages and of the proper methods for their handling and decontamination .

To prevent infection, personnel should wear moisture-proof laboratory coats at all times, wash their hands before and after wearing gloves and at the conclusion of each potential exposure to etiologic agents, refrain from mouth pipetting, and not eat, drink, smoke, or apply cosmetics in the laboratory. Immunization may be appropriate for employees who are exposed often to certain infectious agents, including hepatitis B, yellow fever, rabies, polioviruses, meningococci, Y.pestis, S.typhi, and Francisellatularensis. Universal precautions, body substance isolation, and other mandated practices involve the use of personal protective equipment and engineering controls to minimize laboratory scientists' exposure to blood-borne pathogens, even when the risk of infection is unknown.

4.5.5 Biosafety Levels

Infectious agents are assigned to a biosafety level from 1 to 4 on the basis of their virulence. The containment levels for organisms should correlate with the biosafety level assigned. Biosafety level 1 is for well-defined organisms not known to cause disease in healthy humans; it includes certain nonvirulent E. coli strains (such as K-12) and B. subtilis. Containment level 1 involves standard microbiologic practices, and safety equipment is not needed.

Biosafety level 2, the minimum level for clinical laboratories, is for moderate-risk agents associated with human disease. Containment level 2 includes limited access to the work area, decontamination of all infectious wastes, use of protective gloves, and a biologic safety cabinet for use in procedures that may create aerosols. Examples of biosafety level 2 agents include nematode, protozoan, trematode, and cestode human parasites; all human fungal pathogens except Coccidioides immitis; all members of the Enterobacteriaceae except Y. pestis; Bacillus anthracis; Clostridium tetani; Corynebacterium diphtheriae; Haemophilus species; leptospires; legionellae; mycobacteria other than M. tuberculosis; pathogenic Neisseria species; staphylococci, streptococci, Treponema pallidum; V. cholerae; and hepatitis and influenza viruses. Clinical specimens potentially containing some biosafety level 3 agents, such as Brucella spp., are usually handled using biosafety level 2 containment practices.

Biosafety level 3 is for agents that are associated with risk of serious or fatal aerosol infection. In containment level 3, laboratory access is controlled, special

clothing is worn in the laboratory, and containment equipment is used for all work with the agent. M. tuberculosis, Coccidioides immitis, Coxiella burnetii, and many of the arboviruses are biosafety 3 level agents. Containment level 3 usually is recommended for work with cultures of rickettsiae, brucellae, Y. pestis, and a wide variety of viruses, including human immunodeficiency viruses.

Biosafety level 4 indicates dangerous and novel agents that cause diseases with high fatality rates. Maximum containment and decontamination procedures are used in containment level 4, which is found in only a few reference and research laboratories. Only a few viruses (including Lassa, Ebola, and Marburg viruses) are classified in biosafety level 4

4.6. INDUSTRIAL MICROBIOLOGY

Industrial microbiology includes the use of microorganisms to manufacture food or industrial products in large quantities. Numerous microorganisms are used within industrial microbiology; these include naturally occurring organisms, laboratory selected mutants, or even genetically modified organisms (GMOs). Currently, the debate in the use of genetically modified organisms (GMOs) in food sources is gaining both momentum, with more and more supporters on both sides. However, the use of microorganisms at an industrial level is deeply rooted into today‟s society. The following is a brief overview of the various microorganisms that have industrial uses, and of the roles they play.

Archaea are specific types of prokaryotic microbes that exhibit the ability to sustain populations in unusual and typically harsh environments. Those suriving in the most hostile and extreme settings are known as extremophilearchaea. The isolation and identification of various types of Archaea, particularly the extremophile archaea, have allowed for analysis of their metabolic processes, which have then been manipulated and utilized for industrial purposes.

Extremophile archaea species are of particular interest due to the enzymes and molecules they produce that allow them to sustain life in extreme climates, including very high or low temperatures, extremely acid or base solutions, or when exposed to other harmful factors, including radiation. Specific enzymes which have been isolated and used for industrial purposes include thermostable DNA polymerases from the Pyrococcusfuriosus. This type of polymerase isa common tool in molecular biology; it is capable of withstanding the high temperatures that are necessary to complete

polymerase chain reactions. Additional enzymes isolated from Pyrococcusspecies include specific types of amylases and galactosidases which allow food processing to occur at high temperatures as well.

Corynebacteria are characterized by their diverse origins. They are found in numerous ecological niches and are most often used in industry for the mass production of amino acids and nutritional factors. In particular, the amino acids produced by Corynebacterium glutamicum include the amino acid glutamic acid. Glutamic acid is used as a common additive in food production, where it is known as monosodium glutamate (MSG). Corynebacterium can also be used in steroid conversion and in the degradation of hydrocarbons. Steroid conversion is an important process in the development of pharmaceuticals. Degradation of hydrocarbons is key in the breakdown and elimination of environmental toxins. Items such as plastics and oils are hydrocarbons; the use of microorganisms which exhibit the ability to breakdown these compounds is critical for environmental protection.

Corynebacterium: Corynebacterium species are often used to mass produce amino acids utilized in food processing.Xanthomonas, a type of Proteobacteria, is known for its ability to cause disease in plants. The bacterial species which are classified under Xanthomonas exhibit the ability to produce the acidic exopolysaccharide commonly marketed as xanthan gum, used as a thickening and stabilizing agent in foods and in cosmetic ingredients to prevent separation.

Another type of microorganism utilized by industry includes various species of Aspergillus. Thisgenusincludes several hundred types of mold. Aspergillus has become a key component in industrial microbiology, where it is used in the production of alcoholic beverages and pharmaceutical development. Aspergillus nigeris most commonly used to produce citric acid, which is used in numerous products ranging from household cleaners, pharmaceuticals, foods, cosmetics, photography and construction. Aspergillus is also commonly used in large-scale fermentation in the production of alcoholic beverages such as Japanese sake.

4.6.1. Applications of Microbes

i) In Household Products

You must have seen people add a small amount of curd to fresh milk. In time, this milk at suitable temperature then turns into curd! How does this happen? This is due to the action of microorganisms called Lactic Acid Bacteria (LAB).The small

amount of curd added as a starter culture to milk contains millions of these bacteria, which multiply at suitable temperatures turning milk into curd. LAB also improve the nutritional value of milk by increasing vitamin B_{12}. They also play a significant role in keeping a check on disease-causing microbes in our gut.

Other uses of microorganisms in our household products include the dough used to make idli or dosa. Similarly, the dough used to make bread is fermented using baker‟s yeast (Saccharomyces cerevisiae). The process of fermentation produces CO_2 which gives the bread the puffed-up appearance. Toddy (a drink in southern India) is also the result of microbial activity on sap from palms.Other uses involve fermentation of fish, bamboo-shoots and soybean. Another product made using microorganisms is cheese. For example, the large holes seen in „Swiss cheese‟ is due to the amount of CO_2 produced by the bacterium Propionibacterium sharmanii.

ii) In Industrial Products

Industries also use microbes to make products of value to humans. Since products are made on a large-scale in industries, microorganisms are also grown in bulk in large tanks called „fermentors‟ shown in Fig.4.11.

Fig.4.11. Fermentors

Industries use microbes to make the following products:

(a) Fermented Beverages

The baker‟s yeast that is used to make bread, is also used to ferment malted cereals and fruit juices to make beverages such as beer, whiskey, wine, brandy etc. The

raw material used and the type of processing (distillation or no distillation) gives rise to different alcoholic drinks. For example, processing without distillation produces wine and beer, whereas distillation of fermented broth produces whisky and brandy.

(b) Antibiotics

„Anti" means against and „bio" means life. Microbes produce chemicals that can kill or stop the growth of disease-causing organisms. These are antibiotics. The discovery of antibiotics in the twentieth century was very important to human health.The scientist Alexander Fleming accidentally discovered the first antibiotic – Penicillin. During World War II, Penicillin was extensively used to treat wounded American soldiers. Deadly diseases such as plague, whooping cough, diphtheria which claimed many lives in the past, are now treatable because of antibiotics.

(c) Chemicals, Enzymes And Other Bioactive Molecules

The microbial activity also helps to produce chemicals like organic acids and alcohols. Examples of organisms used are Aspergillus niger(citric acid), Clostridium butylicum (butyric acid), Acetobacter aceti (acetic acid).

The detergents used to remove oily stains contain enzymes such as lipases derived from microbes. Another microbe „Streptococcus" produces Streptokinase that helps to remove blood clots in patients suffering a heart attack.

Microbes (Trichoderma polysporum) also produce bioactive molecules like cyclosporin A. They are used to suppress the immune system during organ transplant procedures. Microbes such as Monascuspurpureus produce statins which help to lower blood cholesterol levels.

iii) In Sewage Treatment

Have you ever wondered what happens to the large amount of sewage (wastewater) humans generate every day? It is treated in Sewage Treatment Plants (STPs) to make it less harmful before releasing into natural water bodies. The microbes present in the sewage itself help in the treatment. It involves the following two stages:

(a) Primary Treatment

This step involves the removal of large and small particles from sewage. First, a process called „sequential filtration" removes the floating debris. Then the process of „sedimentation" removes soil and small pebbles. The solid material that settles is „primary sludge" while the supernatant forms the „effluent". The effluent is then passed through secondary treatment.

(b) Secondary Treatment

It involves the following steps:

i. Mechanical agitation of the effluent in large aeration tanks. This allows growth of useful aerobic microbes into **flocs.** These microbes reduce **BOD** (Biological oxygen demand) of the effluent by consuming the organic matter. BOD is the amount of oxygen consumed if all the organic matter in a litre of sewage is oxidised by bacteria.

ii. Treatment of the sewage water till the BOD is low. (Greater BOD means more polluting power).

iii. Passage of the effluent through the settling tank. Here, „flocs" sediment and generate „**activated sludge**„.

iv. Addition of a small part of the activated sludge to the aeration tank (as inoculum) and the remaining part to „**anaerobic sludge digesters**„. Here, anaerobic bacteria digest other bacteria and fungi and produce gases (methane, CO_2). This is used as biogas and the effluent is released into rivers and streams.

Although urbanisation has increased, the number of sewage treatment plants has not. This has led to the release of untreated sewage into rivers, resulting in their pollution and increase in diseases! Therefore, the Ministry of Environment and Forests has started the **Yamuna Action Plan** and the **Ganga Action Plan** to save these rivers from pollution.

iv) In Biogas Production

Microbial activity produces a mixture of gases (mostly methane). This is biogas and is used as fuel. Different microbes give rise to different gases based on the substrates they use. Anaerobic bacteria feed on cellulose and generate large amounts of methane along with CO_2 and H_2. These bacteria are „methanogens". Methanobacterium, found in the anaerobic sludge (mentioned above) is a common example.

Cattle dung is also rich in these bacteria because they help break down cellulose in the rumen of cattle. Therefore, cattle dung is commonly used to generate biogas. Biogas plants are more common in rural areas because cattle dung is available in large quantities in villages. The **Indian Agricultural Research Institute** (IARI) and the **Khadi and Village Industries Commission** (KVIC) developed the technology of biogas production in India.

A typical biogas plant has the following parts:

- Concrete tank in which a slurry of dung is fed and bio-wastes are added.
- Floating cover on the slurry, this rises as microbes produce gas in the concrete tank.
- An outlet connected to a pipe to supply biogas to nearby households.
- Another outlet to remove the spent slurry and use it as fertiliser.

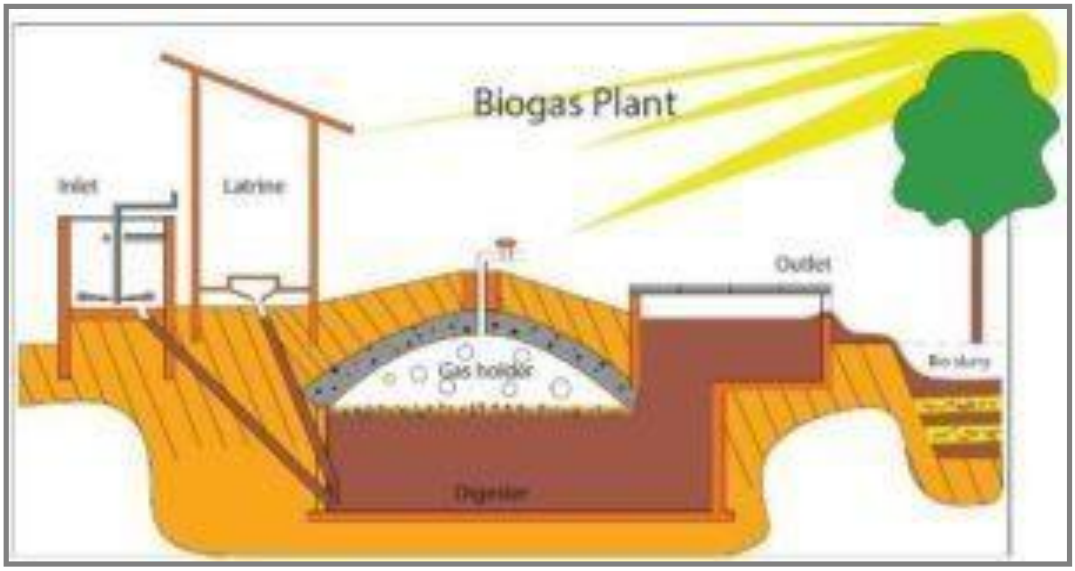


Fig.4.12. Biogas Plant

vi) As Biocontrol Agents

Using pesticides and insecticides not only kills harmful pests but also gets rid of beneficial life forms. Therefore, farmers are now turning to the use of biological methods to control pests and plant diseases. This use of biological methods is „Biocontrol". An example of a microbial biocontrol agent is the bacteria Bacillus thuringiensis that is toxic to butterfly caterpillars but does not harm other insects.Lately, through genetic engineering scientists have introduced toxin genes from this bacteria into plants, making them resistant to attack by pests. Example: Bt-cotton. Another biocontrol agent developed to treat plant diseases is the fungus Trichoderma. Baculoviruses are also excellent biocontrol agents because they attack harmful pests without negatively impacting plants and beneficial insects.

4.7. MEDIA AND STERILIZATION

The purpose of this experiment is that to prepare media from nutrient agar for cultivating microorganisms. Bacteria have special requirements to grow. In order to see bacterial growth, a medium is needed. A MEDIUM is a nutritional environment for bacteria to grow. There are two primary forms of media: Liquid (Broth) and Solid (Agar). The most common solid medium used to grow bacteria in a microbiology lab is Nutrient Agar. The most common liquid medium used in the lab is Nutrient Broth. AGAR is derived from the extract of seaweed (an alga).Agar contains two main components: agarose and agaropectin. Agar contains solidifying factors and therefore has a gelatin like nature. Some agars are used in cooking and preparation of ice cream. The most familiar food is Jell-O. Nutrients such as peptone, tryptone, soy, and beef extract, salt, calcium, magnesium, water, and manganese are added to the agar or broth providing the bacterium with a proper growing environment.

All bacteriological media must first be sterilized before it can be used. Distilled water is added to agar, heated to a boil then autoclaved. An AUTOCLAVE sterilizes the bacterial growth medium so that a pure culture can be obtained. The autoclave sterilizes the medium by subjected it to a temperature of 121° C for 15 to 20 minutes. It uses steam under pressure to obtain this temperature. This will kill any heat resistant bacteria that have contaminated the medium. Once autoclaved, the agar can then be poured into a PETRI PLATE or test tube. When placed in a test tube, it can either be tilted on a slanted board so that it will solidify at a SLANT or remain upright to solidify into a DEEP. Broths are dissolved with water, added to test tubes, capped then autoclaved.

4.7.1 Culture Media

- Media must include source of C, N, P, S, 4 of the 6 major nutrients (CHNOPS), as well as micronutrients. These are usually present as trace contaminants in water, on glassware, or in chemicals used to make media.
- Media can be liquid or solid. Use for different purposes:

    1. Liquid media: easiest to prepare and use. Good for growing quantities of microbes needed for analysis or experiments. Unless inoculated with pure culture, cannot separate different organisms.

    2. Solid media: usually made by adding agar, seaweed extract, to appropriate liquid. 1.5% agar is standard for plates. Agar melts at 80-90 deg. C, will

remain liquid until temperature cools to 40-42 deg. C. Very few microbes can degrade agar, so it is normally not a source of C, and acts as inert gelling medium.

- Synthetic of Defined Media: usually relatively simple media, all components are known. Useful for photoautotrophs, also in some experimental situations where want to select mutants unable to use certain compounds, or for radioisotope labeling. Example: you want to select a microbe that can obtain all its nitrogen from atmospheric N_2. You would prepare synthetic medium with sources of C, P, and S, but no N source. Organisms would be unable to grow unless they can fix nitrogen from air.
- Complex Media: composition of media not completely known. Often made from inexpensive organic materials such as slaughterhouse wastes (tryptic digests called tryptone, trypticase, etc.), soybeans, yeast wastes from brewing (rich source of vitamins), animal blood, etc. All our standard laboratory media in MCB 229 are complex media, such as Tryptone agar, TSA (trypticase soy agar), Nutrient agar, etc.
- Selective Media: media favors the growth of one or more microbes. Example: bile salts inhibit growth of most gram-positive bacteria and some gram-negative bacteria, but enteric bacteria adapted to life in animal gut can grow well. Include bile salts in some media such as EMB, MacConkey agar (will use later in this course) to select for enteric.
- Differential Media: media allows distinguishing between different bacteria that grow. Ex: MacConkey agar has color indicator that distinguishes presence of acid. Bacteria that ferment a particular sugar (e.g., glucose in culture media) will produce acid wastes on plates, turn pH indicator red. Bacteria that cannot ferment the same sugar will grow but not affect pH, so colonies remain white.
- Note that it is possible to design a medium that is both selective and differential.

4.7.2. Sterilization of Media and Equipment:

Sterilization denotes the use of physical or chemical agents to eliminate all viable microbes from a material, whereas disinfection generally refers to the use of germicidal chemical agents to destroy the potential infectivity of a material and need not imply elimination of all viable microbes. Sanitizing refers to procedures used to lower the bacterial content of utensils used for food without necessarily sterilizing them.

Antisepsis usually refers to the topical application of chemicals to a body surface to kill or inhibit pathogenic microbes.

i) Physical Agents:

a) Heat:

Heat is generally preferred for sterilizing materials except those that it would damage. The agent penetrates clumps and reaches sites that might be protected from a chemical disinfectant. Fungi, most viruses and vegetative cells of various pathogenic bacteria are sterilized within a few minutes at 50 to 70 ℃ and the spores of various pathogens at 100 ℃. The spore of some saprophytes, however, can survive boiling for hours. Because absolute sterility is essential for culture media and for the instruments used in major surgical procedures, it has become standard practice to sterilize such materials by steam in an autoclave at a temperature of 121 ℃ (250 ℉) for 15 to 20minutes.

In using an autoclave, it is important that flowing steam be allowed to displace the air before building up pressure , for in steam mixed with air, the temperature is determined by the partial pressure of the water vapour. Thus, if air at 1 atm (15 psi) remains in the chamber and steam is added to provide an additional gauge pressure at 1 atm, the average temperature will be only 100° (that of steam at 1 atm). More over, heating will be uneven because the air will tend to remain at the bottom of the chamber

Pasteurization is now used primarily for milk. It consists of heating at 62 ℃ for 30 minutes or in “flash” pasteurization, at a higher temperature for a fraction of a minute. The total bacterial count is generally reduced by 97% to 99%. Pasteurization is effective because the common milk borne pathogens (tubercle bacillus, Salmonella, Streptococcus, and Brucella) do not form spores.

b) Moist heat and Dry heat:

Sterilization by heat involves protein denaturation and the melting of membrane lipids as a consequence of disruption of multiple weak bonds. Among these, hydrogen bonds between a >C=O and an HN< group are more readily broken if they can be replaced with hydrogen bonds. Accordingly, sterilization requires a higher temperature for dry than for wet material. Reliable sterilization of glassware and instruments in a dry oven requires 160 ℃ for 1 to2 hours. In addition, bacteria and viruses, like isolated enzymes, are more stable in an aqueous medium when the water concentration is reduced by the presence of a high concentration of glycerol or glucose.

The role of water in heat denaturation of proteins is illustrated by the usefulness of steam in pressing woolen fabrics (e.i. in shifting the multiple weak bonds between fibrous molecules of keratin).

c) Freezing:

When a suspension of bacteria is frozen, the crystallization of the water results in the formation of tiny pockets of concentrated solution of salts, which do not themselves crystallize unless the temperature is lowered below the eutectic point (about -20°C for NaCl); at this temperature, the solution becomes saturated, and the salt also crystallizes. The localized high concentrations of salt, and possibly the ice crystals, damage the bacteria, as shown by their increased sensitivity to lysozyme. Only some of the cells are killed, but repeated cycles of freezing and thawing result in the progressive decrease in the viable count.

d) Ultraviolet Radiation:

With radiation of decreasing wavelength, the killing of bacteria first becomes appreciable at 330nm and then increases rapidly. The sterilizing effect of sunlight is attributable mainly to its content of UV light (300-400 nm). Most of the UV light approaching the earth from the sun, and all of that shorter than 290 nm, is screened out by the ozone in the outer regions of the atmosphere; otherwise, organisms could not survive on the earth‟s surface.

e) Ionization radiations:

This mechanism is not convenient for routine laboratory use, but intense source of radioactivity are now being used to sterilize food. Public fear of danger from the irradiation is unwarranted, as the activated mutagenic molecules produced by the irradiation are extremely short-lived.

ii) Mechanical Agents:

a) Ultrasonic and sonic waves:

In the supersonic (ultrasonic) range, with a frequency of 15,000 to several hundred thousand per second, sound waves denature proteins, disperse a variety of materials, and sterilize and fragment bacteria. The effect has not been of practical value as a means of sterilization, but it is useful for disrupting cells for experimental purposes (sonication)

b) Filtration:

Bacteria- free filtrates may be obtained by the use of filters with a maximum pore size not exceeding 400nm.This procedure is used for solutions that cannot tolerate sterilization by heat (e.g. sera an media containing proteins or labile metabolites). The early, rather absorptive filters of asbestos or diatomaceous earth were replaced by unglazed porcelain or sintered glass, and these in turn have been replaced by nitrocellulose membrane filters of graded porosity. Membrane filters can also used to recover bacteria quantitatively for chemical and microbiological analysis.

c) Chemical Agents:

Chemical methods of microbial control: Most of the chemical agents are used for disinfecting and cannot achieve sterility. The term disinfectant is restricted to those that are rapidly bactericidal at low concentrations. The activity of a disinfectant depends upon the pathogen/nature of material being disinfected.

d) Salts:

Pickling in brine or thermal treatment with solid NaCl has been used for many centuries as a means of preserving perishable meats and fish. Bacteria differ widely in susceptibility

e) Heavy Metals:

The various metallic ions can be arranged in a series of decreasing antibacterial activity. With small inocula, Hg + and Ag +. At the head of the list, are effective at less than 1 ppm because of their high affinity for –SH groups. The antibacterial action of Hg 2+ can be reversed readily by sulfhydryl compounds.

f) Phenol and Phenol derivatives:

Phenol (carbolic acid) has an anaesthetic effect at concentration below 1% but at concentrations above 1% it has significant antibacterial effect. Phenol and its derivatives (phenolics), exert antimicrobial effect by damaging plasma membrane, denaturing proteins and inactivating enzymes. The phenolics contain a molecule of phenol that has been chemically altered to reduce its irritating qualities and increase its antibacterial activity in combination with soap or detergent. They are often used as disinfectants. Cresols are a group of phenolic found in Lysol. Another phenolic

derivative a bisphenol -hexachlorophene is ingredient in soaps and lotions used as disinfectants. It is effective against gram positive streptococci, and staphylococci which cause skin infections.

g) Halogens:

Particularly chlorine and iodine are antimicrobial agents effective against all kinds of bacteria, many endospores, fungi, and viruses. Iodine inhibits the microbial protein synthesis by combining with the amino acid tyrosine. The germicidal action of chlorine is due to the formation of hypochlorous acid, which forms when chlorine is mixed with water. A liquid form of compressed chlorine is used for disinfecting drinking water, swimming pools, and sewage. Also hypochlorite solutions (200ppm Cl2) are used to sanitize clean surfaces in the food and the dairy industries and in restaurants.

h) Alcohols:

Effective against bacteria and fungi but not endospores and viruses. Alcohol denatures proteins and dissolves lipid component of cell membranes. Alcohol has the advantage of acting rapidly and evaporating without leaving any residue. Ethanol and isopropanol are the two important alcohols most commonly used.

