St. Mary's College (Autonomous)

(Re-accredited with 'A^{+}' Grade by NAAC)

Thoothukudi – 628001, Tamil Nadu

COMMON SKILL BASED CORE

Computer for Digital Era and Soft Skills

III Year – V Semester

Compilation of Different Chapters from Books on Digital Era and Soft Skills

By

Department of Computer Science & Deans' Office

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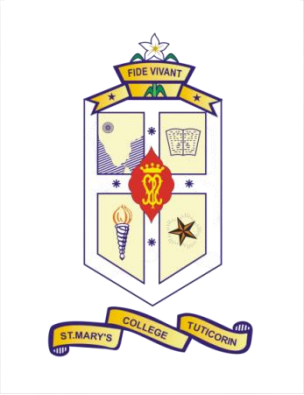

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Semester - V			
Common Skill Based Core : Computer for Digital Era and Soft Skills			
Code : 21UCSB51	**Hrs / Week : 2**	**Hrs / Sem : 30**	**Credits : 2**

Course Outcome

- Identify different types of computer systems.
- Classify various types of software being used.
- Compare various digital payments and use them in day to day life.
- Recognise the innovative technologies IoT and integrate it in various fields.
- Analyze various social networking platforms and use them efficiently.
- Distinguish various cyber attacks and apply preventive measures.
- Understand the various soft skills needed to become successful.
- Analyze self and adapt oneself to work in a team.

Unit I: Fundamentals of Computers:

Introduction to computers- Components of computers-Working principle-Types of computers-Tablet-Notebook-Smart phone-PDA-Impact of computers on society-Types of software.

Unit II: Recent Trends in Computer Science and e-Governance:

IoT - applications- Mobile applications - E-Learning- E-Commerce - digital payments-

Unit III: Social Media:

Face book-Twitter-Linked In-Instagram-Advantages of Social Networking-Issues/Risks of Social Networking-Protecting ourselves from social Networking problems-Cybercrimes-Hacking-Phishing- Cyber Security

Unit IV: Introduction to Soft Skills:

Learning objectives – What are soft skills?-Categories of Soft Skills-Integral Parts of Soft Skills.

Unit V: Understanding Self and Team Building:

Transactional Analysis (TA) - Structural analysis of Ego states- The functional model of Ego states - Egogram-Storkes - Life Position - Egogram and Life Positions Questionnaire-Team and Team Building- Features of effective creative teams

Books for Reference:

1. Peter Norton, Introduction to Computers 6th Edition
2. Charles P Pfleeger, Shari Lawrence Pfleeger, Security in Computing, I Edition, Pearson Education, 2003.
3. E.Balagurusamy, Fundamentals of Computers, McGraw Hill
4. Henry Chan, Raymond Lee, Tharam Dillon, Elizabeth Chang , E-Commerce fundamentals and applications, Wiley Student edition
5. Benita Bhatia Dua, DeepaJeyaraman, Profit with Social Media, CNBC
6. Dr.K.Alex, Soft Skills, S.Chand & Co
7. http://www.digitalindia.gov.in/content/social-media-analytics
8. https://www.researchgate.net/publication/307878962_Introduction_to_E-Governance
9. http://www.ijqr.net/journal/v10
10. https://www.researchgate.net/publication/258339295_FUNDAMENTALS_OF_ COMPUTER _ STUDIES

UNIT-I: Fundamentals of Computers

Introduction to Computers

A computer is an electronic device, operating under the control of instructions stored in its own memory that can accept data (input), process the data according to specified rules, produce information (output) and store the information for future use.

Functionalities of a computer

Any digital computer carries out four functions

- Takes Data as input.
- Stores the data or instruction in its memory and use them when required.
- Processes the data and converts it into useful information.
- Generates the output.

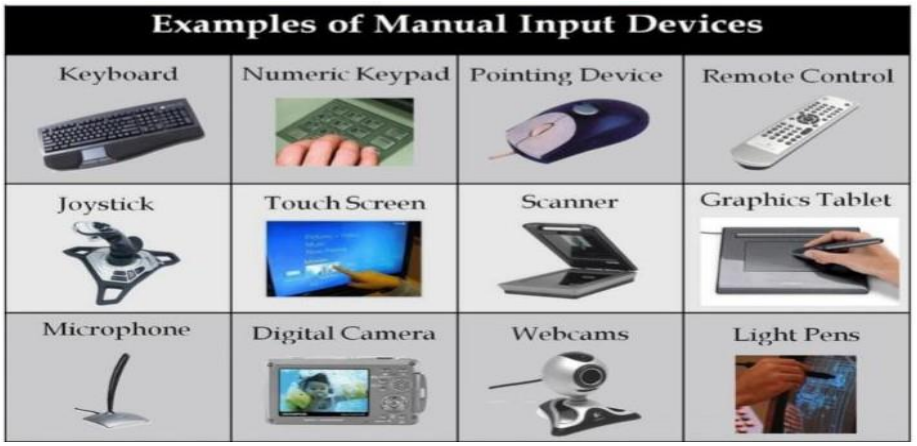
Components of Computers

Any kind of computer consists of Hardware and Software.