4.8 GROWTH KINETICS

Microbial cells use nutrients for growth, energy production and product formation as indicated in the following expression;

Nutrients + microbial cells = cell growth + energy + reaction products

Consider the operation of the "Batch" system shown in the figure given below. This container initially contains a known growth substrate concentration S. The container is well mixed and therefore the dissolved oxygen concentration O2 does not become a limiting factor for microbial growth. Initially a known concentration X of viable microbial cells (i.e. inoculum) is added to the container and, with time, growth substrate S is utilized for cell growth. We therefore over time will observe a decrease in S (negative dS/dt) and a corresponding increase in X (positive dX/dt).

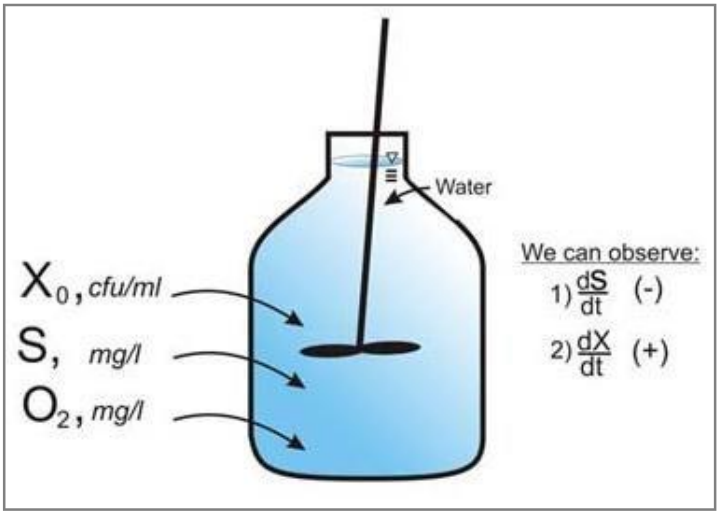


Fig.4.13. Microbial growth and substrate utilization in a well mixed batch container.

A conceptual plot of microbial cell concentration vs time for the batch system is called a growth curve, as shown in Fig 4.14.

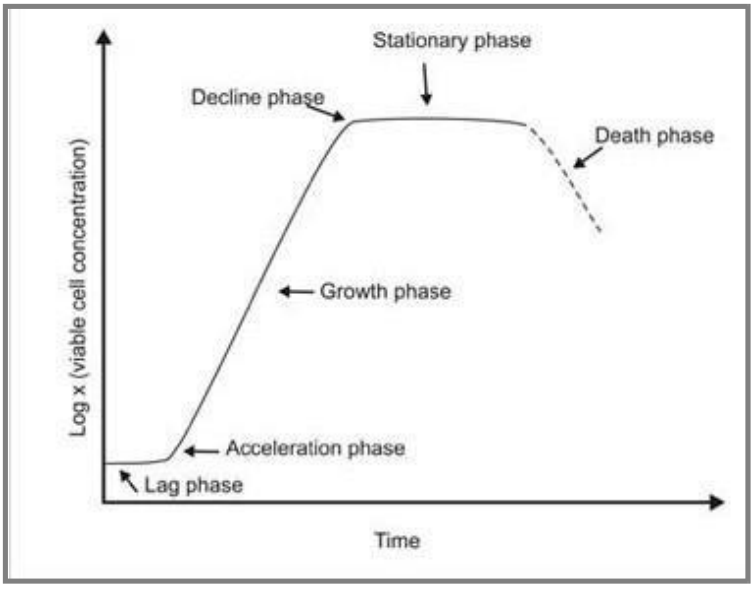


Fig.4.14. Typical growth curve of microbe for a batch system.

By plotting the log of viable cell concentration, X, with time, five distinct phases of the growth curve can be identified; 1) the lag phase which occurs immediately after

inoculation and persists until the cells have acclimated to their new environment, 2) exponential growth phase, during which time cell growth proceeds at an exponential rate (indicated by a straight line on the semi-log plot), 3) a deceleration phase, when essential nutrients are depleted or toxic products begin to accumulate, 4) a stationary phase during which time the net cell growth is approximately zero, and 5) death phase where some cells loose viability or are destroyed by lysis.

Microbial Growth Kinetics

During the lag phase dX/dt and dS/dt are essentially zero. However as exponential growth phase begins it is possible to measure dX/dt and dS/dt values which are very useful for defining important microbial kinetic parameters. Using corresponding observations of dS/dt and dX/dt obtained just after the onset of exponential growth phase in the above figure we can compute the yield coefficient Y_{XS} and the specific growth rate μ as:

Yield coefficient

$$Y = \frac{dX}{dS} = \frac{\text{mass of new cells}}{\text{mass of substrate consumed}}, [\text{dimensionless}] \quad (1)$$

Specific growth rate

$$\mu = \frac{dX}{X_0 dt} = \frac{mass\ of\ cells\ produced}{original\ mass\ of\ cells\ \cdot time}, \left[\frac{1}{time}\right] \quad (2)$$

The yield coefficient, commonly referred to as the substrate-to-biomass yield, is used to convert between cell growth rate dX/dt and substrate utilization rate dS/dt. The yield coefficient and the specific growth rate used to develop three types of microbial growth kinetic relationships; Monod , first order ,and zero order kinetics.

Monod Kinetics

The batch experiment shown in Figure 1 can be repeated by varying initial substrate concentration S over a wide range of valuesresulting in observation of individual μ values which correspond to each substrate concentration. An arithmetic plot of μ vs S will exhibit the general behavior shown in the figure below.

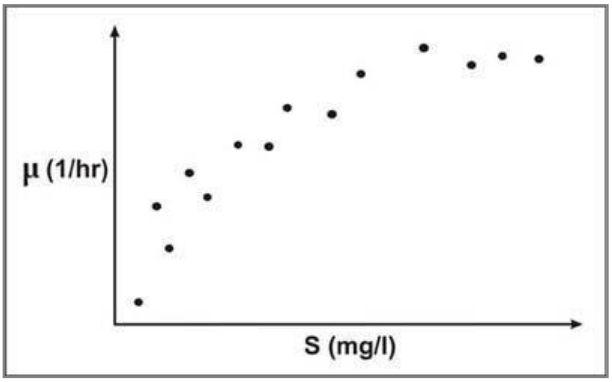


Fig.4.15. Specific growth rate plotted respect to initial substrate concentration in a batch system.

The most widely used expression for describing specific growth rate as a function of substrate concentration is attributed to Monod (1942, 1949). This expression is:

$$\mu = \mu_{max}\left(\frac{S}{S + K_s}\right) \tag{3}$$

Below the figure shows conceptually how the Monod equation is fit to the observed substrate and specific growth rate data in the above figure. In below figure it is seen that μ_{max} is the maximum specific growth rate observed and K_S is the substrate concentration corresponding to 1/2 μ_{max}.

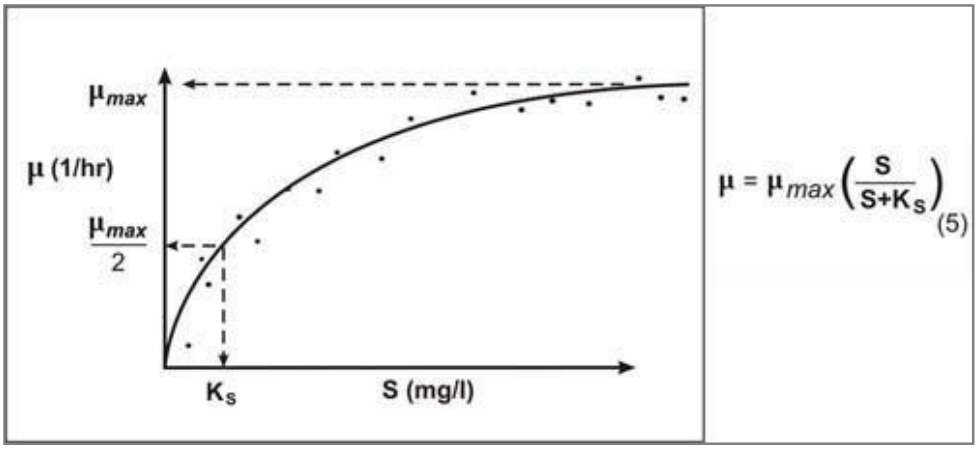


Fig.4.16. Monod Equation fit to observed data.

Monod Kinetics

By combining equations 2 and 3 we can write the following expression for time-rate-of-change of biomass:

$$\frac{dX}{dt} = \mu X_O = \mu_{\max} X_O \left(\frac{S}{S + K_S}\right) \tag{4}$$

Similarly, by combining equations 1 and 3 we can write an expression for substrate utilization rate.

$$\frac{dS}{dt} = \frac{\mu X}{Y} = \frac{\mu_{\max} X_O}{Y}\left(\frac{S}{S + K_S}\right) \tag{5}$$

First Order Kinetics

Equation 5 describes the Monod kinetic relationship for substrate utilization. From Figure 4 it can be seen if $S << K_S$, Equation 5 can be approximated as:

$$\frac{dS}{dt} = \left(\frac{X_O \mu_{max}}{Y K_C}\right) S = K_b S \tag{6}$$

Equation 6 describes the condition where substrate utilization is proportional to substrate concentration (i.e. first order with respect to S).

Zero Order Kinetics

Likewise if $S > K_S$ Equation 5 can be approximated as:

$$\frac{dS}{dt} = \frac{X_O \mu_{max}}{Y} (= constant) \tag{7}$$

Equation 7 describes the condition where substrate utilization rate is a constant (i.e. zero order with respect to S).

5

SENSOR BIOLOGY AND COMMUNICATION SYSTEMS

5.1. SENSORY SYSTEM

Human sensory reception means by which humans react to changes in external and internal environments. Ancient philosophers called the human senses ‒the windows of the soul,‖ and Aristotle described at least five senses sight, hearing, smell, taste, and touch. Aristotle's influence has been so enduring that many people still speak of the five senses as if there were no others. Yet the modern sensory catalog now includes receptors in the muscles, tendons, and joints, which give rise to the kinesthetic sense (that is, the sense of motion), and receptors in the vestibular organs in the inner ear, which give rise to the sense of balance. Within the circulatory system, sensory receptors are found that are sensitive to carbon dioxide in the blood or to changes in blood pressure or heart rate, and there are receptors in the digestive tract that appear to mediate such experiences as hunger and thirst. Some brain cells may also participate as hunger receptors. This is especially true of cells in the lower parts of the brain (such as the hypothalamus) where some cells have been found to be sensitive to changes in blood chemistry (water and other products of digestion) and even to changes in temperature within the brain itself.

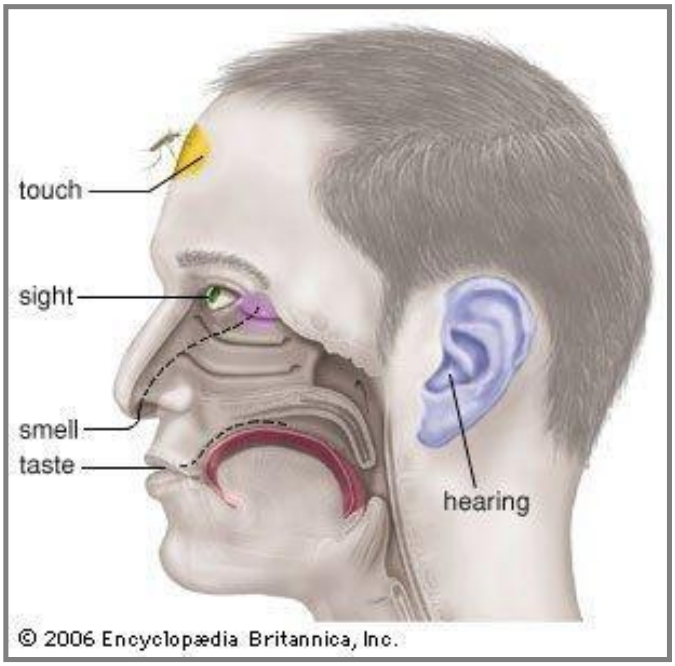


Fig. 5.1. Sensory system in Human Body

5.1.1. General considerations of sensation

a) Basic features of sensory structures

One way to classify sensory structures is by the stimuli to which they normally respond; thus, there are photoreceptors (for light), mechanoreceptors (for distortion or bending), thermoreceptors (for heat), chemoreceptors (e.g., for chemical odours), and nociceptors (for painful stimuli). This classification is useful because it makes clear that various sense organs can share common features in the way they convert (transduce) stimulus energy into nerve impulses. Thus, auditory cells and vestibular (balance) receptors in the ear and some receptors in the skin all respond similarly to mechanical displacement (distortion). Because many of the same principles apply to other animals, their receptors can be studied as models of the human senses. In addition, many animals are endowed with specialized receptors that permit them to detect stimuli that humans cannot sense. The pit viper, for instance, boasts a receptor of exquisite sensitivity to ‑invisible‖ infrared light. Some insects have receptors for ultraviolet light and for pheromones (chemical sex attractants and aphrodisiacs unique to their own species), thereby also exceeding human sensory capabilities.

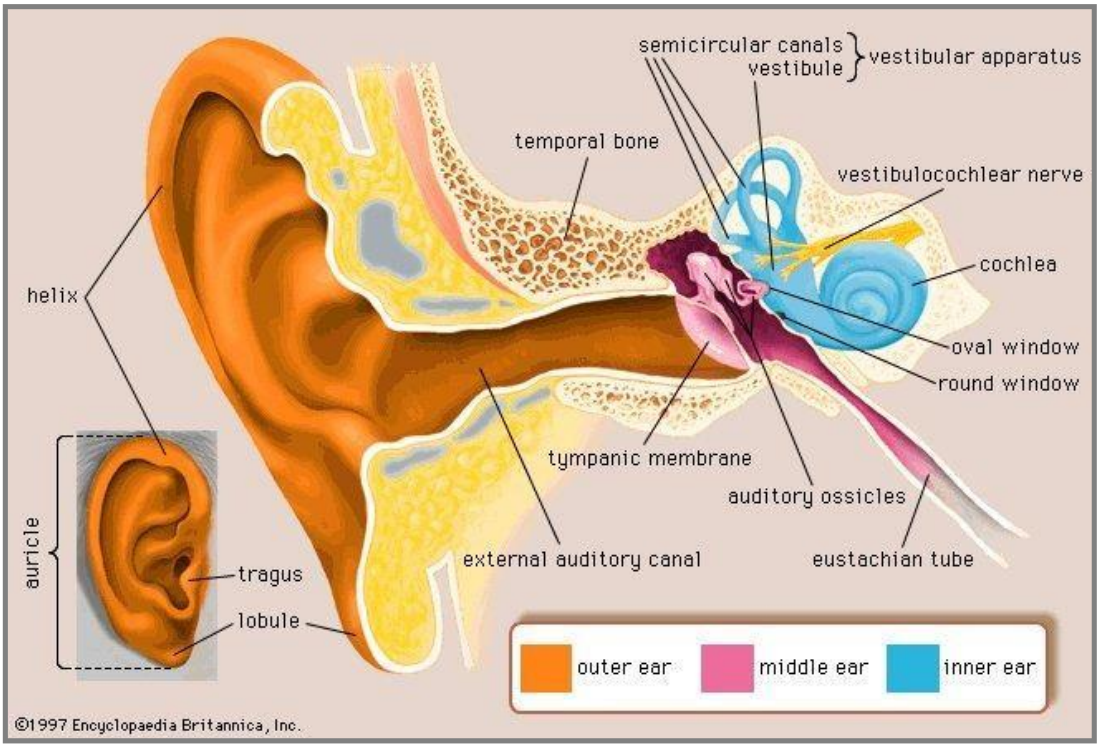


Fig. 5.2. Structure Human Ear

(1) All sense organs contain receptor cells that are specifically sensitive to one class of stimulus energies, usually within a restricted range of intensity. Such selectivity means that each receptor has its own ‑adequate‖ or proper or normal stimulus, as, for example, light is the adequate stimulus for vision. However, other energies (—inadequate‖ stimuli) can also activate the receptor if they are sufficiently intense. Thus, one may ‑see‖ pressure when, for example, the thumb is placed on a closed eye and one sees a bright spot (phosphene) in the visual field at a position opposite the touched place.

(2) The sensitive mechanism for each modality is often localized in the body at a receiving membrane or surface (such as the retina of the eye) where transducer neurons (sensory cells) are located. Often the sensory organ incorporates accessory structures to guide the stimulating energy to the receptor cells; thus, the normally transparent cornea and lens within the eye focus light on the retinal sensory neurons. Retinal nerve cells themselves are more or less shielded from nonvisual sources of energy by the surrounding structure of the eye.

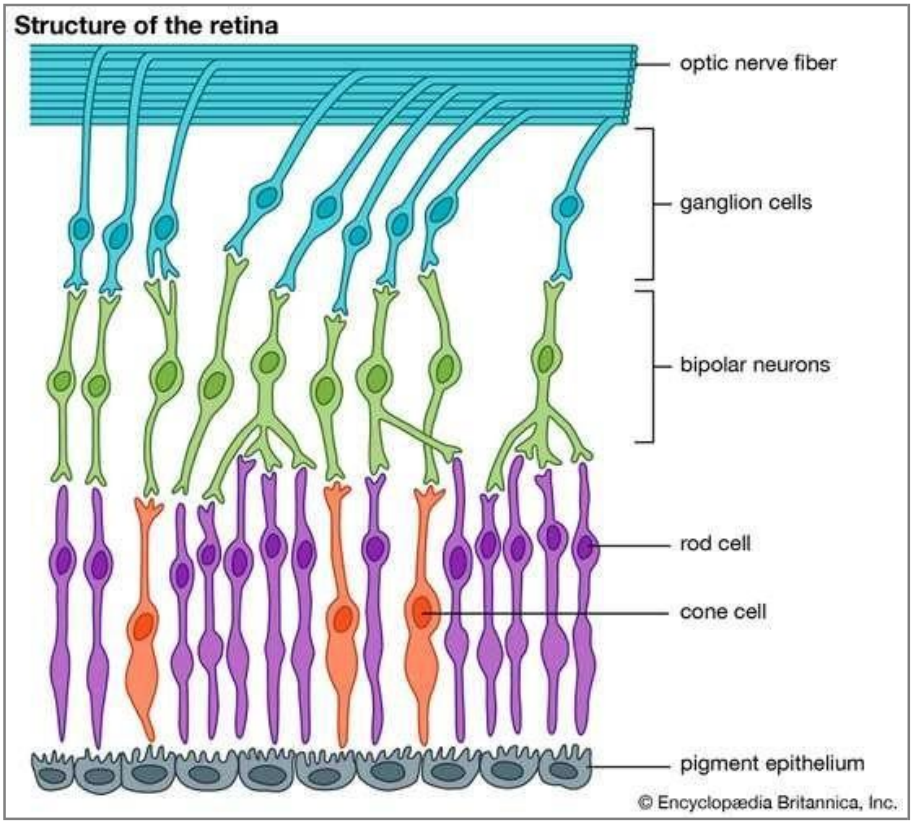


Fig. 5.3. Structure of Retina

(3) The primary transducers or sensory cells in any receptor structure normally connect (synapse) with secondary, ingoing (afferent) nerve cells that carry the nerve impulse. In some receptors, such as the skin, the individual primary cells possess threadlike structures (axons) that may be yards long, winding from just beneath the skin surface through subcutaneous tissues until they reach the spinal cord. Here, each axon from the skin terminates and synapses with the next (second-order) neuron in the chain. By contrast, each primary receptor cell in the eye has a very short axon that is contained entirely in the retina, which synapses with a network of several types of second-order neurons called internuncial cells, which, in turn, synapse with third-order neurons called bipolar cells—all still in the retina. The bipolar-cell axons extend afferently beyond the retina, leaving the eyeball to form the optic nerve, which enters the brain to make further synaptic connections. If this visual system is considered as a whole, the retina may be said to be an extended part of the brain on which light can directly fall.

The arrival of the nerve impulse at the presynaptic terminal stimulates the release of neurotransmitter into the synaptic gap. The binding of the neurotransmitter to receptors on the postsynaptic membrane stimulates the regeneration of the action potential in the postsynaptic neuron.

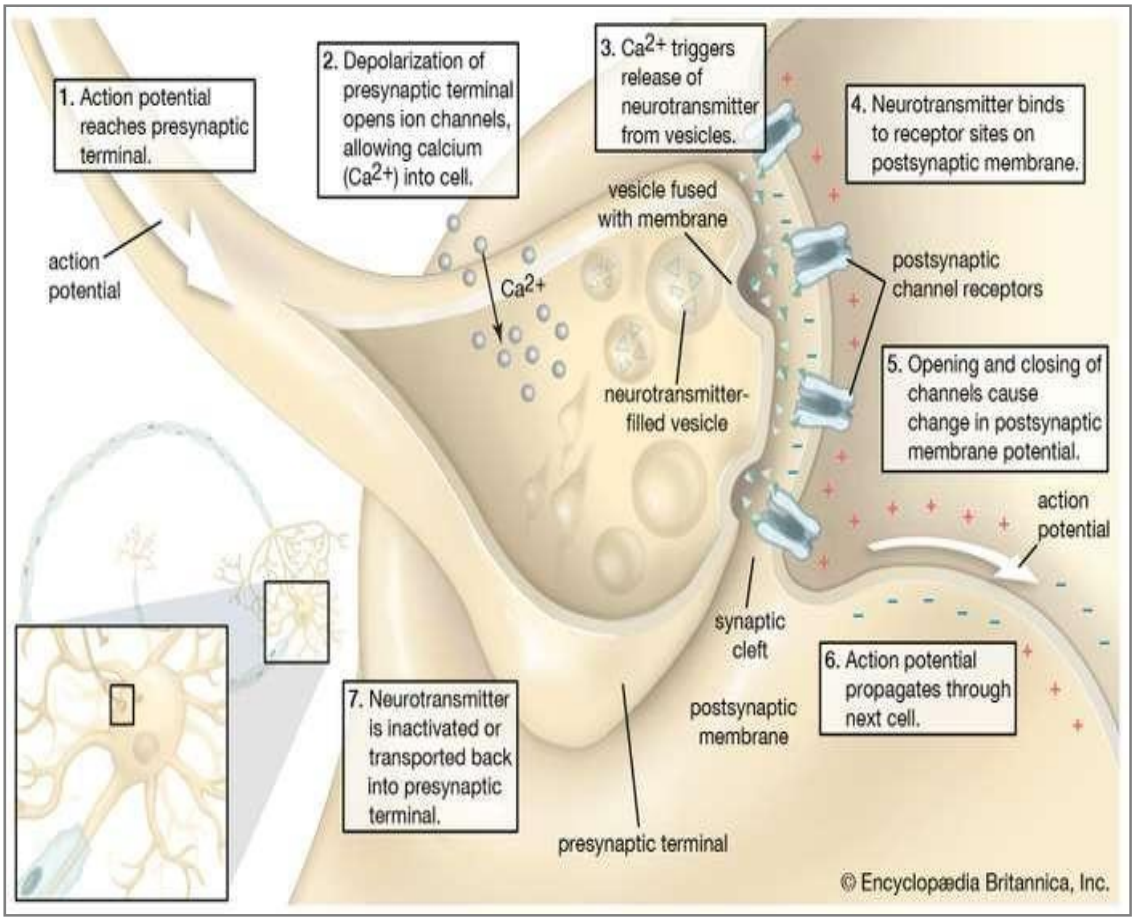


Fig. 5.4. Structure of Neuron

From such afferent nerves, still higher-order neurons make increasingly complex connections with anatomically separate pathways of the brainstem and deeper parts of the brain (e.g., the thalamus) that eventually end in specific receiving areas in the cerebral cortex (the convoluted outer shell of the brain). Different sensory receiving areas are localized in particular regions of the cortex—e.g., occipital lobes in the back of the brain for vision, temporal lobes on the sides for hearing, and parietal lobes toward the top of the brain for tactile function.

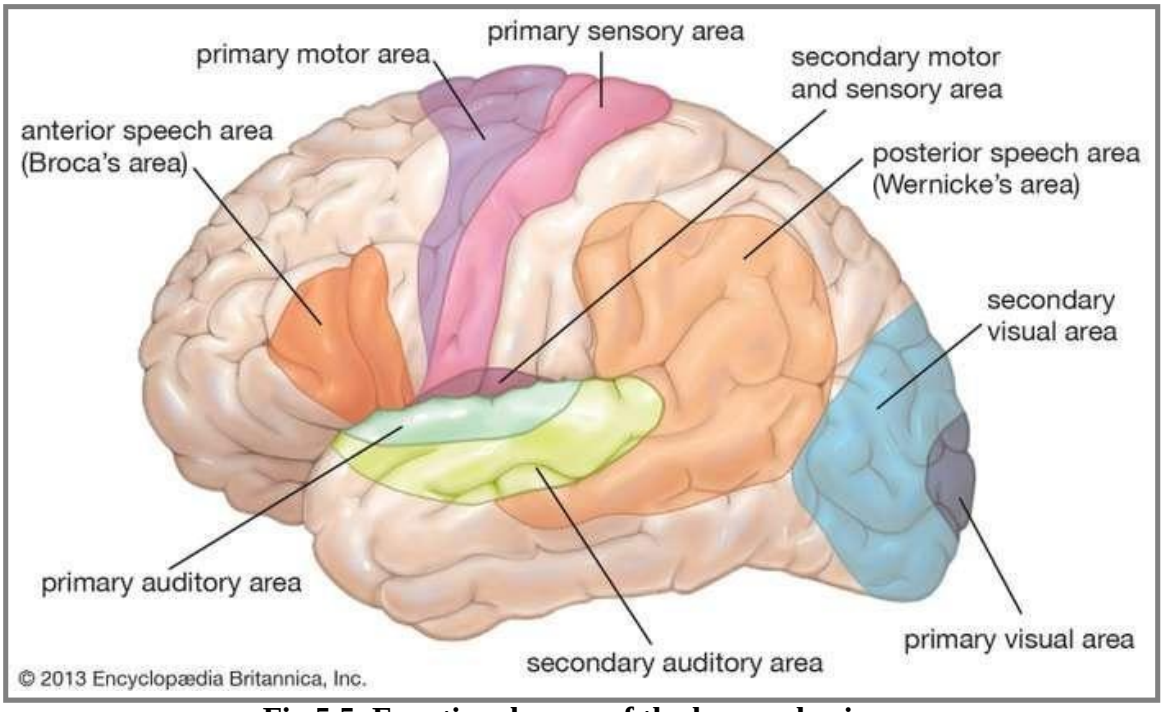


Fig.5.5. Functional areas of the human brain.

b) Approaches to the study of sensing

The science of the human senses is truly interdisciplinary. Philosophers, physicians, anatomists, physical scientists, physiologists, psychologists, and others all study sensory activities. Some of their earliest work was anatomical, an approach that continues to be fruitful. Physical scientists, particularly physicists and chemists, made important contributions to an understanding of the nature of stimulus energies (e.g., acoustic, photic, thermal, mechanical, chemical); in the process, they also performed many fundamental measurements of human sensory function. Hermann von Helmholtz, a 19th-century German scientist who was a physicist, physiologist, and psychologist, studied the way in which sound waves and light are sensed and interpreted. Modern studies of sensation have been enhanced by devices permitting the precise production and control of sensory stimuli. With other instruments, physiologists have been able to probe the electrical signals generated by sensory cells and afferent nerve fibres to provide a biophysical analysis of sensory mechanisms.

Psychophysics embraces the study of the subjective aspects of sensation in terms of objective stimulus energies. One of the oldest and most classical approaches to the study of sensation, psychophysics includes the study of people's reports of their

sensations when they are stimulated: of their ability, for example, to match tones of equal loudness, to detect stimulus differences, and to estimate sensory magnitude or intensity under conditions of controlled stimulation. Psychophysical research continues as an active enterprise particularly among modern psychologists.

The old philosophical notion that the mind is a clean slate or tablet (tabula rasa) until ‑written on‖ by impressions from the senses no longer seems fully tenable; infants, for example, show inborn (innate) ways of sensing or perceiving at birth. In its modern form, the problem of learned versus innate factors in sensory experience is studied in terms of the extent to which the genetically determined structure and function of sense organs and brain depend upon stimulation and experience for their proper maturation. Sensory deprivation in an infant's early life is increasingly being documented as detrimental to the full flowering of mature perceptual and intellectual functions. Since this sort of evidence is may lend some support to the notion of the tabula rasa, modern researchers give credence both to nativistic (based on heredity) and empiricistic (based on learning) interpretations of human sensory function.

Chemical-visceral sensations particularly have hedonic (pleasure-pain) properties. Most people tend to refer to odours and tastes as pleasant or unpleasant; thus, the chemical senses are closely tied to motivations, preferences, and aversions. Although reflex licking or sucking is stimulated by tactile stimulation of the lips and mouth, newborns tend to suck longer and harder when the stimulus has clear hedonic value e.g., avidly turning their lips toward a nipple for a sweet taste. Apparently, one's ‑sweet tooth‖ is largely nativistic, in that it requires little prior learning. The craving for salt (especially heightened under conditions of salt deprivation) likewise appears to be nativistic. The role of taste and smell as innate factors in behaviour may not be quite so influential in humans as in other animals. People's food habits and preferences are strongly related to custom and tradition; that is, they are primarily learned.

In the modern era, the language of communication engineering has been found to be useful in describing human senses. Each sensory modality may be described as a channel that receives stimulus information (input), processes and stores the information (memory), and retrieves it as needed for the effective behaviour (output) of the individual. In addition, devices such as radio, television, radar, and the electron microscope extend the range and power of the senses. In the last analysis, however, all such devices convert (transduce) information back to a form of stimulus energy that is directly perceptible to the unaided senses. For example, a television is a transducer that

converts imperceptible electromagnetic waves into visual and auditory signals. For some special purposes, people may employ alternative sensory channels, as when blind people use Braille or other tactile input as substitutes for missing visual channels. While the chemical senses have little function in symbolic communication among people, the use of perfumes in romantic signaling is a notable exception. In general, however, the chemical senses are more directly involved in physiological survival e.g., warning that a putrid fish is dangerous to eat. Physical well-being also rests heavily on proprioceptors (for sensing bodily position) and on the sense of balance. These structures, monitoring bodily orientation in space, provide crucial sensory feedback for guiding movements.

c) Cutaneous (skin) senses

There is evidence for two pressure senses (for light and for deep stimulation), for two kinds of temperature sensitivity (warm and cold), and for a pain sense. In the 1880s, findings that the human skin is punctate (selectively sensitive at different points) gave clear indication of a dissociation among functions once grouped together as the sense of touch. Mapping the skin with a fine bristle or with a narrow-tipped (warm or cold) cylinder showed that there are different spots of maximum sensitivity to pressure, warm temperatures, and cold temperatures. When stimulated between the spots on the skin, no such sensations were reported. Pain spots also can be located with a finely pointed needle, but the punctate character is less striking since pain seems to be widespread when stimulus intensity is increased. The number of spots is greatest for pain, next for touch, then for cold, and least for warm.

d) Nerve function

Microscopic examination of the skin reveals a variety of nerve terminals including free nerve endings (which are most common), Ruffini endings, and encapsulated endings, such Pacinian corpuscles, Meissner's corpuscles, and Krause end bulbs.

In laboratory animals some nerve endings seem to respond only to one type of stimulus (e.g., to pressure stimuli of very light weight or to slight temperature changes); others exhibit a broad range of sensitivity. Some receptors show combined sensitivity to both temperature and pressure. In some cases only special types of mechanical stimulation (such as rubbing) may be effective. Furthermore, there is extensive overlap in the areas of skin (receptor fields) for individual nerve fibres, suggesting a neural integration of overlapping afferent inputs of skin nerves.

On the other hand, some tactile receptors (e.g., Pacinian corpuscles) respond only to mechanical deformation. A Pacinian corpuscle is an onion-shaped structure of nonneural (connective) tissue built up around the nerve ending that reduces the mechanical sensitivity of the nerve terminal itself. If the onionlike capsule is entirely removed, mechanical sensitivity not only remains but is somewhat greater than when the capsule is present.

In addition to the differences in the sensory end structures of the skin, the afferent nerve fibres (axons) from them also show diversity. The nerve fibres range in size from large myelinated (sheathed) axons of 10 to 15 micrometres (millionths of a metre) in diameter to extremely small unmyelinated fibres measuring only tenths of micrometres across. Fatter axons tend to conduct nerve impulses more rapidly than do small fibres; when axons of different diameters form a single bundle (a nerve), they constitute a so-called mixed nerve. Thus, electrical records from a mixed nerve show what are labeled A (fast), B (medium), and C (slow) components that reflect the typical speeds at which axons of different diameters conduct. Although such specialized capsules, such as Pacinian corpuscles, tend to be associated with larger diameter axons, and temperature-sensitive endings tend to be associated with medium-size fibres, a unique relation of each of the skin modalities with one of the A, B, or C fibre groups cannot be supported. All of the cutaneous senses seem to be associated with some fibres of all diameters; furthermore, the C fibres (once thought to be restricted to the pain function) display quite specific sensitivities to nonpainful stimuli applied to the skin.

A major neural pathway for tactile impulses runs along the back (in the dorsal columns) of the spinal cord. Afferent fibres enter the cord from the cutaneous nerves and ascend without synaptic break in one (the ipsilateral) dorsal column. This is a very rapidly conducting pathway shared by fibres that mediate sensations of deep pressure and kinesthesis. Other tactual, temperature, and pain information crosses the spinal cord close to the level of entry of the sensory fibres and ascends to the brain in contralateral pathways of the cord (the lateral and ventral spinothalamic tracts).

Each of the nerves distributed along the spinal cord contains a sensory bundle that serves a well-defined strip of skin (a dermatome) about 2.5 cm (1 inch) wide or more on the body surface. Successive spinal nerves overlap, so that each place on the skin represents two and sometimes three dermatomes; this yields a segmented pattern of strips over the body from head to toe. All dermatomes feed into a single relay centre (the sensory thalamus) deep within the brain, where there is a precise three-dimensional

layout of tactile sensitivity at the body surface. The neurons in this part of the thalamus (the ventral posterolateral nucleus) are specific to particular skin senses (such as pressure) and form small and precise receptor fields. Pathways from the specific ventral posterolateral thalamus end (or project) in a narrow band of the cerebral cortex (the posterior rolandic cortical sensory area) where there is a point-for-point representation of the body surface on the cortical surface. There is a second more diffuse thalamic system (in the posterior thalamic nuclei) where the receptor fields are large, perhaps bilateral, on the left and right sides, perhaps including one whole side of the body. The receptor fields here and the types of stimuli to which they respond are not clearly delineated. The cortical projection of the posterior thalamic system is less well charted than that of the ventral posterolateral thalamus. Thus, there appears to be a dissociation between tactual structures that are highly specific and those that are more generalized.

The dissociation of cutaneous senses is demonstrated in the course of some diseases; for example, in syringomyelia, degeneration of the central canal of the spinal cord leads to loss of pain and temperature sensitivity. Nevertheless, the patient still can experience pressure. In some instances there may be a complete absence of pain sensitivity with disastrous consequences such as bruises, cuts, or even the loss of body parts. Still other instances of dissociation of pain versus pressure occur in surgical procedures (such as tractotomy) in which spinal tracts or parts of the nerves leading into the brainstem are selectively cut. Such operations are designed specifically to relieve pain without unduly diminishing pressure sensitivity.

e) Tactile psychophysics

The mixture of sensitivities within a given patch of skin provides a basis for the concept of adequate stimulation. Sometimes, for example, a cold spot responds to a very warm stimulus, and one experiences what is called paradoxical cold. The sensation of heat from a hot stimulus presumably arises from the adequate stimulation of warmth receptors combined with the inadequate or inappropriate (although effective) stimulation of cold and pain receptors.

The ability to detect pressure (i.e., pressure threshold) generally appears when a tension of about 0.85 gram per square mm (equivalent to about 1.2 pounds per square inch) of skin surface is applied on the back of the hand. Thus a force of 85 mg applied to a stimulus hair (or bristle) of 0.1 square mm is just about enough to elicit the

experience of pressure. The energy of impact at pressure threshold is much greater than that required for hearing or seeing, the skin requiring approximately 100,000,000 times more energy than the ear and 10,000,000,000 times more energy than the eye. Differential pressure discrimination (the ability to detect just noticeable differences in intensity) requires changes of roughly 14 percent at maximum sensitivity.

Adaptation to pressure is well known; one's awareness of a steadily applied bristle fades and ultimately disappears. As a result people are rarely aware of the steady pressure of their clothing unless movement brings about a change in stimulation. Most dramatic and perhaps best known among tactile experiences is adaptation to thermal stimulation. Continued presentation of a warm or cold stimulus leads to reduction or disappearance of the initial sensation and an increase in threshold values. Total obliteration of thermal sensation through adaptation occurs in the range from about 16 to 42 °C (61 to 108 °F). If one hand is placed in a bowl of hot (40 °C [104 °F]) water and adapted to that, and at the same time the other hand is adapted to cold (20 °C [68 °F]) water, then when both hands are simultaneously placed in lukewarm (30 °C [86 °F]) water, the previously cooled hand feels warm and the other hand feels cold. Both types of temperature receptors show adaptation. Cold receptors are characterized by an electrical discharge on sudden cooling, normally showing no response to sudden warming; similar electrical responses are produced by warmth receptors. Both receptors show steady discharges selectively depending on temperature; maximum discharge typically occurs between 38 and 43 °C (100 and 109 °F) for individual warmth receptors and between 15 and 34 °C (59 and 93 °F) for cold receptors.

Pain is the least understood among all of the human senses. The pattern of stimulation is more crucial in pain than in any other sense. A single brief electric shock to the skin or to an exposed nerve may not elicit the experience of pain; yet it tends to become painful upon repetitive stimulation. Cutaneous pain is often sensed more sharply than is pain associated with deep tissues of the body (e.g., viscera). Certain areas of the body are relatively analgesic (free of pain); for example, one can bite shallowly into the mucous lining of the cheek without discomfort. The organs of the abdominal cavity are usually insensitive to cutting or burning, but traction or stretching of hollow viscera is painful (as when the stomach is distended by gas). Pain also displays sensory adaptation, although the process appears to be more complex than it is for other sensory modalities. Thus, the intensities of headaches, toothaches, and pains from injury often show cyclic fluctuations, possibly from such factors as changes in

blood circulation or in degree of inflammation. The visceral pains, those of dental origin, or of diseased tissues can be reduced by analgesic medications, which tend to be less effective on cutaneous pain. Pain has a strong emotional context. In certain cases, after frontal lobotomies (a type of brain surgery) have been performed, a person may report that he still feels the pain of a pin prick or other irritation but that he does not find it as disturbing or emotionally disruptive as he did before the lobotomy. Many phenomena indicate the powerful role of the brain and spinal cord in sensing potentially painful sensory input. According to one theory, a gate control system in the spinal cord modulates sensory input from the skin to determine whether the input is perceived as painful. This theoretical formulation also may account for moment-to-moment fluctuations in the intensity of perceived pain despite the absence of any stimulus change. Such brain-mediated factors as emotional tension or past psychological experiences are thought to influence pain perception by acting upon this spinal gate control system.

Itching seems to bear the same relation to pain as tickle does to pressure. The experience usually lasts long enough to demand attention and (like tickle) normally leads to a response such as rubbing or scratching the affected area. A number of skin disorders are accompanied by itching, presumably from a fairly low level of irritation in the affected area (which also may be produced in undiseased skin). While a single shock by a low-intensity electrical spark normally produces no sensation, a repetitive pattern of such shocks may induce an itch similar to that produced by an insect bite. Itching also may occur as an aftereffect of the sharp pricking sensation produced by single strong shocks, presumably because the nerves continue to produce a patterned afterdischarge following the cessation of the stimulus.

Nonpainful tactile pattern stimulation is exemplified by vibration. Different frequencies of vibration are readily discriminated, and a tactile communication system employing vibrations on the skin has been devised, particularly for people who cannot see or hear.

5.1.2. Kinesthetic (motion) sense

Even with the eyes closed, one is aware of the positions of his legs and arms and can perceive the movement of a limb and its direction. The term *kinesthesis* (―feeling of motion‖) has been coined for this sensibility.

a) Nerve function

Four types of sensory structures are widely distributed in muscles, tendons, and joints:

(1) neuromuscular spindles consist of small, fine muscle fibres around which sensory fibre endings are wrapped;

(2) Golgi tendon organs consist of sensory nerve fibres that terminate in a branching encapsulated within the tendon;

(3) joint receptors (as in the knee) consist of ‒spray-type‖ Ruffini endings and Golgi-type and Pacinian corpuscles within the joints; and

(4) free nerve endings.

All these receptors combine to provide information on active contraction, passive stretch of muscle fibres, and tension. In passive stretch both the muscle-spindle receptors and the tendon receptors send impulses over their sensory (afferent) nerves; in active contraction the spindles exhibit a silent period of neural activity when tension on the parallel fibres is unloaded, while the tendon receptors discharge just as when stretch is passive.

The muscle spindle is contractile in response to its own small-diameter, gamma motor (efferent) fibre. The receptors and the gamma fibres of the muscle spindle form a neuromuscular loop that ensures that tension on the spindle is maintained within its efficient operating limits. The excitability of the muscle spindle also can be influenced through other neural pathways that control the general level of excitability of the central nervous system (brain and spinal cord). Activity of the descending reticular formation (a network of cells in the brainstem) may enhance the contraction of the spindle and therefore influence its neural discharges.

Muscle and tendon receptors combine to play an intimate and crucial role in the regulation of reflex and voluntary movement. Much of this control is automatic (involuntary) and not directly perceptible except in the aftereffects of movement or change of position. The knee jerk, or patellar reflex, that follows a tap just below the kneecap of a freely hanging leg is one such involuntary reflex. Sensory (afferent) impulses from stretching the receptors (e.g., in the muscles) relay to the spinal cord and activate a path to the motor (efferent) nerves leading back to the same muscle. The knee jerk is a purely spinal reflex response (the brain is not required) which is tested usually

to determine nerve damage or other interference with the spinal cord motor mechanisms. Besides producing loss of knee jerk, a disease like syphilis may lead to locomotor ataxia (a clumsy and stumbling gait) when the bacteria (called a spirochete) attacks the sensory nerves of the cord's dorsal column. The result is that the affected individual has difficulty sensing the position of his limbs. Another general function of the muscle receptors is the maintenance of muscle tone (partial contraction) to permit rapid response (fast reaction time) to stimulation. In normal conditions the muscle has tone and is ready to respond; but, when it is without motor stimulation (deafferented), the muscle is flaccid, showing little tone. Upright posture depends on the tone of opposing (extensor and flexor) muscles in response to the effects of gravity.

The exact contribution of the muscle receptors to sensation is not entirely understood. It seems clear, however, that they are not essential to the sensation of bodily position. The appreciation of passive movement of the limbs probably comes largely from the joints, since, after anesthetizing the overlying skin and muscles, sensibility to the limb movement seems little affected. Very few of the impulses arising from the muscle receptors themselves reach the cerebral cortex; instead, they ascend in the spinal pathways to another part of the brain, the cerebellum, where they interact in the automatic control of bodily movement. Impulses arising from the joint receptors, on the other hand, have been recorded in both the thalamus and cerebral cortex, the degree of angular displacement of a joint being reflected systematically in these structures by the frequency of nerve impulses. Symptoms of some diseases also emphasize the importance of joint sensitivity. When bone disease, for example, destroys only the joint receptors, the ability to appreciate posture and movement is lost.

b) Vestibular sense (equilibrium)

The inner ear contains parts (the nonauditory labyrinth or vestibular organ) that are sensitive to acceleration in space, rotation, and orientation in the gravitational field. Rotation is signaled by way of the semicircular canals, three bony tubes in each ear that lie embedded in the skull roughly at right angles to each other. These canals are filled with fluid called endolymph; in the ampulla of each canal are fine hairs equipped with mechanosensing stereocilia and a kinocilium that project into the cupula, a gelatinous component of the ampulla. When rotation begins, the cupula is displaced as the endolymph lags behind, causing the stereocilia to bend toward the kinocilium and thereby transmit signals to the brain. When rotation is maintained at a steady velocity, the fluid catches up, and stimulation of the hair cells no longer occurs until rotation

suddenly stops, again circulating the endolymph. Whenever the hair cells are thus stimulated, one normally experiences a sensation of rotation in space. During rotation one exhibits reflex nystagmus (back-and-forth movement) of the eyes. Slow displacement of the eye occurs against the direction of rotation and serves to maintain the gaze at a fixed point in space; this is followed by a quick return to the initial eye position in the direction of the rotation. Stimulation of the hair cells in the absence of actual rotation tends to produce an apparent ‒swimming‖ of the visual field, often associated with dizziness and nausea.

Two sacs or enlargements of the vestibule (the saccule and utricle) react to steady (static) pressures (e.g., those of gravitational forces). Hair cells within these structures, similar to those of the semicircular canal, possess stereocilia and a kinocilium. They also are covered by a gelatinous cap in which is embedded small granular particles of calcium carbonate, called otoliths, that weigh against the hairs. Unusual stimulation of the vestibular receptors and semicircular canals can cause sensory distortions in visual and motor activity. The resulting discord between visual and motor responses and the external space (as aboard a ship in rough waters) often leads to nausea and disorientation (e.g., seasickness). In space flight abnormal gravitational and acceleratory forces may contribute to nausea or disequilibrium.

In some diseases (e.g., ear infections), irritation of vestibular nerve endings may cause the affected individual to be subject to falling as well as to spells of disorientation and vertigo. Similar symptoms may be induced by flushing hot and cold water into the outer opening of the ear, since the temperature changes produce currents in the endolymph of the semicircular canals. This effect is used in clinical tests for vestibular functions and in physiological experiments. Externally applied electrical currents may also stimulate the nerve endings of the vestibule. When a current is applied to the right mastoid bone (just behind the ear), nystagmus to the right tends to occur with a reflex right movement of the head; movement tends to the left for the opposite mastoid. Destruction of the labyrinth in only one ear causes vertigo and other vestibular symptoms, such as nystagmus, inaccurate pointing, and tendency to fall.

c) Taste (gustatory) sense

The sensory structures for taste are the taste buds, clusters of cells contained in goblet-shaped structures called papillae that open by a small pore to the mouth cavity. A single taste bud contains about 50 to 75 slender taste receptor cells, all arranged in a

banana-like cluster pointed toward the gustatory pore. Taste receptor cells, which differentiate from the surrounding epithelium, are replaced by new cells in a turnover period as short as 7 to 10 days. The various types of cells in the taste bud appear to be different stages in this turnover process. Slender nerve fibres entwine among and make contact usually with many cells. Taste buds are located primarily in fungiform (mushroom-shaped), foliate, and circumvallate (walled-around) papillae of the tongue or in adjacent structures of the palate and throat. Many gustatory receptors in small papillae on the soft palate and back roof of the mouth in adults are particularly sensitive to sour and bitter tastes, whereas the tongue receptors are relatively more sensitive to sweet and salty tastes. Some loss of taste sensitivity suffered among denture wearers may occur because of mechanical interference of the dentures with taste receptors on the roof of the mouth. Taste buds on the human tongue exhibit sensitivity to specific tastes.

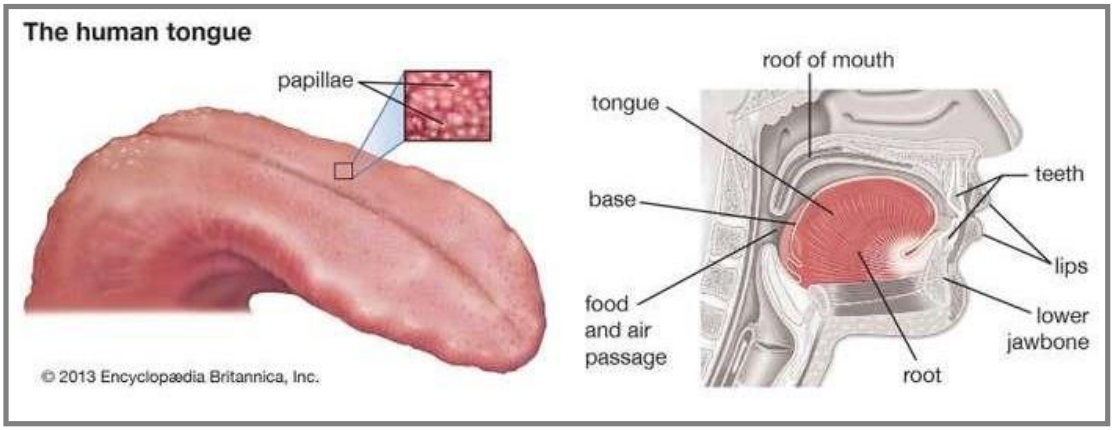


Fig. 5.6. Human Tongue

d) Nerve supply

There is no single sensory nerve for taste. The anterior (front) two-thirds of the tongue is supplied by one nerve (the lingual nerve), the back of the tongue by another (the glossopharyngeal nerve), and the throat and larynx by certain branches of a third (the vagus nerve), all of which subserve touch, temperature, and pain sensitivity in the tongue, as well as taste. The gustatory fibres of the anterior tongue leave the lingual nerve to form the chorda tympani, a slender nerve that traverses the eardrum on the way to the brainstem. When the chorda tympani at one ear is cut or damaged (by injury to the eardrum), taste buds begin to disappear and gustatory sensitivity is lost on the

anterior two-thirds of the tongue on the same side. The taste fibres from all the sensory nerves from the mouth come together in the medulla oblongata. Here and at all levels of the brain, gustatory fibres run in distinct and separate pathways, lying close to the pathways for other modalities from the tongue and mouth cavity. From the medulla, the gustatory fibres ascend by a pathway to a small cluster of cells in the thalamus and then to a taste-receiving area in the anterior cerebral cortex.

e) Physiological basis of taste

No simple relationship has been found between the chemical composition of stimuli and the quality of gustatory experience except in the case of acids. The taste qualities of inorganic salts (such as potassium bromide) are complex; epsom salt (magnesium sulfate) is commonly sensed as bitter, while table salt (sodium chloride) is typical of sodium salts, which usually yield the familiar saline taste. Sweet and bitter tastes are elicited by many different classes of chemical compound.

Theorists of taste sensitivity classically posited only four basic or primary types of human taste receptors, one for each gustatory quality: salty, sour, bitter, and sweet. Yet, recordings of sensory impulses in the taste nerves of laboratory animals show that many individual nerve fibres from the tongue are of mixed sensitivity, responding to more than one of the basic taste stimuli, such as acid plus salt or acid plus salt plus sugar. Other individual nerve fibres respond to stimuli of only one basic gustatory quality. Most numerous, however, are taste fibres subserving two basic taste sensitivities; those subserving one or three qualities are about equal in number and next most frequent; fibres that respond to all four primary stimuli are least common. Mixed sensitivity may be only partly attributed to multiple branches of taste nerve endings. In humans, tastes of sugars, synthetic sweeteners, weak salt solutions, and some unpleasant medications are blocked by gymnemic acid, a drug obtained from *Gymnema* bushes native to India. Among some laboratory animals, gymnemic acid blocks only the nerve response to sugar, even if the fibre mediates other taste qualities. Such a multiresponsive fibre still can transmit taste impulses (e.g., for salt or sour), so that blockage by the drug can be attributed to chemically specific sites or cells in the taste bud.

In some animals (e.g., the cat), specific taste receptors appear to be activated by water; these water receptors are inhibited by weak saline solutions. Water taste might be considered a fifth gustatory quality in addition to the basic four.

f) The qualities of taste

Sour

The hydrogen ions of acids (e.g., hydrochloric acid) are largely responsible for the sour taste; however, although a stimulus grows more sour as its hydrogen ion (H^+) concentration increases, this factor alone does not determine sourness. Weak organic acids (e.g., the acetic acid in vinegar) taste more sour than would be predicted from their hydrogen ion concentration alone; apparently the rest of the acid molecule affects the efficiency with which hydrogen ions stimulate.

Salt

Although saltiness is often associated with water-soluble salts, most such compounds (except sodium chloride) have complex tastes such as bitter-salt or sour-salt. Salts of low molecular weight are predominantly salty, while those of higher molecular weight tend to be bitter. The salts of heavy metals such as mercury have a metallic taste, although some of the salts of lead (especially lead acetate) and beryllium are sweet. Both parts of the molecule (e.g., lead and acetate) contribute to taste quality and to stimulating efficiency. The following is a series for degree of saltiness, in decreasing order: ammonium (most salty), potassium, calcium, sodium, lithium, and magnesium salts (least salty).

Sweet

Except for some salts of lead or beryllium, sweetness is associated largely with organic compounds (such as alcohols, glycols, sugars, and sugar derivatives). Sensitivity to synthetic sweeteners (e.g., saccharin) is especially remarkable; the taste of saccharin can be detected in a dilution 700 times weaker than that required for cane sugar. The stereochemical (spatial) arrangement of atoms within a molecule may affect its taste; thus, slight changes within a sweet molecule will make it bitter or tasteless (*see* the fig. 5.7). Several theorists have proposed that the common feature for all of the sweet stimuli is the presence in the molecule of a proton acceptor, such as the OH (hydroxyl) components of carbohydrates (e.g., sugars) and many other sweet-tasting compounds. It has also been theorized that such molecules will not taste sweet unless they are of appropriate size.

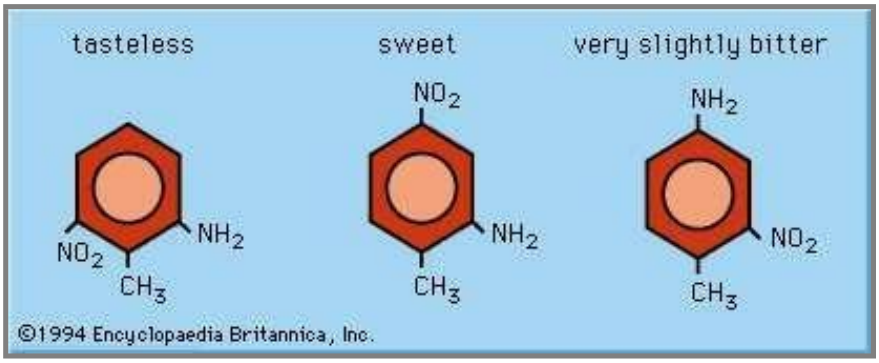


Fig.5.7. Effects of molecular arrangement on taste sensation.

Bitter

The experience of a bitter taste is elicited by many classes of chemical compounds and often is associated with sweet and other gustatory qualities. Among bitter substances are such alkaloids (often toxic) as quinine, caffeine, and strychnine. Most of these substances have extremely low taste thresholds and are detectable in very weak concentrations. The size of such molecules is theoretically held to account for whether or not they will taste bitter. An increase in molecular weight of inorganic salts or an increase in length of chains of carbon atoms in organic molecules tends to be associated with increased bitterness.

A substantial minority of people exhibit specific taste blindness, an inability to detect as bitter such chemicals as phenylthiocarbamide (PTC). Taste blindness for PTC and other carbamides appears to be hereditary (as a recessive trait), occurring in about a third of Europeans and in roughly 40 percent of the people in Western India. Taste blindness for carbamides is not correlated with insensitivity to other bitter stimuli.

g) Factors affecting taste sensitivity

Fluids of extreme temperature, especially those that are cold, may produce temporary taste insensitivity. People generally seem to taste most acutely when the stimulus is at or slightly below body temperature. When the tongue and mouth are first adapted to the temperature of a taste solution, sugar sensitivity increases with temperature rise, salt and quinine sensitivity decrease, and acid sensitivity is relatively unchanged. Gustatory adaptation (partial or complete disappearance of taste sensitivity) may occur if a solution is held in the mouth for a period of time. The effect of one adapting stimulus on the sensitivity to another one (cross adaptation) is especially common with substances that are chemically similar and that elicit the same taste

quality. Adaptation to sodium chloride will reduce one's ability to sense the saltiness of a variety of the inorganic salts but will leave undiminished or even enhance such qualities as bitterness, sweetness, or sourness that were part of the taste of the salt before adaptation. Likewise, adaptation by one acid may reduce sensitivity to the sourness of other acids.

Adaptation studies often are complicated by so-called contrast effects; for example, people say that distilled water tastes sweet following their exposure to a weak acid. Water may take on other taste qualities as well; following one's adaptation to a sour-bitter chemical such as urea, water may taste salty. Adaptation tends to diminish or enhance the effect of a subsequent stimulus depending on whether the two stimuli normally elicit the same or a contrasting taste. Thus, the adapted sweetness of water and all normally sweet-tasting substances are enhanced after one has tasted acid (sour). The bitterness of tea and coffee or the sourness of lemon are masked or suppressed by sugar or saccharin.

The human gustatory difference threshold (for a just noticeable difference in intensity) is approximately a 20 percent change in concentration. For very weak taste stimuli, however, the threshold sensitivity is less.

5.1.3. Smell (olfactory) sense

In humans the olfactory receptors are located high in the nasal cavity. The yellow-pigmented olfactory membrane covers about 2.5 square cm (0.4 square inch) on each side of the inner nose. Olfactory receptors are long thin cells ending in 6 to 12 delicate hairs called cilia that project into and through the mucus that normally covers the nasal epithelium, or lining. The end of each receptor narrows to a fine nerve fibre, which, along with many others, travels through a channel in the bony roof of the nasal cavity and enters either of two specialized structures called olfactory bulbs, stem like projections under the front part of the brain to end in a series of intricate basketlike clusters called glomeruli. Each glomerulus receives impulses from about 26,000 receptors and sends them on through other cells, eventually to reach higher olfactory centres at the base of the brain. Fibres also cross from one olfactory bulb to the other.

Odorous molecules are carried to the olfactory region by slight eddies in the air during quiet breathing, but vigorous sniffing brings a surge of air into the olfactory region. Odour sensitivity may be impaired by blocking the nasal passages mechanically, as when membranes are congested by infection.

Pain endings of the trigeminal nerve fibres are widely distributed throughout the nasal cavity, including the olfactory region. Relatively mild odorants, such as orange oil, as well as the more obvious irritants, such as ammonia, stimulate such nerve endings as well as the olfactory receptors.

a) Olfactory qualities

The vocabulary of odour is rich with names of substances that elicit a great variety of olfactory qualities. One of the best-known published psychological attempts at classification was in 1916 on the basis of more than 400 different scents on human subjects. On the basis of the apparent similarities of perceived odour quality or confusions in naming, it was concluded that there were six main odour qualities: fruity, flowery, resinous, spicy, foul, and burned.

Electrical activity can be detected with fine insulated wires inserted into the olfactory bulb. Portions of the olfactory bulb toward the anterior or oral region in the rabbit are found to be more sensitive to water-soluble substances, whereas the more posterior parts of the olfactory bulb are more sensitive to fat-soluble substances. In addition, when very fine electrodes are used, individual cells (mitral cells) are sensitive to different groups of chemicals. Evidence for the existence of only a few primary receptors, however, does not emerge from such studies; a variety of different combinations of sensitivity has been found. Similarly, recordings from the primary receptor nerve fibres reveal different patterns of sensitivity. Electrical recording of this type also shows that olfactory sensitivity can be enhanced by a painful stimulus, such as a pinch on the foot. This appears to be a reflex that serves to enhance the detection of dangerous stimuli in the environment. Different parts of the olfactory neural pathways seem to be selectively tuned to discriminate different classes of olfactory information. For example, the third- and fourth-order olfactory neurons found beyond the olfactory bulb of the rat seem particularly concerned with distinguishing the odour of sexually receptive females. These neurons appear to be especially important in the preference the male rat shows for the smell of urine from the female in heat.

b) Odourous substances

To be odorous, a substance must be sufficiently volatile for its molecules to be given off and carried into the nostrils by air currents. The solubility of the substance also seems to play a role; chemicals that are soluble in water or fat tend to be strong odorants. No unique chemical or physical property that can be said to elicit the experience of odour has yet been discovered.

Only seven of the chemical elements are odorous: fluorine, chlorine, bromine, iodine, oxygen (as ozone), phosphorus, and arsenic. Most odorous substances are organic (carbon-containing) compounds in which both the arrangement of atoms within the molecule as well as the particular chemical groups that comprise the molecule influence odour. Stereoisomers (i.e., different spatial arrangements of the same molecular components) may have different odours. On the other hand, a series of different molecules that derive from benzene all have a similar odour. It is of historic interest that the first benzene derivatives studied by chemists were found in pleasant-smelling substances from plants (such as oil of wintergreen or oil of anise), and so the entire class of compounds was labelled aromatic. Subsequently, other so-called aromatic compounds were identified that have less-attractive odours.

The scent of flowers and roots (such as ginger) depends upon the presence of minute quantities of highly odorous essential oils. Although the major odour constituents can be identified by chemical analysis, some botanical essences are so complex that their odours can be duplicated only by adding them in small amounts to synthetic formulations.

c) Odour sensitivity

In spite of the relative inaccessibility of the olfactory receptor cells, odour stimuli can be detected at extremely low concentrations. Olfaction is said to be 10,000 times more sensitive than taste. A threshold value for the odorant ethyl mercaptan (found in rotten meat) has been cited in the range of 1/400,000,000th of a milligram per litre of air. A just-noticeable difference in odour intensity may be apparent when there is a 20 percent increase in odorant strength, but at low concentrations as much as a 100 percent increase in concentration may be required. Temperature influences the strength of an odour by affecting the volatility and therefore the emission of odorous particles from the source; humidity also affects odour for the same reasons. Hunting dogs can follow a spoor (odour trail) most easily when high humidity retards evaporation and dissipation of the odour. Perfumes contain chemicals called fixatives, added to retard evaporation of the more volatile constituents. The temporary anosmia (absence of sense of smell) following colds may be complete or partial; in the latter case, only the odours of certain substances are affected. Paranosmia (change in perceived odour quality) also may occur during respiratory infections. Changes in sensitivity are reported to occur in women during the menstrual cycle, particularly in regard to certain odorants (steroids) related to sex hormones. Olfactory sensitivity also is said to become more acute during hunger.

Adaptation to odours is so striking that the stench of a junkyard or chemical laboratory ceases to be a nuisance after a few minutes have passed. Olfactory adaptation, as measured by a rise in threshold, is especially pronounced for stronger odours. Cross adaptation (between different odours) may take place; thus, eucalyptus oil may be difficult to detect after one becomes adapted to the smell of camphor. Adaptation was long regarded solely as the result of changes in the olfactory receptor; however, the receptor cells in the nose seem to adapt only partially. Rhythmic discharges continue in the olfactory bulb long after one ceases to detect an odour. Apparently, some olfactory adaptation may occur in the brain as well as in the sense organ.

d) Effects on behaviour

Mammals in the wild state appear to utilize their odour glands for sexual attraction. Rats show a preference for the branch of a maze that has been scented with the odour of a sexually receptive female. It is likely that some rudiments of these effects operate in humans. The most sexually provocative perfumes have a high proportion of musk or a musklike odour. Genuine musk is derived from the sexual glands of the musk deer and is chemically related to human sex hormones; odour sensitivity in humans varies with the menstrual cycle.

Among laboratory animals the secretion of reproductive hormones can be markedly influenced by odour stimulation. This seems to be an innate physiological process rather than the result of learning. When the odour of a strange male is presented to a recently mated female, pregnancy block occurs. The normal hormonal changes following copulation are blocked under these conditions, and the fertilized egg fails to survive. A related study of the periodicity and length of the menstrual cycle in women exposed to the normal odours of men suggests that there may be similar effects among humans. Human behaviour, though it is molded and shaped by custom and culture, has many of its roots in basic sensual appetites.

5.2. Circulatory system

The circulatory system can be compared to a system of interconnected, one-way roads that range from superhighways to back alleys. Like a network of roads, the job of the circulatory system is to allow the transport of materials from one place to another. As described in Fig 5.8, the materials carried by the circulatory system include

hormones, oxygen, cellular wastes, and nutrients from digested food. Transport of all these materials is necessary to maintain homeostasis of the body. The main components of the circulatory system are the heart, blood vessels, and blood. Each of these components is described in detail below.

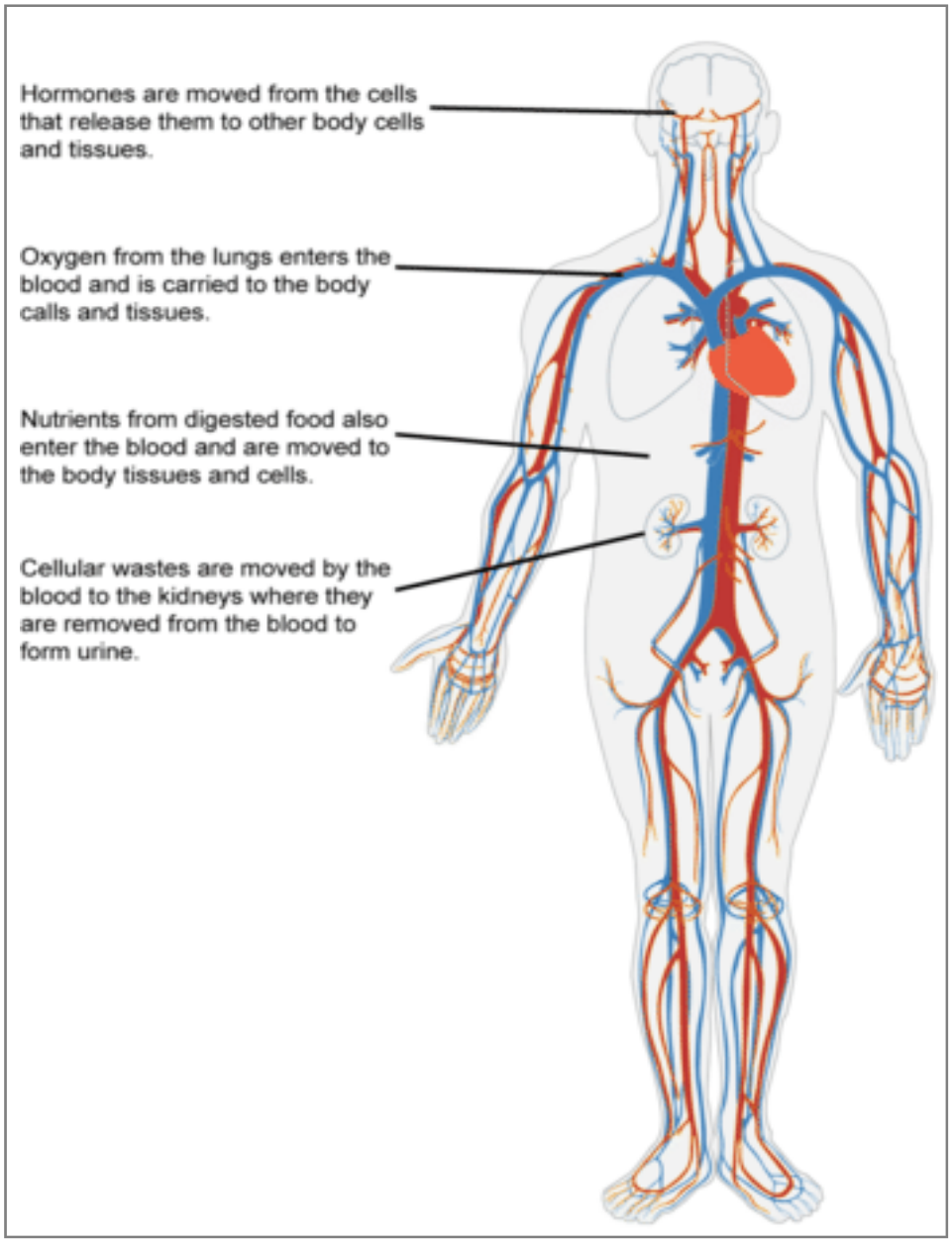


Fig.5.8. Circulatory system of human body

5.2.1 The Heart

The heart is a muscular organ in the chest. It consists mainly of cardiac muscle tissue and pumps blood through blood vessels by repeated, rhythmic contractions. The heart has four chambers, as shown in fig. 5.9 : two upper atria (singular, atrium) and two lower ventricles. Valves between chambers keep blood flowing through the heart in just one direction.

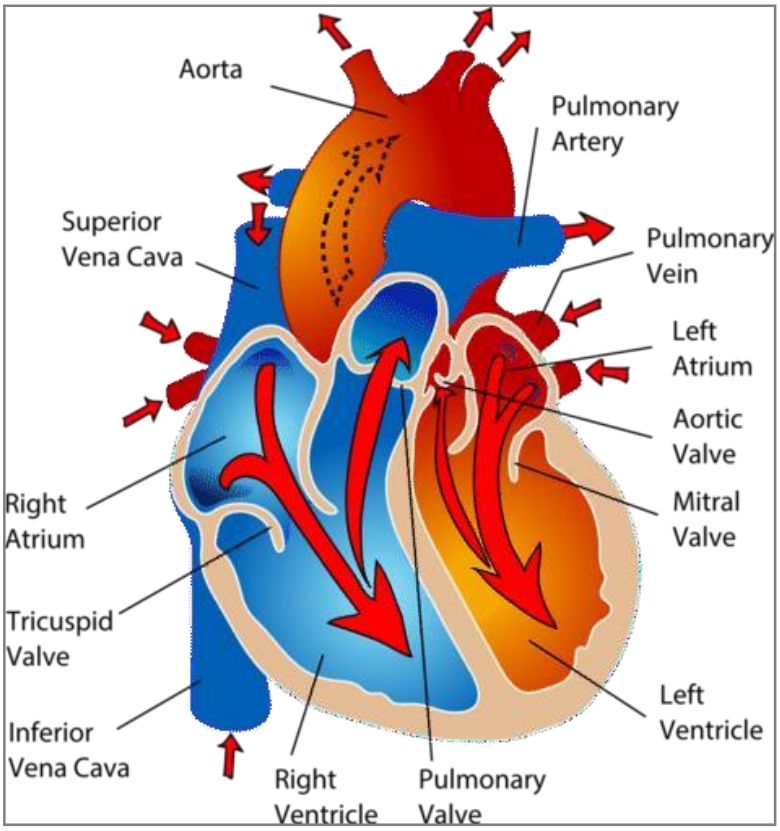


Fig.5.9. Human Heart

a) Blood Flow Through the Heart

Blood flows through the heart in two separate loops, which are indicated by the arrows in figure above.

1. Blood from the body enters the right atrium of the heart. The right atrium pumps the blood to the right ventricle, which pumps it to the lungs. This loop is represented by the blue arrows in figure above.

2. Blood from the lungs enters the left atrium of the heart. The left atrium pumps the blood to the left ventricle, which pumps it to the body. This loop is represented by the red arrows in figure above.

b) Heartbeat

Unlike skeletal muscle, cardiac muscle contracts without stimulation by the nervous system. Instead, specialized cardiac muscle cells send out electrical impulses that stimulate the contractions. As a result, the atria and ventricles normally contract with just the right timing to keep blood pumping efficiently through the heart..

c) Blood Vessels

Blood vessels form a network throughout the body to transport blood to all the body cells. There are three major types of blood vessels: arteries, veins, and capillaries. All three are shown in fig. 5.10 and described below.

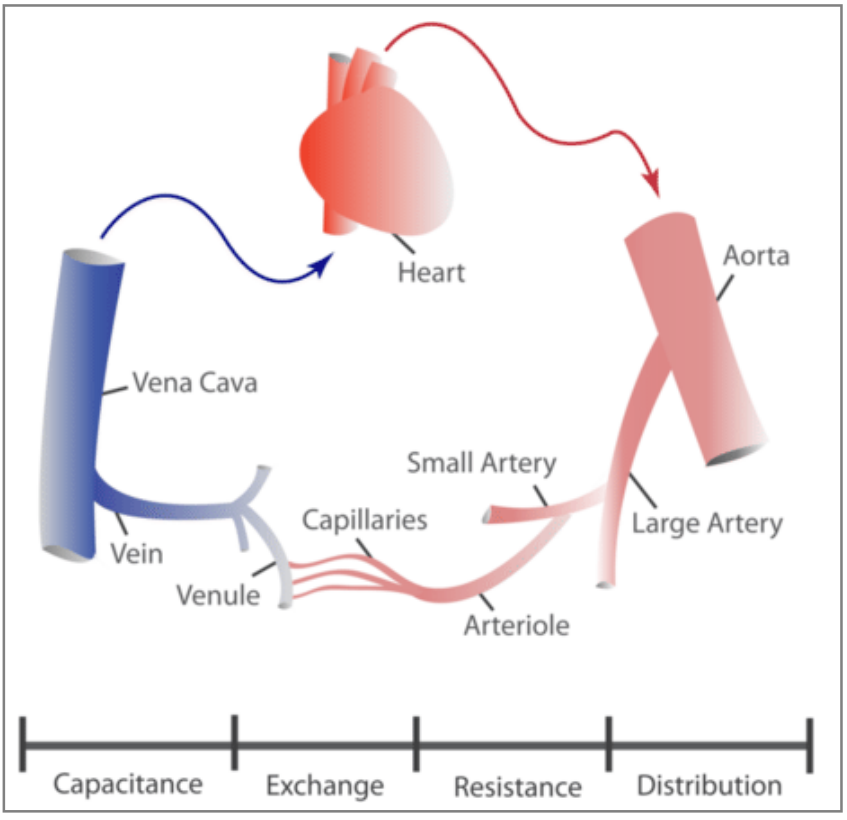


Fig.5.10. Blood vessels network

i. **Arteries** are muscular blood vessels that carry blood away from the heart. They have thick walls that can withstand the pressure of blood being pumped by the heart. Arteries generally carry oxygen-rich blood. The largest artery is the aorta, which receives blood directly from the heart.

ii. **Veins** are blood vessels that carry blood toward the heart. This blood is no longer under much pressure, so many veins have valves that prevent backflow of blood. Veins generally carry deoxygenated blood. The largest vein is the inferior vena cava, which carries blood from the lower body to the heart.

iii. **Capillaries** are the smallest type of blood vessels. They connect very small arteries and veins. The exchange of gases and other substances between cells and the blood takes place across the extremely thin walls of capillaries.

d) Blood Vessels and Homeostasis

Blood vessels help regulate body processes by either constricting (becoming narrower) or dilating (becoming wider). These actions occur in response to signals from the autonomic nervous system or the endocrine system. Constriction occurs when the muscular walls of blood vessels contract. This reduces the amount of blood that can flow through the vessels (see fig. 5.11). Dilation occurs when the walls relax. This increases blood flows through the vessels.

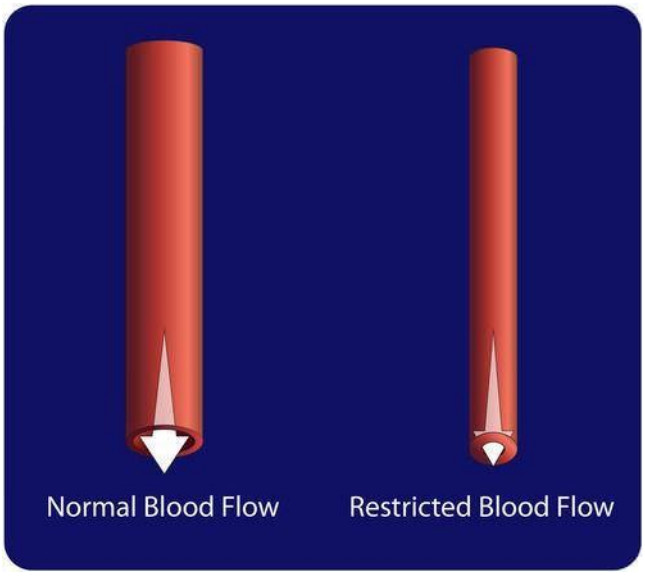


Fig.5.11. Blood Flow

Constriction and dilation allow the circulatory system to change the amount of blood flowing to different organs. For example, during a fight-or-flight response, dilation and constriction of blood vessels allow more blood to flow to skeletal muscles and less to flow to digestive organs. Dilation of blood vessels in the skin allows more blood to flow to the body surface so the body can lose heat. Constriction of these blood vessels has the opposite effect and helps conserve body heat.

e) Blood Vessels and Blood Pressure

The force exerted by circulating blood on the walls of blood vessels is called blood pressure. Blood pressure is highest in arteries and lowest in veins. When you have your blood pressure checked, it is the blood pressure in arteries that is measured. High blood pressure, or hypertension, is a serious health risk but can often be controlled with lifestyle changes or medication.

f) Pulmonary and Systemic Circulations

The circulatory system actually consists of two separate systems: pulmonary circulation and systemic circulation.

1. Pulmonary Circulation

Pulmonary circulation is the part of the circulatory system that carries blood between the heart and lungs (the term *pulmonary* means ‑of the lungs‖). It is illustrated in fig. 5.12. Deoxygenated blood leaves the right ventricle through pulmonary arteries, which transport it to the lungs. In the lungs, the blood gives up carbon dioxide and picks up oxygen. The oxygenated blood then returns to the left atrium of the heart through pulmonary veins.

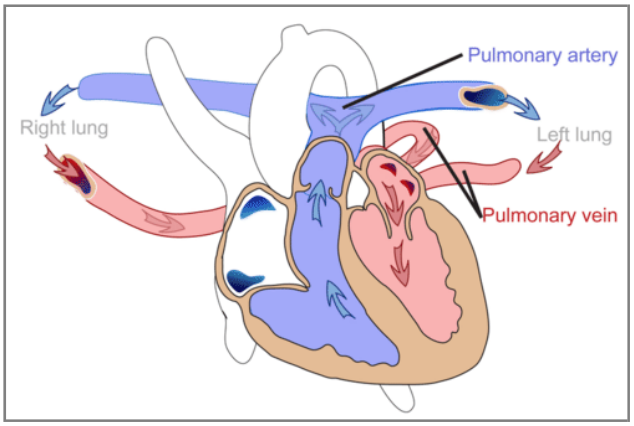


Fig. 5.12. Pulmonary Circulation

2. Systemic Circulation

Systemic circulation is the part of the circulatory system that carries blood between the heart and body. It is illustrated in fig 5.13. Oxygenated blood leaves the left ventricle through the aorta. The aorta and other arteries transport the blood throughout the body, where it gives up oxygen and picks up carbon dioxide. The deoxygenated blood then returns to the right atrium through veins.

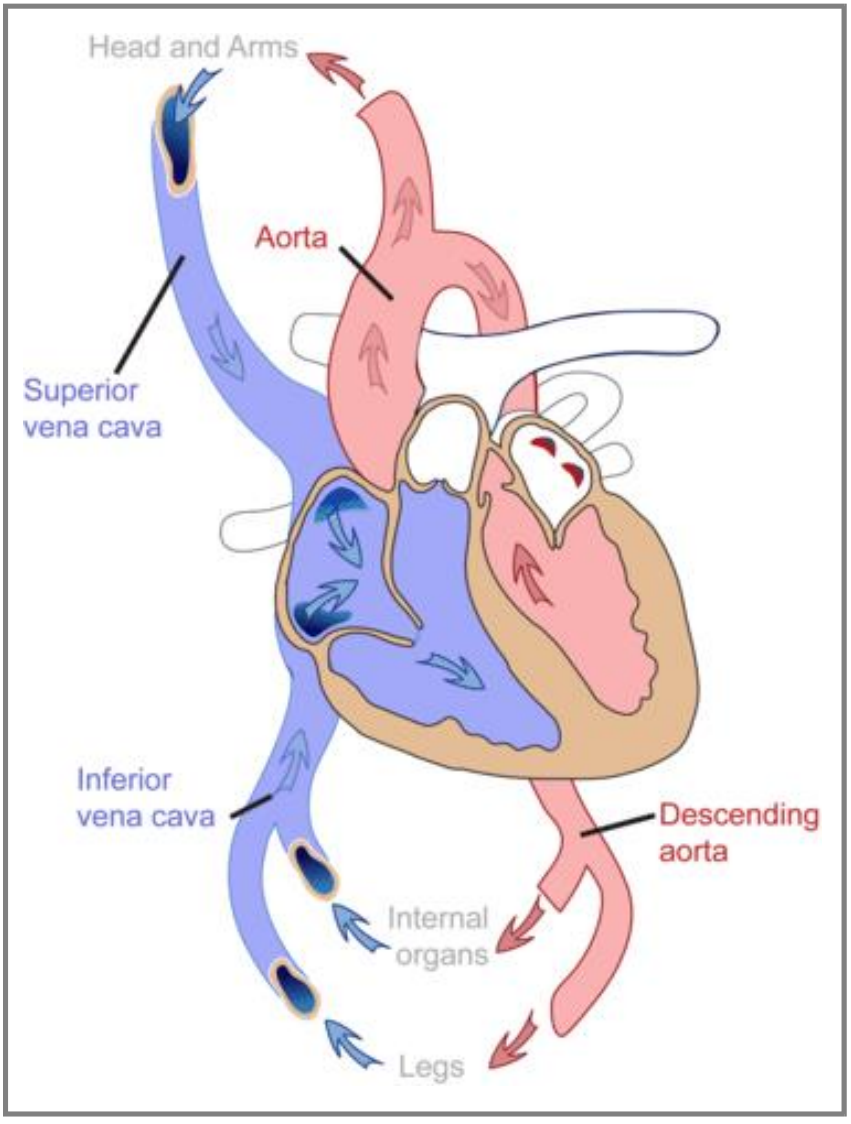


Fig. 5.13. Systemic circulation

g) Cardiovascular Disease

Diseases of the heart and blood vessels, called cardiovascular diseases **(CVD)**, are very common. The leading cause of CVD is atherosclerosis.

Atherosclerosis

Atherosclerosis is the buildup of plaque inside arteries (see fig. 5.14). Plaque consists of cell debris, cholesterol, and other substances. Factors that contribute to plaque buildup include a high-fat diet and smoking. As plaque builds up, it narrows the arteries and reduces blood flow.

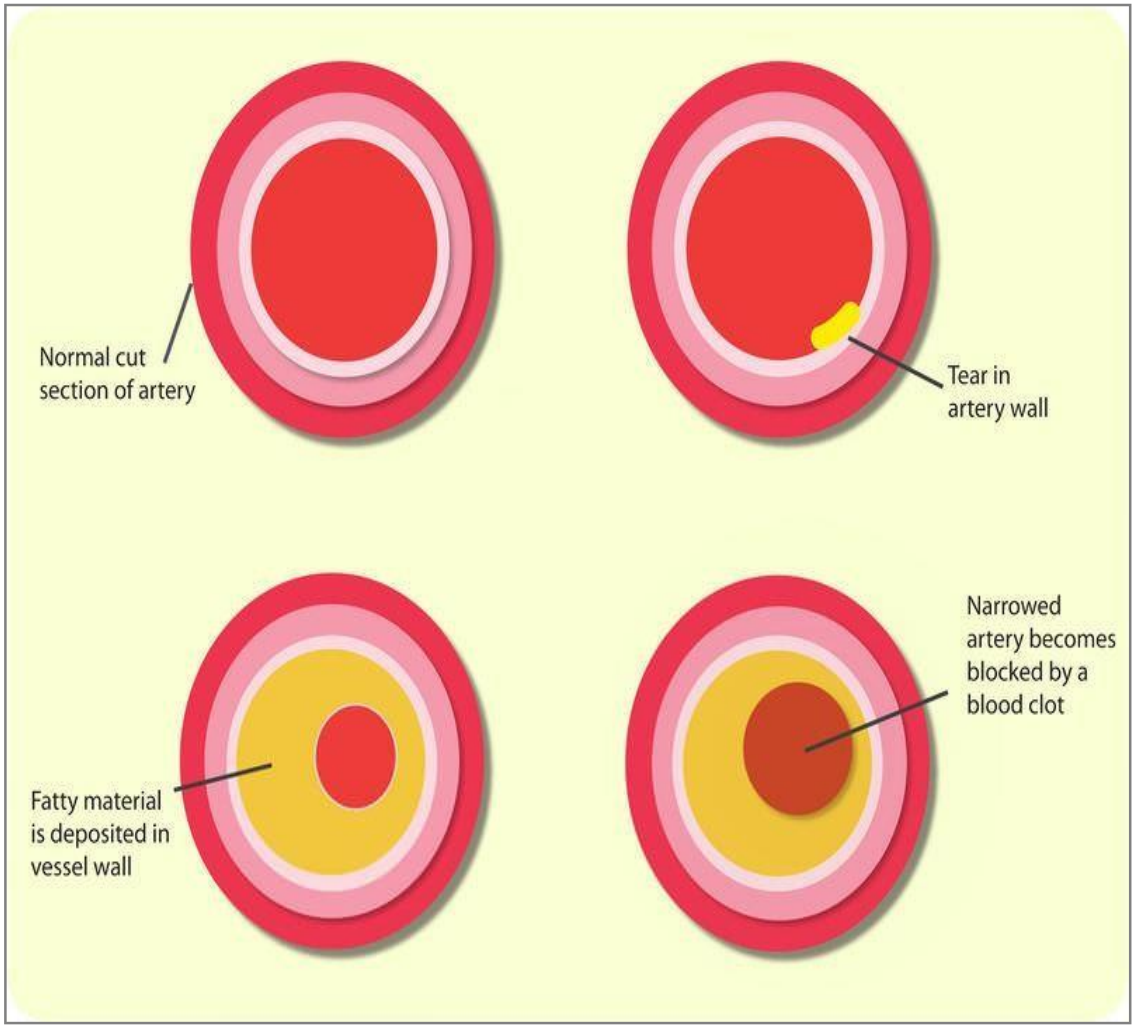


Fig 5.14. Cardiovascular Disease

The fatty material inside the artery on the right is plaque. Notice how much narrower the artery has become. Less blood can flow through it than the normal artery.

h) Coronary Heart Disease

Atherosclerosis of arteries that supply the heart muscle is called coronary heart disease. This disease may or may not have symptoms such as chest pain. As the disease progresses, there is an increased risk of heart attack. A **heart attack** occurs when the blood supply to part of the heart muscle is blocked and cardiac muscle fibers die. Coronary heart disease is the leading cause of death of adults in the U.S.

Preventing Cardiovascular Disease

Many factors may increase the risk of developing coronary heart disease and other CVDs. The risk of CVDs increases with age and is greater in males than females at most ages. Having a close relative with CVD also increases the risk. These factors cannot be controlled, but other risk factors can, including smoking, lack of exercise, and high-fat diet. By making healthy lifestyle choices, you can reduce your risk of developing CVD.

i) Blood

Blood is a fluid connective tissue. It circulates throughout the body through blood vessels by the pumping action of the heart. Blood in arteries carries oxygen and nutrients to all the body's cells. Blood in veins carries carbon dioxide and other wastes away from the cells to be excreted. Blood also defends the body against infection, repairs body tissues, transports hormones, and controls the body's pH.

j) Composition of Blood

The fluid part of blood is called plasma. It is a watery golden-yellow liquid that contains many dissolved substances and blood cells. Types of blood cells in plasma include red blood cells, white blood cells, and platelets (see fig 5.15).

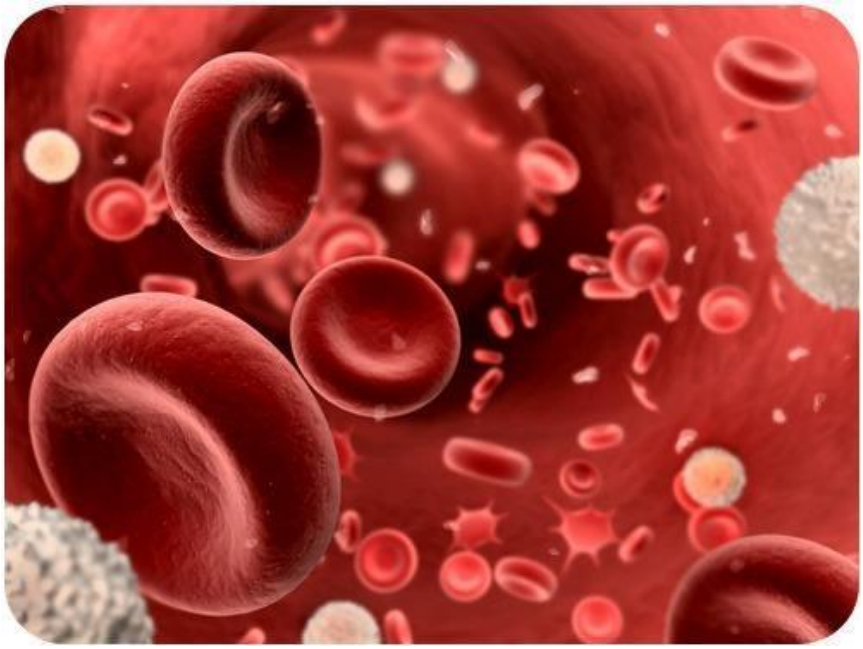


Fig.5.15. Blood cells

- The trillions of **red blood cells** in blood plasma carry oxygen. Red blood cells contain hemoglobin, a protein with iron that binds with oxygen.
- **White blood cells** are generally larger than red blood cells but far fewer in number. They defend the body in various ways. For example, white blood cells called phagocytes swallow and destroy microorganisms and debris in the blood.
- **Platelets** are cell fragments involved in blood clotting. They stick to tears in blood vessels and to each other, forming a plug at the site of injury. They also release chemicals that are needed for clotting to occur.

k) Blood Type

Blood type is a genetic characteristic associated with the presence or absence of certain molecules, called antigens, on the surface of red blood cells. The most commonly known blood types are the ABO and Rhesus blood types.

(1) ABO blood type is determined by two common antigens, often referred to simply as antigens A and B. A person may have blood type A (only antigen A), B (only antigen B), AB (both antigens), or O (no antigens).

- Rhesus blood type is determined by one common antigen. A person may either have the antigen (Rh^+) or lack the antigen (Rh^-).

Blood type is important for medical reasons. A person who needs a blood transfusion must receive blood that is the same type as his or her own. Otherwise, the transfused blood may cause a potentially life-threatening reaction in the patient's bloodstream.

5.3. EXCRETORY SYSTEM

Excretion is the process of removing wastes and excess water from the body. It is one of the major ways the body maintains homeostasis. Although the kidneys are the main organs of excretion, several other organs also excrete wastes. They include the large intestine, liver, skin, and lungs. All of these organs of excretion, along with the kidneys, make up the **excretory system**. This lesson focuses on the role of the kidneys in excretion. The roles of the other excretory organs are summarized below:

- The large intestine eliminates solid wastes that remain after the digestion of food.
- The liver breaks down excess amino acids and toxins in the blood.
- The skin eliminates excess water and salts in sweat.
- The lungs exhale water vapor and carbon dioxide.

5.3.1. Urinary System

The kidneys are part of the urinary system, which is shown in Fig 5.15. The main function of the urinary system is to filter waste products and excess water from the blood and excrete them from the body. The kidneys are the chief organs of the urinary system.

a. Kidneys and Nephrons

The kidneys are a pair of bean-shaped organs just above the waist. A cross-section of a kidney is shown in fig 5.16. The function of the kidney is to filter blood and form urine. **Urine** is the liquid waste product of the body that is excreted by the urinary system. **Nephrons** are the structural and functional units of the kidneys. A single kidney may have more than a million nephrons!

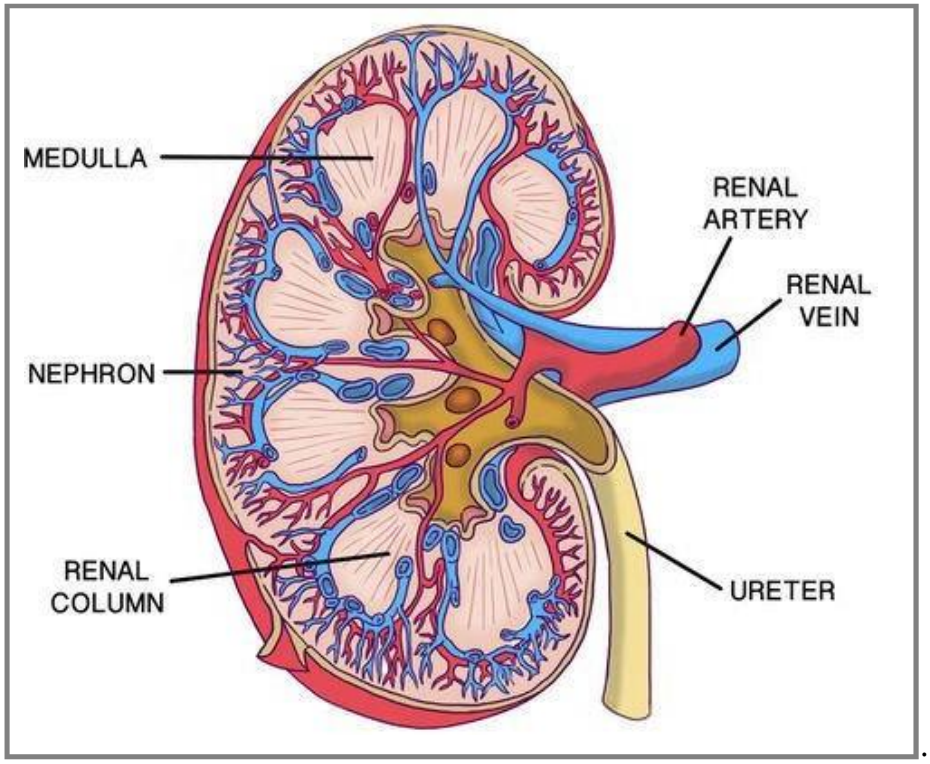


Fig.5.16. A cross-section view of Kidney

b. Filtering Blood and Forming Urine

As shown in Fig.5.17, each nephron is like a tiny filtering plant. It filters blood and forms urine in the following steps:

1. Blood enters the kidney through the renal artery, which branches into capillaries. When blood passes through capillaries of the glomerulus of a nephron, blood pressure forces some of the water and dissolved substances in the blood to cross the capillary walls into Bowman's capsule.

2. The filtered substances pass to the renal tubule of the nephron. In the renal tubule, some of the filtered substances are reabsorbed and returned to the bloodstream. Other substances are secreted into the fluid.

3. The fluid passes to a collecting duct, which reabsorbs some of the water and returns it to the bloodstream. The fluid that remains in the collecting duct is urine.

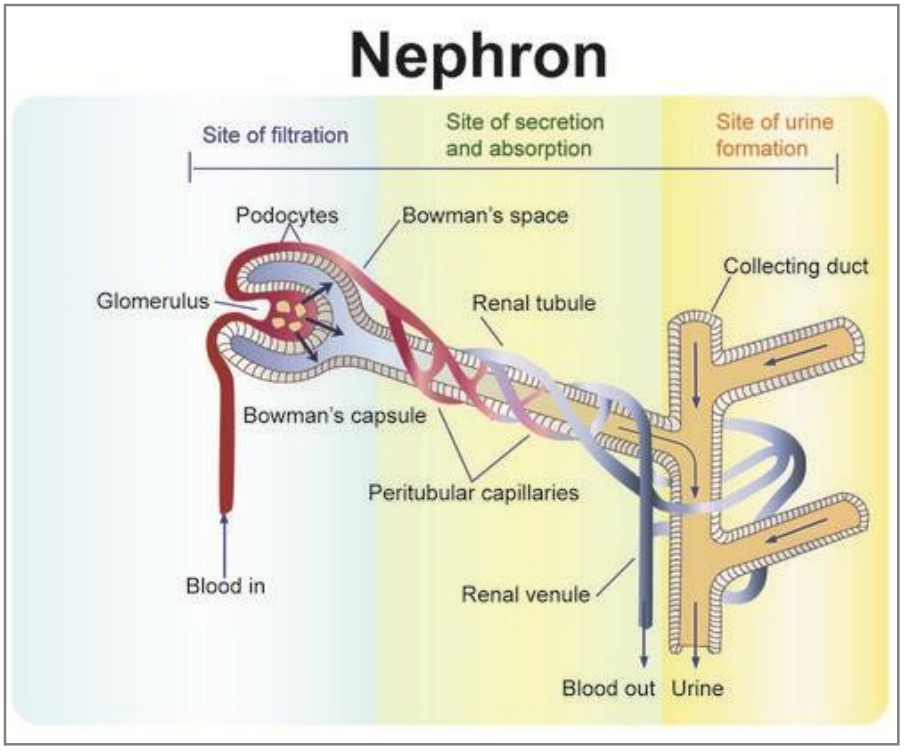


Fig.5.17. Parts of a Nephron

c. Excretion of Urine

From the collecting ducts of the kidneys, urine enters the ureters, two muscular tubes that move the urine by peristalsis to the bladder (see figure above). The bladder is a hollow, sac-like organ that stores urine. When the bladder is about half full, it sends a nerve impulse to a sphincter to relax and let urine flow out of the bladder and into the urethra. The urethra is a muscular tube that carries urine out of the body. Urine leaves the body through another sphincter in the process of **urination**. This sphincter and the process of urination are normally under conscious control.

d. Kidneys and Homeostasis

The kidneys play many vital roles in homeostasis. They filter all the blood in the body many times each day and produce a total of about 1.5 liters of urine. The kidneys control the amount of water, ions, and other substances in the blood by excreting more or less of them in urine. The kidneys also secrete hormones that help maintain homeostasis. Erythropoietin, for example, is a kidney hormone that stimulates bone marrow to produce red blood cells when more are needed. The kidneys themselves are

also regulated by hormones. For example, antidiuretic hormone from the hypothalamus stimulates the kidneys to produce more concentrated urine when the body is low on water.

e. Kidney Disease and Dialysis

A person can live a normal, healthy life with just one kidney. However, at least one kidney must function properly to maintain life. Diseases that threaten the health and functioning of the kidneys include kidney stones, infections, and diabetes.

- Kidney stones are mineral crystals that form in urine inside the kidney. They may be extremely painful. If they block a ureter, they must be removed so urine can leave the kidney and be excreted.
- Bacterial infections of the urinary tract, especially the bladder, are very common. Bladder infections can be treated with antibiotics prescribed by a doctor. If untreated, they may lead to kidney damage.
- Uncontrolled diabetes may damage capillaries of nephrons. As a result, the kidneys lose much of their ability to filter blood. This is called **kidney failure**. The only cure for kidney failure is a kidney transplant, but it can be treated with dialysis. **Dialysis** is a medical procedure in which blood is filtered through a machine (see figure below).

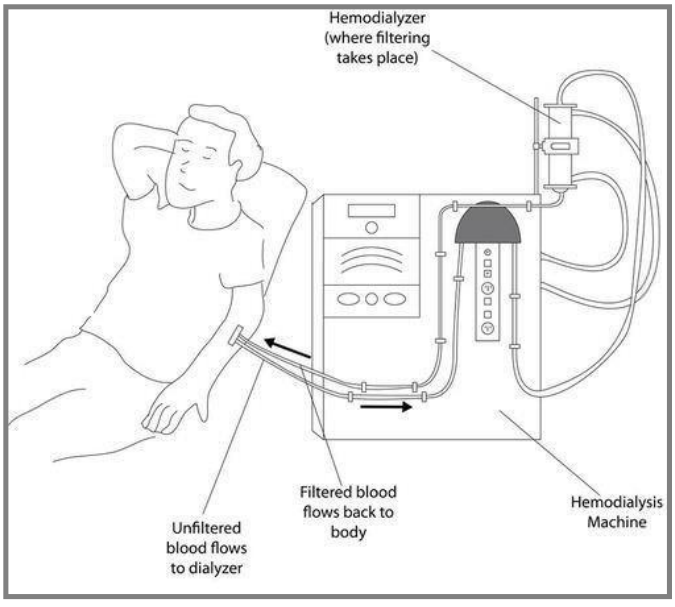


Fig.5.18. Dialysis machine filters a patient's blood.

5.4. Hormonal Regulation

Maintaining a proper water balance in the body is important to avoid dehydration or over-hydration (hyponatremia). The water concentration of the body is monitored by osmoreceptors in the hypothalamus, which detect the concentration of electrolytes in the extracellular fluid. The concentration of electrolytes in the blood rises when there is water loss caused by excessive perspiration, inadequate water intake, or low blood volume due to blood loss. An increase in blood electrolyte levels results in a neuronal signal being sent from the osmoreceptors in hypothalamic nuclei. The anterior pituitary is composed of glandular cells that secrete protein hormones. The pituitary gland has two components: anterior and posterior. The posterior pituitary is an extension of the hypothalamus. It is composed largely of neurons that are continuous with the hypothalamus.

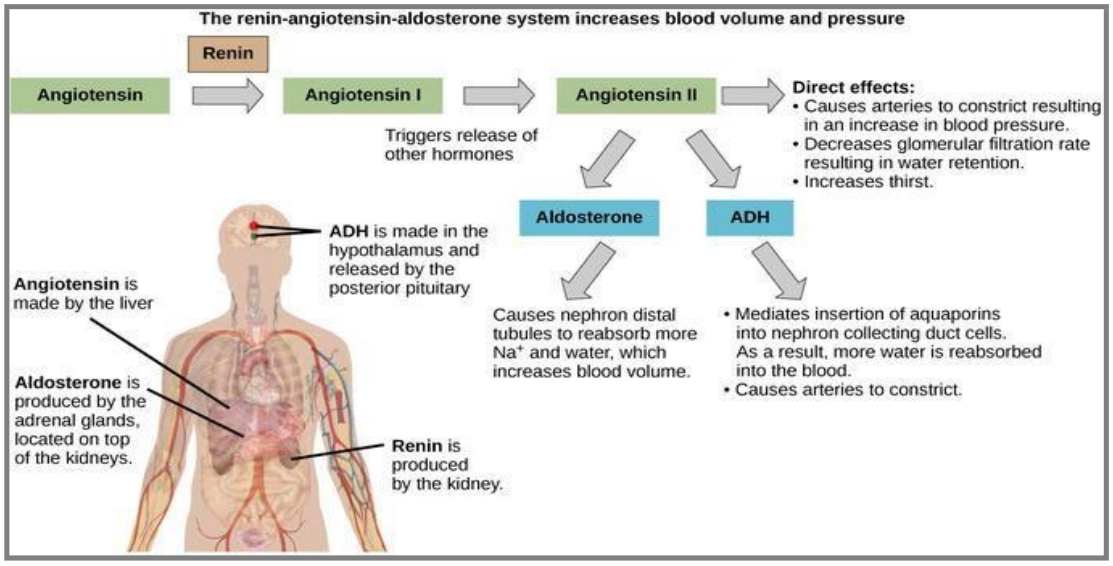
Antidiuretic Hormone (ADH)

The hypothalamus produces a polypeptide hormone known as antidiuretic hormone (ADH), which is transported to and released from the posterior pituitary gland. The principal action of ADH is to regulate the amount of water excreted by the kidneys. As ADH (which is also known as vasopressin) causes direct water reabsorption from the kidney tubules, salts and wastes are concentrated in what will eventually be excreted as urine. The hypothalamus controls the mechanisms of ADH secretion, either by regulating blood volume or the concentration of water in the blood. Dehydration or physiological stress can cause an increase of osmolarity above threshold levels, which, in turn, raises ADH secretion and water retention, causing an increase in blood pressure. ADH travels in the bloodstream to the kidneys where it changes the kidneys to become more permeable to water by temporarily inserting water channels, aquaporins, into the kidney tubules. Water moves out of the kidney tubules through the aquaporins, reducing urine volume. The water is reabsorbed into the capillaries, lowering blood osmolarity back toward normal. As blood osmolarity decreases, a negative feedback mechanism reduces osmoreceptor activity in the hypothalamus; ADH secretion is reduced. ADH release can be reduced by certain substances, including alcohol, which can cause increased urine production and dehydration.

Chronic underproduction of ADH or a mutation in the ADH receptor results in diabetes insipidus. If the posterior pituitary does not release enough ADH, water cannot be retained by the kidneys and is lost as urine. This causes increased thirst, but water taken in is lost again and must be continually consumed. If the condition is not severe, dehydration may not occur, but severe cases can lead to electrolyte imbalances due to dehydration.

Another hormone responsible for maintaining electrolyte concentrations in extracellular fluids is aldosterone, a steroid hormone that is produced by the adrenal cortex. In contrast to ADH, which promotes the reabsorption of water to maintain proper water balance, aldosterone maintains proper water balance by enhancing Na^+ reabsorption and K^+ secretion from extracellular fluid of the cells in kidney tubules. Because it is produced in the cortex of the adrenal gland and affects the concentrations of minerals Na^+ and K^+, aldosterone is referred to as a mineralocorticoid, a corticosteroid that affects ion and water balance. Aldosterone release is stimulated by a decrease in blood sodium levels, blood volume, or blood pressure, or an increase in blood potassium levels. It also prevents the loss of Na^+ from sweat, saliva, and gastric juice. The reabsorption of Na^+ also results in the osmotic reabsorption of water, which alters blood volume and blood pressure.

Aldosterone production can be stimulated by low blood pressure, which triggers a sequence of chemical release. When blood pressure drops, the renin-angiotensin-aldosterone system (RAAS) is activated. Cells in the juxtaglomerular apparatus, which regulates the functions of the nephrons of the kidney, detect this and release renin. Renin, an enzyme, circulates in the blood, reacting with a plasma protein produced by the liver called angiotensinogen. When angiotensinogen is cleaved by renin, it produces angiotensin I, which is then converted into angiotensin II in the lungs. Angiotensin II functions as a hormone, causing the release of the hormone aldosterone by the adrenal cortex, resulting in increased Na^+ reabsorption, water retention, and an increase in blood pressure. Angiotensin II, in addition to being a potent vasoconstrictor, also causes an increase in ADH and increased thirst, both of which help to raise blood pressure.



Action of aldosterone: ADH and aldosterone increase blood pressure and volume. Angiotensin II stimulates release of these hormones. Angiotensin II, in turn, is formed when renin cleaves angiotensin. This increases water retention and blood pressure.

i. Hormonal Regulation of Metabolism

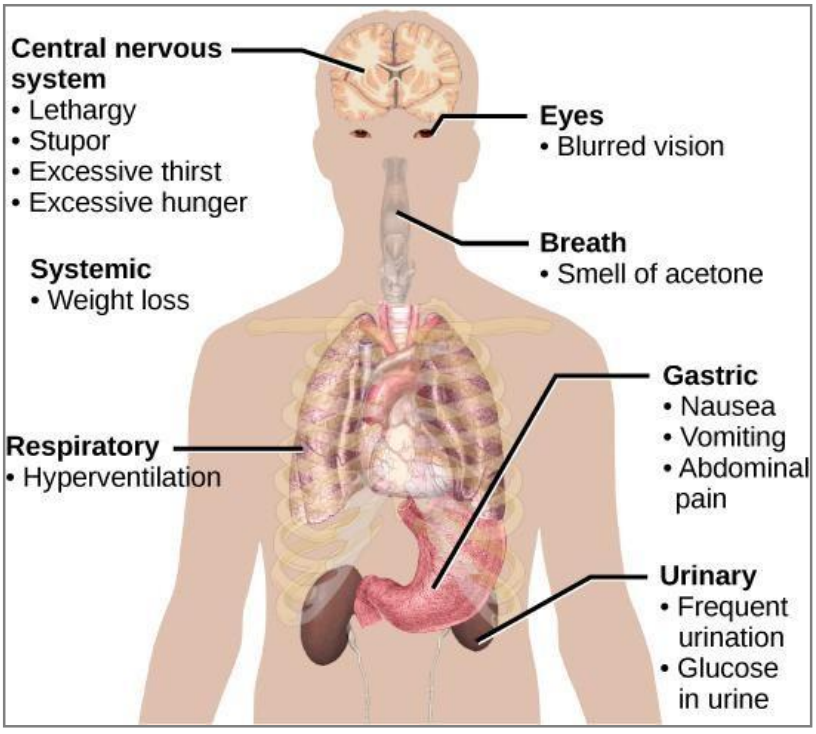
Blood glucose levels vary widely over the course of a day as periods of food consumption alternate with periods of fasting. Insulin and glucagon are the two hormones primarily responsible for maintaining homeostasis of blood glucose levels. Additional regulation is mediated by the thyroid hormones.

a. Regulation of Blood Glucose Levels: Insulin and Glucagon

Cells of the body require nutrients in order to function. These nutrients are obtained through feeding. In order to manage nutrient intake, storing excess intake, and utilizing reserves when necessary, the body uses hormones to moderate energy stores. Insulin is produced by the beta cells of the pancreas, which are stimulated to release insulin as blood glucose levels rise (for example, after a meal is consumed). Insulin lowers blood glucose levels by enhancing the rate of glucose uptake and utilization by target cells, which use glucose for ATP production. It also stimulates the liver to convert glucose to glycogen, which is then stored by cells for later use. As insulin binds to its target cell via insulin receptors and signal transduction, it triggers the cell to incorporate glucose transport proteins into its membrane. This allows glucose to enter

the cell, where it can be used as an energy source. These actions mediated by insulin cause blood glucose concentrations to fall, called a hypoglycemic, or –low sugar‖ effect, which inhibits further insulin release from beta cells through a negative feedback loop.

Impaired insulin function can lead to a condition called diabetes mellitus, which has many effects on the body. It can be caused by low levels of insulin production by the beta cells of the pancreas, or by reduced sensitivity of tissue cells to insulin. This prevents glucose from being absorbed by cells, causing high levels of blood glucose, or hyperglycemia (high sugar). High blood glucose levels make it difficult for the kidneys to recover all the glucose from nascent urine, resulting in glucose being lost in urine. High glucose levels also result in less water being reabsorbed by the kidneys, causing high amounts of urine to be produced; this may result in dehydration. Over time, high blood glucose levels can cause nerve damage to the eyes and peripheral body tissues, as well as damage to the kidneys and cardiovascular system. Oversecretion of insulin can cause hypoglycemia, low blood glucose levels. This causes insufficient glucose availability to cells, often leading to muscle weakness. It can sometimes cause unconsciousness or death if left untreated.



Diabetes mellitus: Diabetes mellitus can cause a wide range of symptoms, including nausea, vomiting, blurred vision, lethargy, a frequency in urination, and high levels of glucose in the urine.

When blood glucose levels decline below normal levels, for example between meals or when glucose is utilized during exercise, the hormone glucagon is released from the pancreas. Glucagon raises blood glucose levels, eliciting what is called a hyperglycemic effect, by stimulating the breakdown of glycogen to glucose in skeletal muscle cells and liver cells in a process called glycogenolysis. Glucose can then be utilized as energy by muscle cells and released into circulation by the liver cells. Glucagon also stimulates absorption of amino acids from the blood by the liver, which then converts them to glucose. This process of glucose synthesis is called gluconeogenesis. Rising blood glucose levels inhibit further glucagon release by the pancreas via a negative feedback mechanism. In this way, insulin and glucagon work together to maintain homeostatic glucose levels.

b. The regulation of blood glucose levels by insulin and glucagon:

As the levels of glucose in the blood rise, insulin stimulates the cells to take up more glucose and signals the liver to convert the excess glucose to glycogen, a form in which it can be stored for later use. When the levels of glucose in the blood fall, glucagon responds by stimulating the breakdown of glycogen into glucose and signals the production of additional glucose from amino acids.

Regulation of Blood Glucose Levels: Thyroid Hormones

The basal metabolic rate, which is the amount of calories required by the body at rest, is determined by two hormones produced by the thyroid gland: thyroxine, also known as tetraiodothyronine or T4, and triiodothyronine, also known as T3. T3 and T4 release from the thyroid gland are stimulated by thyroid-stimulating hormone (TSH), which is produced by the anterior pituitary. These hormones affect nearly every cell in the body except for the adult brain, uterus, testes, blood cells, and spleen. They are transported across the plasma membrane of target cells where they bind to receptors on the mitochondria, resulting in increased ATP production. In the nucleus, T3and T4activate genes involved in energy production and glucose oxidation. This results in increased rates of metabolism and body heat production. This is known as the hormone's calorigenic effect.

Disorders can arise from both the underproduction and overproduction of thyroid hormones. Hypothyroidism, underproduction of the thyroid hormones, can cause a low metabolic rate leading to weight gain, sensitivity to cold, and reduced mental activity, among other symptoms. In children, hypothyroidism can cause cretinism, which can lead to mental retardation and growth defects. Hyperthyroidism, the overproduction of thyroid hormones, can lead to an increased metabolic rate, which may cause weight loss, excess heat production, sweating, and an increased heart rate.

ii. Hormonal Control of Blood Calcium Levels

Blood levels of calcium are regulated by the parathyroid hormone, which acts on the bones, kidneys, and intestines to keep levels constant. Regulation of blood calcium concentrations is important for generation of muscle contractions and nerve impulses, which are electrically stimulated. If calcium levels get too high, membrane permeability to sodium decreases and membranes become less responsive. If calcium levels get too low, membrane permeability to sodium increases and convulsions or muscle spasms may result.

Blood calcium levels are regulated by parathyroid hormone (PTH), which is produced by the parathyroid glands. PTH is released in response to low blood calcium levels. It increases calcium levels by targeting the skeleton, the kidneys, and the intestine. In the skeleton, PTH stimulates osteoclasts, which are cells that cause bone to be reabsorbed, releasing calcium from bone into the blood. PTH also inhibits osteoblasts, cells which deposit bone, reducing calcium deposition in bone. In the intestines, PTH increases dietary calcium absorption and in the kidneys, PTH stimulates re-absorption of the calcium. While PTH acts directly on the kidneys to increase calcium re-absorption, its effects on the intestine are indirect. PTH triggers the formation of calcitriol, an active form of vitamin D, which acts on the intestines to increase absorption of dietary calcium. PTH release is inhibited by rising blood calcium levels.

Regulation of blood calcium levels:

Parathyroid hormone (PTH) is released in response to low blood calcium levels. It increases blood calcium levels by stimulating the resorption of bones, increasing calcium resorption in the kidneys, and indirectly increasing calcium absorption in the intestines.

Hyperparathyroidism results from an overproduction of PTH, which leads to excessive amounts of calcium being removed from bones and introduced into blood circulation. This may produce structural weakness of the bones, which can lead to deformation and fractures, plus nervous system impairment due to high blood calcium levels. Hypoparathyroidism, the underproduction of PTH, results in extremely low levels of blood calcium, which causes impaired muscle function and may result in tetany (severe sustained muscle contraction).

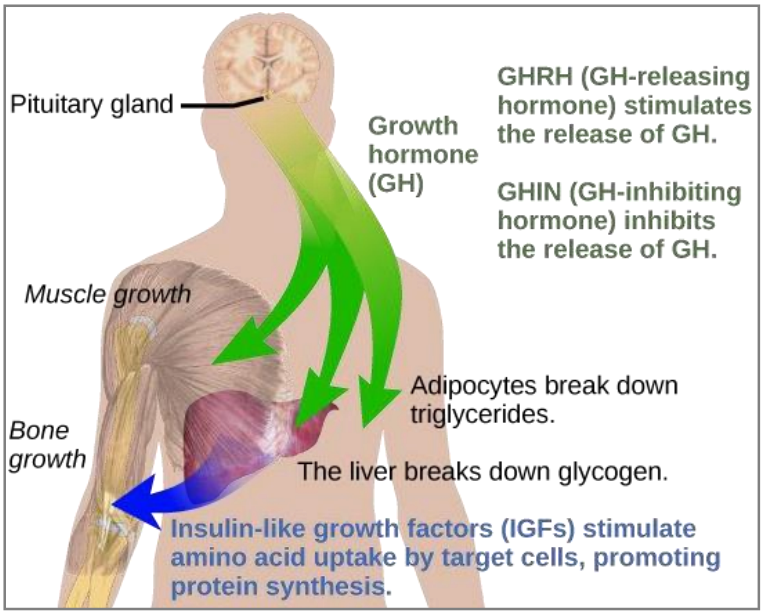
The hormone calcitonin, which is produced by the parafollicular (or C) cells of the thyroid, has the opposite effect on blood calcium levels as PTH. Calcitonin decreases blood calcium levels by inhibiting osteoclasts, stimulating osteoblasts, and stimulating calcium excretion by the kidneys. This results in calcium being added to the bones to promote structural integrity. Calcitonin is most important in children (when it stimulates bone growth), during pregnancy (when it reduces maternal bone loss), and during prolonged starvation (because it reduces bone mass loss). In healthy, nonpregnant, unstarved adults, the role of calcitonin is unclear.

iii. Hormonal Regulation of Growth

Body growth is controlled by growth hormone (GH), produced by the anterior pituitary, and IGF-1, whose production is stimulated by GH. Hormonal regulation is required for the growth and replication of most cells in the body. Growth hormone (GH), produced by the anterior portion of the pituitary gland, accelerates the rate of protein synthesis, particularly in skeletal muscle and bones. Effects of growth hormone on the tissues of the body can generally be described as anabolic (building up). Like most other protein hormones, GH acts by interacting with a specific receptor on the surface of cells. Increased height during childhood is the most widely-known effect of GH. Height appears to be stimulated by at least two mechanisms: Because polypeptide hormones are not fat-soluble, they cannot penetrate cell membranes. Thus, GH exerts some of its effects by binding to receptors on target cells, where it activates a pathway that directly stimulates division and multiplication of chondrocytes of cartilage.

GH also stimulates, through another pathway, the production of insulin-like growth factor 1 (IGF-1), a hormone homologous to proinsulin. The liver, a major target organ of GH for this process, is the principal site of IGF-1 production. IGF-1 has growth-stimulating effects on a wide variety of tissues. IGFs stimulate the uptake of amino acids from the blood, allowing the formation of new proteins, particularly in

skeletal muscle cells, cartilage cells, and other target cells. This is especially important after a meal, when glucose and amino acid concentration levels are high in the blood. GH levels are regulated by two hormones produced by the hypothalamus. GH release is stimulated by growth hormone-releasing hormone (GHRH) and is inhibited by growth hormone-inhibiting hormone (GHIH), also called somatostatin. IGF-1 also has stimulatory effects on osteoblast and chondrocyte activity to promote bone growth.



Effects of growth hormone:

Growth hormone directly accelerates the rate of protein synthesis in skeletal muscle and bones. Insulin-like growth factor 1 (IGF-1) is activated by growth hormone and also allows formation of new proteins in muscle cells and bone.

A balanced production of growth hormone is critical for proper development. Underproduction of GH in adults does not appear to cause any abnormalities, but in children it can result in pituitary dwarfism, in which growth is reduced. Pituitary dwarfism is characterized by symmetric body formation. In some cases, individuals are under 30 inches in height. Over secretion of growth hormone can lead to gigantism in children, causing excessive growth. In some documented cases, individuals can reach heights of over eight feet. In adults, excessive GH can lead to acromegaly, a condition in which there is enlargement of bones in the face, hands, and feet that are still capable of growth.

iv. Hormonal Regulation of Stress

The adrenal glands respond to either short-term or long-term stressors by releasing different hormones that act differently on the body. When a threat or danger is perceived, the body responds by releasing hormones that will ready it for the ―fight-or-flight‖ response. The effects of this response are familiar to anyone who has been in a stressful situation: increased heart rate, dry mouth, and hair standing erect.

The sympathetic nervous system regulates the stress response via the hypothalamus. Stressful stimuli cause the hypothalamus to signal the adrenal medulla (which mediates short-term stress responses) via nerve impulses, and the adrenal cortex, which mediates long-term stress responses via the hormone adrenocorticotropic hormone (ACTH), which is produced by the anterior pituitary.

a. Short-term Stress Response

Interactions of the endocrine hormones have evolved to ensure the body's internal environment remains stable. Stressors are stimuli that disrupt homeostasis. The sympathetic division of the vertebrate autonomic nervous system has evolved the fight-or-flight response to counter stress-induced disruptions of homeostasis. In the initial alarm phase, the sympathetic nervous system stimulates an increase in energy levels through increased blood glucose levels. This prepares the body for physical activity that may be required to respond to stress: to either fight for survival or to flee from danger.

b. Fight-or-flight response:

When an animal feels threatened, epinephrine and norepinephrine released by the adrenal medulla prepare the body to fight a threat or flee from it by breaking down stores of glycogen, which provides an immediate boost of energy. When presented with a stressful situation, the body responds by calling for the release of hormones that provide a burst of energy. The hormones epinephrine (also known as adrenaline) and norepinephrine (also known as noradrenaline) are released by the adrenal medulla. Epinephrine and norepinephrine increase blood glucose levels by stimulating the liver and skeletal muscles to break down glycogen and by stimulating glucose release by liver cells. Additionally, these hormones increase oxygen availability to cells by increasing the heart rate and dilating the bronchioles. The hormones also prioritize body function by increasing blood supply to essential organs, such as the heart, brain, and skeletal muscles, while restricting blood flow to organs not in immediate need, such as the skin, digestive system, and kidneys. Epinephrine and norepinephrine are collectively called catecholamines.

c. Long-term Stress Response

Some stresses, such as illness or injury, can last for a long time. Glycogen reserves, which provide energy in the short-term response to stress, are exhausted after several hours and cannot meet long-term energy needs. If glycogen reserves were the only energy source available, neural functioning could not be maintained once the reserves became depleted due to the nervous system's high requirement for glucose. In this situation, the body has evolved a response to counter long-term stress through the actions of the glucocorticoids, which ensure that long-term energy requirements can be met. The glucocorticoids mobilize lipid and protein reserves, stimulate gluconeogenesis, conserve glucose for use by neural tissue, and stimulate the conservation of salts and water.

Long-term stress response differs from short-term stress response. The body cannot sustain the bursts of energy mediated by epinephrine and norepinephrine for long times. Instead, other hormones come into play. In a long-term stress response, the hypothalamus triggers the release of ACTH from the anterior pituitary gland. The adrenal cortex is stimulated by ACTH to release steroid hormones called corticosteroids. Corticosteroids turn on transcription of certain genes in the nuclei of target cells. They change enzyme concentrations in the cytoplasm and affect cellular metabolism.

There are two main corticosteroids: glucocorticoids, such as cortisol, and mineralocorticoids, such as aldosterone. These hormones target the breakdown of fat into fatty acids in the adipose tissue. The fatty acids are released into the bloodstream for other tissues to use for ATP production. The glucocorticoids primarily affect glucose metabolism by stimulating glucose synthesis. Glucocorticoids also have anti-inflammatory properties through inhibition of the immune system. For example, cortisone is used as an anti-inflammatory medication; however, it cannot be used long term as it increases susceptibility to disease due to its immune-suppressing effects. Mineralocorticoids function to regulate ion and water balance of the body. The hormone aldosterone stimulates the reabsorption of water and sodium ions in the kidney, which results in increased blood pressure and volume.

Hypersecretion of glucocorticoids can cause a condition known as Cushing's disease, characterized by a shifting of fat storage areas of the body. This can cause the accumulation of adipose tissue in the face and neck, and excessive glucose in the blood. Hyposecretion of the corticosteroids can cause Addison's disease, which may result in bronzing of the skin, hypoglycemia, and low electrolyte levels in the blood.

5.5. DEFENSE MECHANISM

Immunity, if defined broadly, encompasses all mechanisms and responses used by the body to defend itself against foreign substances, microorganisms, toxins, and noncompatible living cells. Such immunity may be conferred by the immune system itself, or by the protective role of other generalized host defensive mechanisms. Every aspect of immunity and host defense is dependent upon a proper supply and balance of nutrients.

The generalized primary forms of host defense are termed "innate," "inborn," or "nonspecific" immunity. These initial defensive mechanisms guard the body by contributing protective responses that are effective against a diverse variety of threats. Nonspecific defensive mechanisms may be active or passive in nature. Although nonspecific immunity does not require participation of the immune system per se, it may trigger secondary immune system actions.

The second, or subsequent, form of host defense is termed "adaptive," "acquired," or "antigen-specific" immunity. This form of protection is delivered by the immune system itself, with its complex and highly interactive network of lymphocyte species and their products. The immune system is characterized by antigen specificity and antigen-related memory. Beginning at birth and continuing throughout life, the immune system's immunological repertoire expands as a myriad of new and different antigens are encountered.

a. Generalized (Nonspecific) Host Defenses

Nonspecific host defenses are provided by both passive and active mechanisms. These mechanisms are involved in defining host susceptibility or resistance to infection, trauma, or other disease threats, and they may be drastically impaired by diverse forms of malnutrition.

i. Passive Defensive Measures

Passive defenses include anatomical barriers and pathways (skin and mucous membranes, fascial planes, body spaces, tubular structures); exogenous body secretions (mucin, saliva, bronchial fluids, gastric HCI, properdin, opsonins, lysozyme, etc.); physicochemical environments within normal tissues; normal ciliary activity; normal physiological factors (age, sex, race, circadian rhythms); normal microbiological flora in various locations; and even occupational and environmental factors.

Passive defenses can be compromised by malnutrition, injury or trauma, fatigue, specific illnesses (for example, diabetes, leukemia, Hodgkin's disease, alcoholism), prescribed or addictive drugs (for example, corticosteroids, antimetabolites, antimicrobials, hallucinogens, crack cocaine), and medically implanted foreign bodies (for example, vascular prostheses, catheters, drains).

ii. Active Nonspecific Defenses

Nonspecific active defensive measures include a diverse variety of physiological responses (for example, elevated body temperatures tachycardia, vomiting and diarrhea, pituitary-adrenal activation), phagocytic cell activation, creation of inflammatory reactions, formation of nitric oxide from arginine, and a stereotyped pattern of acute-phase reactions (including fever, myalgias, arthralgias, headache and somnolence, anorexia, and a markedly altered pattern of protein synthesis and breakdown in liver and muscle, respectively). In contrast to the synthesis of antigen-specific antibodies by the immune system, nonspecific active humoral defense mechanisms include the production of cytokines, hormones, acute-phase plasma proteins, and sometimes the activation of protein components of the complement, kinin, and coagulation systems. However, it is important to point out that not all of these components may come into play in any one inflammatory or nonantigen-specific response.

b. Cytokines

Cytokines are small peptides that function as intercellular signals and mediators. Cytokines are produced by many different types of cells throughout the body. Most cytokines have a diverse variety of actions, depending on the cells they stimulate. Cytokines involved in immune function include interleukins, interferons, colony stimulating factors, and a variety of other closely related mediators. In addition to their multiplicity of actions, cytokines tend to have great redundancy, with overlapping actions being common. Moreover, certain cytokines can stimulate the synthesis and release of other cytokines.

In their role as active participants in nonspecific immunity, a group of "proinflammatory cytokines" (that is, interleukin-1 [IL-1], IL-6, IL-8, tumor necrosis factor [TNF], and interferon-gamma [INF-γ]) initiate acute-phase reactions, launch immune system activities, trigger central nervous system (CNS) responses, and stimulate the release (or suppression) of hormones. Proinflammatory cytokines also participate in inflammatory reactions. Interferons have both antiviral and

immunological properties. INF-γ (produced by lymphocytes) also participates in inflammatory and acute-phase processes. Yet another category of cytokines, colony stimulating factors, whose main functions are as hematopoietic factors, may also play a role in inflammation in select responses.

Although new information about the cytokines is still being generated, the fundamental importance of their diverse functions is now fully recognized. As an example of cytokine biology, when macrophages or monocytes are activated (by microorganisms, antigen-antibody complexes, toxins, chemicals, etc.), they quickly respond by producing IL-1, TNF, and other cytokines. These cytokines travel via the blood or interstitial fluid between cells to interact with specific receptor proteins located on the walls of many different target cells throughout the body. The union of a cytokine with its specific cellular receptor leads to the activation of phospholipase enzymes within the target cell wall and the subsequent release into the cell of arachidonic acid (from n-6 polyunsaturated fatty acids [PUFAs] in the plasma membrane), or eicosapentaenoic acid (from n-3 PUFA).

Then, depending on the enzymes (cyclooxygenases or lipoxygenases) contained within a target cell, the arachidonic and eicosapentaenoic acids (EPAs) are converted into eicosanoids (for example, prostaglandins, leukotrienes, thromboxanes, lipoxins) of different potencies. These eicosanoid messenger-effector molecules, in turn, initiate target cell-specific responses (some of which can be blocked by glucocorticoids, aspirin, or ibuprofen).

Although these cytokine-induced responses are generally protective in nature, an excess production and/or activity of cytokines can be harmful. In fact, an excess of proinflammatory cytokines can lead to hypotensive shock, multiorgan failure, and death. The body possesses an elaborate system of checks and balances to control the production and activity of individual cytokines, a system far more complicated than those that regulate endocrine functions.

The effects of cytokines can be inhibited by a number of different mechanisms. Cytokines with inhibitory actions can block the synthesis and release of other cytokines. In addition, target cells release cytokine receptors into the plasma, and these soluble free receptors can intercept and inactivate the cytokine before it reaches the target cell. Blocking proteins can obstruct cell wall receptors so that cytokines cannot exert their cellular effects. Finally, hormones such as the glucocorticoids can inhibit the intracellular effects of certain cytokines.

Every aspect of cytokine biology requires proper nutrition. A full range of essential nutrients is required to (1) permit the replication of cytokine-producing cells; (2) allow the activation of these cells and the subsequent synthesis and release of cytokines into plasma; (3) allow target cells to synthesize receptor proteins and cytokine-related enzymes; (4) provide a full spectrum of cell wall PUFAs and permit their multistep conversion into eicosanoids; (5) enable target cells to respond appropriately to specific eicosanoid stimuli; and (6) allow the simultaneous development of mechanisms used for controlling excess cytokine activity.

c. Acute-Phase Reactions

Acute-phase reactions constitute an interrelated group of physiologic and metabolic changes that occur in response to generalized acute infectious illnesses, trauma, severe inflammatory processes, tissue injury, and other medical and surgical diseases. Acute-phase reactions are initiated by proinflammatory cytokines and generally have an acute onset.

Acute-phase reactions include fever, generalized malaise, somnolence, anorexia, arthralgia or myalgia, skeletal muscle proteolysis, endocrine system participation, water and salt retention, and cachexia accompanied by negative body balances of nitrogen, phosphate, magnesium, and zinc. Acute-phase reactions are accompanied by a stimulated production of white blood cells and by immune system activation, and are characterized metabolically by a transient intolerance to glucose, hypertriglyceridemia, the sequestration of iron and zinc, and a diminished production of hemoglobin. Numerous other metabolic responses include a massive reprioritization of hepatocyte functions that involves the synthesis of acute-phase reactant glycoproteins, hepatic enzymes, and metallothioneins, concomitant with a depressed production of plasma albumin.

Acute-phase proteins are synthesized within hours; they include C-reactive protein (CRP), haptoglobin, ceruloplasmin, orosomucoid, α_1-antitrypsin, serum amyloid A protein, fibrinogen, and others.

Proinflammatory cytokines, especially IL-1 and TNF, trigger all of these physiological and cellular events. Cytokine actions within the CNS trigger the onset of fever (by causing the release of prostaglandins within the temperature-regulating center), anorexia, somnolence, and the release (or suppression) of hormones produced in the CNS and pituitary gland.

d. Inflammatory Reactions

Localized inflammatory reactions serve to control and confine infectious microorganisms, to attract cells and their products to localized areas of injury, and to initiate the healing process. Inflammatory reactions involve many pathophysiological processes and cell types.

Inflammatory reactions are characterized by heat and redness, swelling, and pain. Despite these noxious symptoms, inflammatory reactions serve to localize a disease process and prevent it from becoming generalized.

Inflammatory reactions initially include dilation of local blood vessels, vascular congestion, and the binding of white blood cells to the endothelium. At the same time, immunoglobulin G (IgG) molecules become attached to mast cells and basophils, activating them to release histamine and other inflammation-producing complement activation aids in this process. Polymorphonuclear (PMN) leukocytes, monocytes, and other cells penetrate blood vessel endothelium to enter the area of inflammation; this penetration is abetted by the release of chemoattractants.

These initial inflammatory reactions are accompanied by extensive cellular activation that may include the following: the release of prostaglandins, defensins, cathepsins, and thromboxanes from phagocytes; release of lysozyme from macrophages or monocytes; release of histamine from basophils; release of cationic and basic proteins by eosinophils; deposition of fibrin; binding of iron to PMN-synthesized lactoferrin; microbicidal killing; and destruction of many participating cells with localized release of their contents, including enzymes and free oxygen radicals.

The healing process involves the proliferation of fibroblasts, the synthesis of collagen and glucosaminoglycans, phagocytic removal of inflammatory debris, and eventual disappearance of vascular congestion and edema.

The Complement System

As one of the principal mediators of the inflammatory reaction, the complement system participates in both adaptive and cell-mediated immunological responses. It assists in phagocytosis, chemotaxis, cellular activation, the respiratory burst of phagocytes, anaphylaxis, increased capillary permeability, and damage to microorganism surfaces. It is a highly adaptive system of profound importance, but its activity can be curtailed by malnutrition.

Actions of complement fragments after complement component C3 is activated via either the classic or the alternate pathway, C3 cleavage fragments have a variety of actions. C3b is also the starting point for the lytic pathway.

The complement system consists of a group of 17 plasma proteins that, when the system is activated, are cleaved and/or linked in a sequential manner (termed the complement cascade). Induction of the cascade by antibodies produces the "classic activation pathway," and initiation by endotoxins or microbial antigens produces the "alternate activation pathway."

In the classic complement activation pathway, aggregated antigen—antibody complexes bind to C1 (first complement component), converting it to a protease enzyme that activates C4 and C2, in turn, to produce fragments C4b and C2a. These fragments then form a complex that activates C3.

In the alternate pathway, the presence of microbial cell wall lipopolysaccharides activates plasma proteins, including properdin, to trigger the cleavage of C3 to C3b. Each complement pathway leads to the activation of C3, which in turn is cleaved into the biologically active molecules C3a (anaphylatoxin) and C3b. The effector molecule C3b is the starting point of the lytic pathway, which proceeds via the participation of C5, C6, C7, C8, and C9. Lytic components can stimulate chemotaxis or produce lesions in cell membranes.

e. Antigen-Specific Host Defenses

Assisting the nonspecific defenses of the host, the immune system provides additional defensive measures by focusing on and reacting to the highly specific molecular structures of antigens (unique molecular components of microorganisms, food, tissues, inert substances, chemicals, etc.).

The immune system is divided into two major branches that provide (1) antigen-specific cell-mediated immunity (CMI) and (2) humoral immunity. CMI is controlled by the thymus gland and provided by T-lymphocytes and natural killer (NK) cells. Humoral immunity is generated by B-lymphocytes. When stimulated appropriately by an antigen plus IL-2 and other cytokines, B-cells undergo clonal expansion and/or are converted into plasma cells, the cellular factories that produce antigen-specific antibodies. The ability of the immune system to recognize and respond to foreign antigens has three phases:

(1) In the initial *cognitive phase*, foreign antigens become bound to specific receptors on the cell wall of mature lymphocytes. B-lymphocytes express specific antibodies on their surfaces that can bind soluble foreign proteins, polysaccharides, or lipids, while T-lymphocytes express receptors that recognize only short peptide sequences on foreign antigens (including those located on the surfaces of other cells).

(2) In the *activation phase*, lymphocytes that have recognized a foreign antigen proliferate, leading to the creation of lymphocyte clones. This involves both of the major arms, or branches, of the immune system. In the *humoral arm*, humoral immunity is generated by B-cells, which, when stimulated by an antigen plus IL-2 and other cytokines, differentiate into antibody-secreting plasma cells. Simultaneously, in the CMI arm of the immune system, antigen-stimulated T-cells differentiate from null cells to helper or suppressor cells or into killer cells. The CMI arm is controlled by the thymus gland and its zinc-containing hormones. The CMI serves to support the *humoral arm* of the immune system, recruit other defensive cells, and kill any cell recognized as foreign. Responding T- and B-lymphocytes eventually migrate to sites of antigen administration or antigen penetration into body tissues.

(3) In the third, *effector phase*, newly secreted antibodies serve to eliminate the foreign antigen and also to activate the complement cascade, stimulate the degranulation of mast cells, and initiate the release of mediators from other cells. Activated T-cells secrete cytokines that enhance the functions of B-cells and phagocytes and stimulate nonspecific inflammatory responses.

The immune system performs its unique functions in two anatomically distinct but interrelated loci. A systemic component generates both CMI and humoral responses whenever it is penetrated by a foreign antigen. A second and separate (but cofunctioning) surface component of the immune system recognizes foreign antigens on body surfaces (including the respiratory and intestinal mucosa) and produces antibodies for secretion in tears and in mucosal, dermal, and intestinal fluids.

Other functioning aspects of the immune system can generate inappropriate or exaggerated tissue-damaging responses. Hypersensitivity or allergic reactions, or autoimmunity, result in damaging long-term immune responses as the body makes antibodies against its own tissues.

f. Cell-Mediated Immunity

CMI protects the specific, genetically determined tissue type of the body (host) from anything foreign. Through CMI, the body recognizes and defends against infusions of incompatible blood cells or transplanted tissues. Constant surveillance by NK-cells is maintained to detect any body cells that may undergo malignant mutations and, if possible, destroy them. NK-lymphocytes arise from "previously uncommitted or null" cells and can, with the help of IL-2, lyse individual blood borne tumor stem cells without prior sensitization or major histocompatibility complex (MHC). NK-cells also function in graft-versus-host reactions and have been implicated in antibacterial and antiviral defense mechanisms.

Antibody responses to T-cell-dependent antigens require direct physical contact (regulated by genetically determined MHC proteins and adhesion molecules on cell surfaces) between T-helper cells, antigen-presenting cells, and B-cells, and the activation of B-cells to produce appropriate antibodies. CMI includes the lymphocytic secretion of and response to a variety of cytokines. CMI also is involved in eliciting delayed hypersensitivity reactions, that is, reactions that result 24–48 h after contact with an antigen to which the body has been exposed previously. CMI, particularly T-cell number and function, is a major target of malnutrition. Generalized protein-energy malnutrition (PEM) may cause severe atrophy of lymphoid tissues, especially in their T-cell areas. Deficiencies of other nutrients, including vitamins A and B6 and the minerals iron, zinc, copper, and selenium, also can produce CMI dysfunction. Zinc is of special concern because, in addition to its role in nucleic acid synthesis and the activity of many metalloenzymes, it is a component of the thymic hormones and is essential for their functions. Severe generalized malnutrition also causes the disappearance of clinical allergies and hypersensitivities.

g. T-Cells

T-cells are long-lived lymphocytes that circulate continually throughout the body, periodically returning (homing) to the site of their individual origins. At all locations, T-cell activities are influenced by the thymus gland through the effects of its thymic hormones. T-cells have diverse functions and activities, many of which are triggered when a native T-cell is first stimulated by a new foreign antigen. These functional responses develop in three distinct phases: (1) the cognitive phase, (2) the activation phase, and (3) the effector phase.

Many different subsets of T-cells exist. Each T-cell subset has unique (but sometimes overlapping) actions and can be identified by specific cluster of differentiation (CD) antigens (glycoproteins localized on their exterior cell membranes). Subsets include CD4+ T-helper cells, CD8+ T-suppressor cells, and killer cells. T-cells may recognize both specific antigens presenting on membranes of body cells and antigen fragments associated with MHC proteins on these membranes.

CD4+ T-cells recognize antigen fragments associated with Class II MHC molecules in *antigen-presenting cells* (APCs). APCs engulf (endocytose) and process antigens and link recognizable antigenic fragments to the Class II MHC molecules on their surfaces, which stimulates initiating or helping activities.

When CD4+ cells recognize complexes of antigen and Class II (MHC) molecules on the surface of a macrophage, they activate the macrophage, stimulating it to destroy engulfed organisms. However, if the complexes are on the surface of a B-lymphocyte, the CD4+ cells release cytokines, including IL-2, that lead to B-cell activation, clonal expansion, and antibody production.

CD8+ T-cells recognize antigen fragments associated with Class I MHC molecules on cell surfaces. Cells infected by a virus may exhibit viral peptide antigens linked to surface Class I molecules. CD8+ T-cells thus can recognize and destroy virally infected cells.

h. Humoral Immunity

Humoral immunity involves the production of antibodies against specific antigens. Like CMI, humoral immunity has both a systemic component (which produces serum antibodies IgG, IgM, and IgA) and a surface component (which produces secretory IgA).

B-Cells

B-cells are primarily responsible for humoral immunity. They recognize both intact, "whole," extracellular antigens and processed antigens delivered by antigen-presenting cells. B-cells receive help from CD4+ T-helper cells and stimulatory cytokines. During B-cell interactions with antigen-presenting cells, the role of MHC molecules is essential. There are many genetically determined MHC haplotypes that vary in their efficiency, resulting in differences in the quantity and quality of subsequent immune responses.

Following the presentation of an antigen to a naive B-cell, the B-cell undergoes clonal expansion to produce daughter cells that will respond to the same antigen whenever it is encountered in the future. Antigen-activated B-cells, stimulated further by IL-2 and other cytokines, mature into plasma cells, the major antibody-producing cells.

i. Antibodies

Antibodies are bifunctional (immunoglobulin) molecules created to interact with specific antigens. The primary molecular structure of antibodies consists of four peptides (chains) connected by sulfhydryl bonds and arranged in the shape of a Y. Two heavy chains form the Y, and two light chains are attached to the arms of the Y. These arms are termed the Fab (fragment-antibody) regions, and they serve to interact with (bind to) specific intact antigens, either free or on the surface of a microorganism or cell. The stem of the Y is termed the Fc region, and it interacts with receptors on lymphocytes and other cells, or directly with complement.

There are several types of antibodies (immunoglobulins): IgG, IgM, IgA, secretory IgA, IgD, and IgE. After the first exposure of a B-cell to a new foreign antigen, systemic antibody production focuses predominantly on IgM, with relatively little IgG produced. Subsequent exposures to the same antigen result in predominant production of IgG. In contrast, the formation of a secretory antibody by the mucosal immune system involves the initial production of IgA molecules, the joining of two IgAs by a small protein J (joiner) peptide, and actual secretion of the IgA dimer by epithelial or mucosal cells, which add another secretory fragment or peptide to the process.

Antibodies have a variety of distinct functions such as the direct neutralization of circulating antigens and the formation of immune complexes with circulating antigens. After creation of an immune complex in plasma, the Fc regions of the antibody can act as adapters that cross-link the antigen–antibody complex to Fc receptors on the surface of host phagocytic cells (that is, macrophages and PMN leukocytes) that express Fc receptors. This linkage then can lead to the cellular uptake and destruction of the antigen–antibody complex:

- Recognition and sensitization of "foreign" cell targets or parasites for attack by cytotoxic killer cells that possess Fc receptors. This is called antibody-dependent cell-mediated cytotoxicity and is used by eosinophils and large, granular lymphocytes.

- Participation in inflammatory reactions. IgG antibodies can bind to and sensitize mast cells and basophils via their Fc receptors. This binding activates the cells to release inflammation-producing mediators, such as histamine, that contribute to the local inflammatory reaction.
- Activation of complement. This is followed by the release of proinflammatory mediators.

The effects of malnutrition on humoral immunity are far less pronounced than on CMI. Atrophy of B-cell areas of lymphoid tissues is relatively small, and preexisting antibodies continue to be produced. In fact, a slightly paradoxical increase in plasma antibody concentrations is a common finding in children with severe PEM. Antibody responses to new antigens, such as those in vaccines, can generally be detected, although the titers and activities of such new antibodies may be reduced.

j. Hypersensitivity (or Allergic) Reactions

When immune responses are inappropriate or exaggerated and lead to tissue damage, the terms "hypersensitivity" or "allergy" are used. These responses occur only in certain individuals, as a result of second (or numerous) contacts with a particular antigen (antigens that produce these reactions are generally termed allergens). Hypersensitivity responses can occur in a variety of distinct but sometimes overlapping types, as listed below.

- *Type I* reactions are immediate reactions that result from the interaction of an allergen with IgE-sensitized mast cells and basophils. Histamine and other mediators are released, causing acute responses in the skin (weal and flare reactions, urticaria), eyes, nose, bronchial tree, and so forth. These reactions are commonly called allergies. If of extreme, immediate, life-threatening severity, they are termed anaphylactic responses.
- *Type II* reactions are cytolytic or cytotoxic reactions triggered by the interaction of IgG or IgM with antigens on the surface of specific cells or tissues. These reactions lead to tissue destruction, as seen in autoimmune diseases; the rejection of transplanted foreign tissue; or skin diseases such as pemphigus.
- *Type III* reactions are immune complex reactions that are also triggered by IgG or IgM and are caused by the interaction of soluble antigen–antibody complexes with complement. If these complexes are deposited within certain tissues, they

can cause localized damage. In the kidney, they induce glomerulonephritis, and in the skin, diseases such as erythema multiforme and erythema induratum. Interaction with fungal antigens causes farmer's lung disease. Skin testing with the inciting allergen produces Arthus reactions of 5- to 24-h duration.

- *Type IV* reactions are delayed-type dermal hypersensitivity reactions to the intracutaneous injection of tuberculin or other antigens. If positive, these modified inflammatory responses progress slowly and peak in 24 to 48 h. In deeper tissues, these reactions induce granulomatous responses that are characteristic of tuberculosis.

Severe malnutrition, by resulting in immunological dysfunction, can cause both hypersensitivity and allergic reactions to disappear.

5.6. HUMAN DISORDER AND DISEASE

5.6.1 Monogenic diseases

Monogenic diseases result from modifications in a single gene occurring in all cells of the body. Though relatively rare, they affect millions of people worldwide. Scientists currently estimate that over 10,000 of human diseases are known to be monogenic. Pure genetic diseases are caused by a single error in a single gene in the human DNA. The nature of disease depends on the functions performed by the modified gene. The single-gene or monogenic diseases can be classified into three main categories:

- Dominant
- Recessive
- X-linked

All human beings have two sets or copies of each gene called –allele‖; one copy on each side of the chromosome pair. Recessive diseases are monogenic disorders that occur due to damages in both copies or allele. Dominant diseases are monogenic disorders that involve damage to only one gene copy. X linked diseases are monogenic disorders that are linked to defective genes on the X chromosome which is the sex chromosome. The X linked alleles can also be dominant or recessive. These alleles are expressed equally in men and women, more so in men as they carry only one copy of X chromosome (XY) whereas women carry two (XX).

Monogenic diseases are responsible for a heavy loss of life. The global prevalence of all single gene diseases at birth is approximately 10/1000. In Canada, it has been estimated that taken together, monogenic diseases may account for upto 40% of the work of hospital based paediatric practice.

a) Thalassaemia

Thalassaemia is a blood related genetic disorder which involves the absence of or errors in genes responsible for production of haemoglobin, a protein present in the red blood cells. Each red blood cell can contain between 240 and 300 million molecules of haemoglobin. The severity of the disease depends on the mutations involved in the genes, and their interplay.

A haemoglobin molecule has sub-units commonly referred to as alpha and beta. Both sub-units are necessary to bind oxygen in the lungs properly and deliver it to tissues in other parts of the body. Genes on chromosome 16 are responsible for alpha subunits, while genes on chromosome 11 control the production of beta subunits. A lack of a particular subunit determines the type of thalassaemia (eg. a lack of alpha subunits results in alpha-thalassemia). The lack of subunits thus corresponds to errors in the genes on the appropriate chromosomes. There can be various gradations of the disease depending on the gene and the type of mutations.

Prevalence:

The alpha and beta thalassaemias are the most common inherited single-gene disorders in the world with the highest prevalence in areas where malaria was or still is endemic. The burden of this disorder in many regions is of such a magnitude that it represents a major public health concern. For example in Iran, it is estimated that about 8,000 pregnancies are at risk each year. In some endemic countries in the Mediterranean region, long-established control programs have achieved 80-100% prevention of newly affected births.

Diagnosis/ prognosis:

Diagnosis of thalassaemia can be made as early as 10-11 weeks in pregnancy using procedures such as amniocentesis and chorionic villi sampling. Individuals can also be tested for thalassaemia through routine blood counts. Thalassaemic patients may have reduced fertility or even infertility. Early treatment of thalessaemia has proved to be very effective in improving the quality of life of patients. Currently, genetic testing

and counselling, and prenatal diagnosis play an increasingly important role in informing individual as well as professional decisions around the prevention, management and treatment of this disease.

b) Sickle cell anemia

Sickle-cell anemia is a blood related disorder that affects the haemoglobin molecule, and causes the entire blood cell to change shape under stressed conditions. In sickle cell anaemia, the haemoglobin molecule is defective. After haemoglobin molecules give up their oxygen, some may cluster together and form long, rod-like structures which become stiff and assume sickle shape.

Unlike healthy red blood cells, which are usually smooth and donut-shaped, sickled red blood cells cannot squeeze through small blood vessels. Instead, they stack up and cause blockages that deprive organs and tissues of oxygen-carrying blood. This process produces periodic episodes of pain and ultimately can damage tissues and vital organs and lead to other serious medical problems. Normal red blood cells live about 120 days in the bloodstream, but sickled red cells die after about 10 to 20 days. Because they cannot be replaced fast enough, the blood is chronically short of red blood cells, leading to a condition commonly referred to as anemia.

Prevalence:

Sickle cell anemia affects millions throughout the world. It is particularly common among people whose ancestors come from Sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and Mediterranean countries such as Turkey, Greece, and Italy. In the Unites States, it affects around 72,000 people, most of whose ancestors come from Africa. The disease occurs in about 1 in every 500 African-American births and 1 in every 1000 to 1400 Hispanic-American births. About 2 million Americans, or 1 in 12 African Americans, carry the sickle cell allele.

Diagnosis/ prognosis:

The sickle cell disease can be diagnosed in a simple blood test. In many cases, sickle-cell anemia is diagnosed when new-borns are screened. Vaccines, antibiotics, and folic acid supplements are administered, in addition to pain killers. Blood transfusions and surgery are used in severe cases. The only known cure at present is a bone marrow transplant.

c) Haemophilia

Haemophilia is a hereditary bleeding disorder, in which there is a partial or total lack of an essential blood clotting factor. It is a lifelong disorder, that results in excessive bleeding, and many times spontaneous bleeding, which, very often , is internal. Haemophilia A is the most common form, referred to as classical haemophilia. It is the result of a deficiency in clotting factor 8, while haemophilia B (Christmas Disease) is a deficiency in clotting factor 9. This illness is a sex-linked recessive disorder.

Prevalence:

Due to the sex-linkage of the disorder, there is a greater prominence in males than in females. About a third of new diagnoses are where there is no previous family history. It appears world-wide and occurs in all racial groups. About 6,000 people are affected with haemophilia in the UK. There are about 5400 people in the UK with haemophilia A and about 1100 with haemophilia B.

Diagnosis/ prognosis:

Blood tests can determine the presence of the haemophilia condition, and more specifically whether it is a type A or a type B disease. Usually, infants do not show signs before 9 months of age. Administration of clotting factors help affected individuals to live with the disease. There are various lifestyle changes that one can make as a haemophiliac, and though a serious disease, it can be tolerable with proper precautions and therapy. The prospects for youngster with haemophilia are excellent. Only a few decades ago, children with haemophilia had a significantly reduced life expectancy. They were often crippled with arthritis and joint deformity by their teens and had to attend special schools for disabled people. Many recent studies have documented a greatly increased life-expectancy among people suffering from haemophilia in developed countries over the last few decades. Children with haemophilia now face few limitations. They certainly attend normal schools, most jobs are open to them, and full participation in society through employment, marriage and having children is now the norm. It is anticipated, however, that the number of people with haemophilia in developed countries will increase steadily over the next few decades.

d) Cystic Fibrosis

Cystic Fibrosis is a genetic disorder that affects the respiratory, digestive and reproductive systems involving the production of abnormally thick mucus linings in the lungs and can lead to fatal lung infections. The disease can also result in various obstructions of the pancreas, hindering digestion. An individual must inherit two defective cystic fibrosis genes, one from each parent, to have the disease. Each time two carriers of the disease conceive, there is a 25 percent chance of passing cystic fibrosis to their children ; a 50 percent chance that the child will be a carrier of the cystic fibrosis gene; and a 25 percent chance that the child will be a non-carrier.

Prevalence:

The incidence of CF varies across the globe. Although it is severely underdiagnosed in Asia, existing evidence indicates that the prevelance of CF is rare. In the European Union 1 in 2000-3000 new borns is found to be affected by CF . In the United States of America the incidence of CF is reported to be 1 in every 3500 births.

Diagnosis/ prognosis:

People with CF have a variety of symptoms including: very salty-tasting skin; persistent coughing, at times with phlegm; wheezing or shortness of breath; an excessive appetite but poor weight gain; and greasy, bulky stools. Symptoms vary from person to person, in part, due to the more than 1,000 mutations of the CF gene, several of which have been identified and sequenced by researchers.The sweat test is the standard diagnostic test for CF. This simple and painless procedure measures the amount of salt in the sweat. A high salt level indicates CF. Although the results of this test are valid any time after a baby is 24 hours old, collecting a large enough sweat sample from a baby younger than 3 or 4 weeks old may be difficult. The sweat test can also confirm the diagnosis in older children and adults. If pancreatic enzyme levels are reduced, an analysis of the person's stool may reveal decreased or absent levels of the digestive enzymes (trypsin and chymotrypsin) or high levels of fat. If insulin secretion is reduced, blood sugar levels are high. Pulmonary function tests may show that breathing is compromised. Also, a chest x-ray may suggest the diagnosis. Relatives other than the parents of a child with cystic fibrosis may want to know if they're likely to have children with the disease. Genetic testing on a small blood sample can help determine who has a defective cystic fibrosis gene. Unless both parents have at least one such gene, their children will not have cystic fibrosis. If both parents carry a

defective cystic fibrosis gene, each pregnancy has a 25 percent chance of producing a child with cystic fibrosis. During pregnancy, an accurate diagnosis of cystic fibrosis in the fetus is usually possible.

The severity of cystic fibrosis varies greatly from person to person regardless of age; the severity is determined largely by how much the lungs are affected. However, deterioration is inevitable, leading to debility and eventually death. Nonetheless, the outlook has improved steadily over the past 25 years, mainly because treatments can now postpone some of the changes that occur in the lungs. Half of the people with cystic fibrosis live longer than 28 years. Long-term survival is somewhat better in males, people who don't have pancreatic problems, and people whose initial symptoms are restricted to the digestive system. Despite their many problems, people with cystic fibrosis usually attend school or work until shortly before death. Gene therapy holds great promise for treating cystic fibrosis.

According to the CF Foundation's National Patient Registry, the median age of survival for a person with CF is currently 33.4 years. Only thirty years ago, a CF patient was not expected to reach adulthood. Many people even live into their fifties and sixties.

As more advances have been made in the treatment of CF, the number of adults with CF has steadily grown. Today, nearly 40 percent of the CF population is age 18 and older. Adults, however, may experience additional health challenges including CF-related diabetes and osteoporosis. CF also can cause reproductive problems - more than 95 percent of men with CF are sterile. But, with new technologies, some are becoming fathers. Although many women with CF are able to conceive, limited lung function and other health factors may make it difficult to carry a child to term.

e) Tay sachs disease

Tay-Sachs disease is a fatal genetic disorder in which harmful quantities of a fatty substance called Ganglioside GM2 accumulate in the nerve cells in the brain. This is caused by a decrease in the functioning of the Hexosaminidase A enzyme. Abnormal Hexosaminidase A enzyme activity causes an accumulation of fat in nerve cells, leading to paralysis, dementia, blindness, psychoses, and even death. Though the degradation of the central nervous system begins at the fetal stage, observations such as loss of peripheral vision and motor co-ordination are not seen until about 6 months of age. This disease is autosomal recessive which means that an individual must inherit two

defective genes, one from each parent, to inherit this disease. According to the age of onset there are two existing forms of Tay-Sachs disease.

- Infantile Tay-Sachs disease
- Late onset Tay-Sachs disease (chronic GM2-gangliosidosis)

Prevalence:

The frequency of the condition is much higher in in Ashkenazi Jews of Eastern European origin than in others. Approximately one in every 27 Jews in the United States of America is a carrier of the TSD gene. There is also a noticeable incidence of TSD in non-Jewish French Canadians living near the St. Lawrence River and in the Cajun community of Louisiana. By contrast, the carrier rate in the general population as well as in Jews of Sephardic origin is about one in 250.

Among Jews of Sephardic origin and in the general, non-Jewish population, the carrier rate is about 1 in 250. There are certain exceptions. French-Canadian and the Cajun community of Louisiana have the same carrier rate as Ashkenazi Jews, one in 27. Also, individuals with ancestry from Ireland are at increased risk for the Tay-Sachs gene. Current research indicates that among Irish Americans, the carrier rate is about one in 50.

Diagnosis/ prognosis:

The diagnosis for Tay- Sachs disease (TSD) can be made via a blood test in which the Hex A enzyme can be measured in either the serum, the white blood cells, or in the skin fibroblast. Over the past 25 years, carrier screening and genetic counselling within high-risk populations have greatly reduced the number of children born with TSD in these groups. Therefore, a great percentage of the babies born with Tay-Sachs Disease today are born to couples who were not previously thought to be at significant risk.

Prenatal tests that can diagnose Tay-Sachs in the fetus before birth are available. These procedures are referred to as Amniocentesis and Chorionic Villus Sampling. Amniocentesis sampling is performed between the 15th and 16th week of pregnancy. The procedure involves inserting a needle into the mother's abdomen and obtaining a sample of the fluid that surrounds the baby. In Chorionic Villus Sampling a sample of cells from the placenta is retrieved by the doctor during the 10th and 12th week of pregnancy, and tested for the presence of Hex A.

f) Fragile X syndrome

The Fragile X syndrome is caused by a "fragile" site at the end of the long arm of the X-chromosome. It is a genetic disorder that manifests itself through a complex range of behavioural and cognitive phenotypes. It is the result of genetic mutation which varies considerably in severity among patients. Fragile X syndrome is the most common cause of inherited mental retardation. Although it is a X-linked recessive trait with variable expression and incomplete penetrance, 30% of all carrier women are affected.

Prevalence:

According to the Fragile X association of Southern California, Fragile X syndrome is the single most common inherited cause of mental impairment affecting 1 in 3600 males and 1 in 4000 to 6000 females with full mutation worldwide. Some studies also suggest that fragile X affects 1 in every 2000 males and 1 in every 4000 females of all races and ethnic groups. Studies have also revealed that 1 in 259 women of all races carry fragile X and could pass it to their children. The number of men who are carriers is thought to be 1 in 800 of all races and ethnicity. Carrier females have a 30% to 40>% chance of giving birth to a retarded male child and a 15 to 20% chance of having a retarded female.

Diagnosis/ prognosis:

The diagnosis of Fragile-X syndrome is made through the detection of errors in the FMR1 gene. Over 99% of individuals have a full mutant FMR1 gene. Tests used for diagnosis include chromosome analysis and various protein tests. Diagnosis is usually made when young, and there is no current cure for this illness. Early diagnosis of the syndrome call allow for therapeutic interventions like speech therapy, occupational therapy, psychotherapy and special education, that can considerably improve the quality of the patients' life.

g) Huntington's disease

Huntington’s disease is a degenerative brain disorder, in which afflicted individuals lose their ability to walk, talk, think, and reason. They easily become depressed, and lose their short-term memory capacity. They may also experience a lack of concentration and focus. This disease begins between ages 30-45, and every individual with the gene for the disease will eventually develop the disease. Huntington's is an autosomal dominant genetic disorder which means that if one parent

carriers the defective Huntington's gene, his/her offspring have a 50/50 chance of inheriting the disease.

Prevalence:

Huntington's disease (HD) affects males and females equally and crosses all ethnic and racial boundaries. It typically begins in mid-life, between the ages of 30 and 45, though onset may occur as early as the age of 2. Children who develop the juvenile form of the disease rarely live to adulthood. There is a 50/50 chance of inheriting the fatal gene from the parents. Everyone who carries the gene will develop the disease. In Western countries, it's estimated that about five to seven people per 100,000 are affected by HD.

Diagnosis/ prognosis:

There is no treatment or cure for Huntington's Disease, and the patient eventually becomes completely dependent on others for daily functioning. Individuals may also die due to other secondary complications such as choking, infection, or heart failure. Children who are diagnosed with Huntington's Disease do not usually live to reach adulthood.

5.6.2. Genes and noncommunicable diseases

Most diseases involve many genes in complex interactions, in addition to environmental influences. An individual may not be born with a disease but may be at high risk of acquiring it. This is called as genetic predisposition or susceptibility. The genetic susceptibility to a particular disease due to the presence of one or more gene mutations, and/or a combination of alleles need not necessarily be abnormal.

WHO's department of Noncommunicable Diseases and Mental Health (NMH) has done extensive work on major noncommunicable diseases, like cancer, diabetes, cardiovascular disease, asthma, and some mental illnesses. In some cases, such as cancer, individuals are born with genes that are altered by lifestyle habits or exposure to chemicals. Cancer, for example, may involve tumour-suppressor genes, genes which suppress tumour formation, which lose their function, thus giving rise to carcinomas. Cardiovascular disease tends to manifest itself in specific ways unique to various communities. For example, African communities tend to have strokes as a result of cardiovascular disease, while south asians tend to have heart attacks.

Understanding genetic predisposition to disease and knowledge of lifestyle modifications that either exacerbate the condition or that lessen the potential for

diseases (i.e., no smoking or drinking) is necessary for the public to make informed choices. This section on genetic predisposition to disease aims to provide descriptions of major diseases that have a genetic predisposition. It also contains resources for further information from the World Health Organization and other sources.

h) Cancer

Cancer occurs because of mutations in the genes responsible for cell multiplication and repair. The changes which a cell undergoes in the process of malignant transformation is a reflection of the sequential acquisition of these genetic alterations. This multi-step process is not an abrupt transition from normal to malignant, but may take over 20 years or more. The mutation of critical genes, including supressor genes, oncogenes and genes involved in DNA repair, leads to genetic instability and to progressive loss of differentiation. Tumours enlarge because cancer cells lack the ability to balance cell division by cell death (apoptosis) and by forming their own vascular system (angiogenesis) . The transformed cells lose their ability to interact with each other and exhibit uncontrolled growth, invade neighbouring tissues and eventually spread through the blood stream or the lymphatic system to distant organs.

Prevalence:

According to the 2002 World Health Report, about 7.1 million deaths are attributed to cancer each year. The most prevalent of these cancers include lung, stomach, colon, liver, breast and oesophagus cancer, in that order of occurrence. Combined, these cancers are responsible for over 4.2 million deaths. Furthermore, according to the World Cancer Report (IARC 2003) deaths due to cancer will increase by 50% in the next 20 years. Even though generally considered as an illness of the developed countries cancer is a world wide health problem. In 2000 54% of new cancer cases occurred in developing countries. Due to demographic changes and changes in life style this percentage is expected to rise in the near future.

Diagnosis / prognosis:

The roles that genes play differ greatly, ranging from genes that completely determine the disease state (disease genes) to genes that interact with other genes and environment factors in causing cancer (susceptibility genes). Studies have shown that the primary determinants of most cancers are lifestyle factors, such as tobacco, dietary and exercise habits, environment carcinogens and infectious agents, rather than inherited genetic factors. In fact, inherited cancer syndromes caused by high penetrance

genes transmitted In fact, the proportion of cancers caused by high penetrance genes is low, about less than 5% for breast cancer and less for most other cancer types except retinoblastoma in children.

Inherited mutations of the BRCA 1 gene account for a small proportion of all breast cancers, but affected family members have a greater than 70% lifetime risk for developing breast cancer or ovarian cancer. Identification of a germline mutations by genetic testing allows for preventive measures, clinical management and counselling. Since the prevalence of germline mutations such as BRCA1 is very low in most societies, the introduction of mass screening to identify people at risk to develop cancer is not recommended.

It is now appreciated that so-called metabolic polymorphisms, that is differences in the way people metabolize chemical carcinogens, explain differences in the susceptibility of individuals to cancer, and that these are controlled in cells by mutations in specific genes. A major research endeavour is now under way to characterize these genetic polymorphisms. It is already clear that there are a multiplicity of such genetic changes, that they are caused by genes of low penetrance, and that the classic Mendelian laws do not apply. However, it seems likely that collectively they explain much of innate susceptibility to cancer, and that therefore their potential contribution to the occurrence of cancer is large. It may eventually be possible to identify those individuals at special risk of tobacco or diet-associated cancers, and also those susceptible to the effects of environmental contaminants.

It is also anticipated, but not yet shown, that genetic tests may eventually provide information that will be used to determine the best course of treatment for some cancers. Some cancers currently classified as a single disease may ultimately be classified into different types, each best managed by a different therapeutic strategy.

In conclusion genetics may eventually play an important role in the control of cancer, including:

- identification of individuals at risk for a specific cancer, leading to preventive or screening strategies for an individual or family members.
- identification of the subtype of a cancer so that treatment can be tailored to target that specific disease.

i) Diabetes

Diabetes is a disease in which the body does not produce or properly use insulin. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. The cause of diabetes continues to be a mystery, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. There are three major classes of diabetes

Type 1 diabetes

This results from the body's failure to produce insulin, the hormone that "unlocks" the cells of the body, allowing glucose to enter and fuel them.

Type 2 diabetes

This results from insulin resistance which implies that the body fails to properly use insulin, combined with relative insulin deficiency. As a result of this the cells may be starved for energy and over time, high blood glucose levels may damage the eyes, kidneys, nerves or the heart.

Gestational diabetes

This is a type of diabetes, or high blood sugar, that only pregnant women get. If a woman gets high blood sugar when she's pregnant, she has gestational diabetes. It is one of the top health concerns related to pregnancy. If not treated, gestational diabetes can cause problems for mothers and babies.

Prevalence:

It is estimated that about 180 million people in the world suffer from diabetes today and more than two-thirds of them live in developing countries. The largest number of persons with diabetes live in India (32 million), China (21 million) and USA (18 million). The vast majority of persons with diabetes have Type 2 diabetes (90-95%). In developed countries about one-third of the persons with diabetes are unaware that they have diabetes, while the proportion of undiagnosed diabetes is even higher in developing countries. This is because classical symptoms of diabetes, like excessive thirst and involuntary weight loss, are often absent in Type 2 diabetes.

Diagnosis / prognosis:

Diabetes is diagnosed by measuring the sugar in the blood. Diabetes is a potentially life-threatening condition if left untreated. If inadequately treated, it can lead

to blindness, kidney failure, heart disease and limb amputation. The mainstay of diabetes treatment is diet and regular physical exercise, but the majority of persons with diabetes will need medication as well.

j) Cardiovascular disease

Major cardiovascular diseases (CVD) include coronary heart disease, cerebrovascular disease, heart failure, rheumatic heart disease and congenital heart disease. The major risk factors associated with cardiovascular diseases are cigarette smoking, unhealthy diet, physical inactivity, hypertension, diabetes and high blood cholesterol. CVD may also result from a variety of genetic causes, including single-gene mutations, the interaction of multiple genes and environmental factors. Economic transition, urbanisation, industrialization and globalisation bring about lifestyle changes that promote heart disease. Life expectancy in developing countries is rising sharply and people are exposed to these risk factors for longer periods

Prevalence:

Heart diseases have no geographical, gender or socioeconomic boundaries. CVD is an important cause of global mortality and in five of the six WHO regions it is the leading causes of mortality. Of the estimated 16.6 million deaths attributed to CVD worldwide, 80% is in developing countries. By 2010, CVD is estimated to be the leading cause of death in developing countries. Patients with established coronary heart disease or cardiovascular disease are at high risk for subsequent coronary and cerebral events Survivors of myocardial infarction are at increased risk of recurrent infarction and have an annual death rate five to six times higher than that of people of the same age who do not have coronary disease.

Diagnosis / prognosis:

Cost effective interventions for prevention and control of the CVD epidemic are available and if effectively implemented have the potential to halve the CVD burden in a short time frame of five years. The mapping of the human genome has contributed to a better understanding of the genetic causal factors associated with CVD. This will allow the development of more precise and effective treatments and management of the CVD in the future.

k) Asthma

Asthma is a disease in which the airways become blocked or narrowed. These effects are usually temporary, but they cause shortness of breath, breathing trouble, and other symptoms. When encountering a triggering particle, there is an inflammation of the linings, a constriction of the airways and mucous production. This results in difficulty in breathing, and may even block the airways completely. If an asthma episode is severe, a person may need emergency treatment to restore normal breathing. An asthma episode is triggered by things in the environment. These triggers vary from person to person, but common ones include cold air; exercise; allergens such as dust mites, mould, pollen, Cigarette smoke, animal dander or cockroach debris; and some types of viral infections.

Symptoms of asthma are coughing while exercising or post-exercise, wheezing, shortness of breath, and tightness in the chest.

There are many types of asthma:

- Exercise-induced
- Allergy induced
- Childhood asthma
- Occupational asthma
- Chronic asthma

The cause of asthma is not exactly known, but scientists believe that causes may include: genetic heredity, lifestyles, smoking, pollution, and viral infections.

Prevalence:

In 2001, 20.3 million people currently had asthma of which 6.3 million were children under age 18 years. Over recent years, the number of people suffering from asthma has increased at an alarming rate. Since the 1980's the incidence of asthma has more than doubled and the American Lung Association believes it will double again by the year 2020.

Diagnosis / prognosis:

Asthma, though it can be a life-threatening condition, can also be managed if asthma patients are keenly observant regarding what triggers their asthma. Identification of the asthma triggers is an important step in living with asthma. Once patients know what triggers their asthma attacks, they can make sure they avoid these triggers in their daily life and be prepared when they know they may encounter them.