Hardware:

Computer hardware is the collection of physical elements that constitutes a computer system. Computer hardware refers to the physical parts or components of a computer such as the monitor, mouse, keyboard, computer data storage, hard drive disk (HDD), system unit (graphic cards, sound cards, memory, motherboard and chips), etc. all of which are physical objects that can be touched.

Input Devices: An input device is a piece of hardware used to provide data to a computer. It allows input of raw data to the computer for processing. Most common are keyboard and mouse.



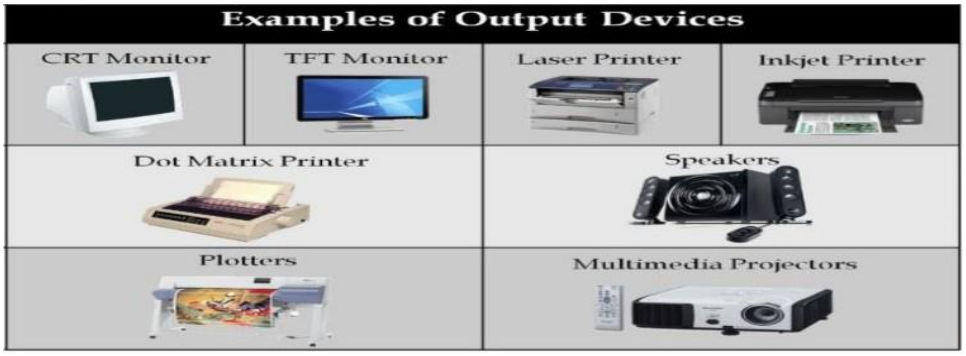
Central Processing Unit (CPU): A CPU is the brain of a computer. It is responsible for all functions and processes. The CPU is the most important element of a computer system.
The CPU is comprised of three main parts:

* **Arithmetic-Logic Unit (ALU):** An arithmetic logic unit (**ALU)** is a digital circuit used to perform arithmetic and logic operations. It represents the fundamental building block of the **central processing unit (CPU)** of a computer. Modern CPUs contain very powerful and complex ALUs.

* **Control Unit (CU):** The control unit (CU) is a component of a computer's central processing **unit** (CPU) that directs the operation of the processor. It tells the computer's memory, arithmetic and logic unit and input and output devices how to respond to the instructions that have been sent to the processor. It directs the operation of the other units by providing timing and control signals.

* **Registers:** It is a temporary storage place for instruction or data. They are not part of the memory, but they are a special additional storage location that offers the advantage of speed.

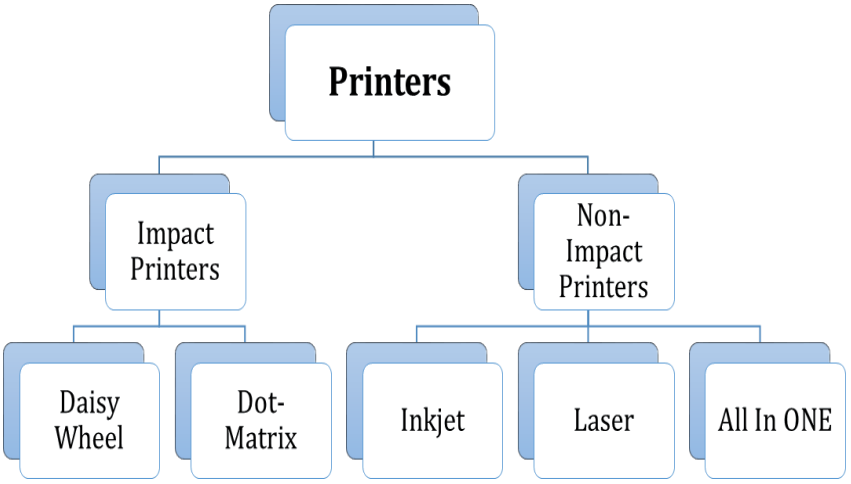
Output devices: An output device is any piece of computer hardware equipment used to communicate the results of data processing carried out by an information processing system (such as a computer) which converts the electronically generated information into human readable form. Most prominent output devices are monitors and printers.



Basic types of monitors are

a) Cathode Ray Tube (CRT) b) Liquid Crystal Displays (LCD) c) Light-Emitting diode (LED)

and various types of printers are



Software:

Software is a generic term for organized collection of instructions that enables a user to interact with the computer. Software is normally classified into two major categories: **System software** that provides the basic non task - specific functions of the computer, and **Application software** which is used by users to accomplish specific tasks.

System software is responsible for controlling, integrating, and managing the individual hardware components of a computer system so that other software and the users of the system see it as a functional unit without having to be concerned with the low-level details such as transferring data from memory to disk, or rendering text onto a display.

Application software is used to accomplish specific tasks other than just running the computer system. Application software may consist of a single program, such as a browser (Google Chrome, Internet Explorer) or a collection of programs (often called a software package) that work closely together to accomplish a task, such as a spreadsheet or text processing system.

Working Principle of Computers:

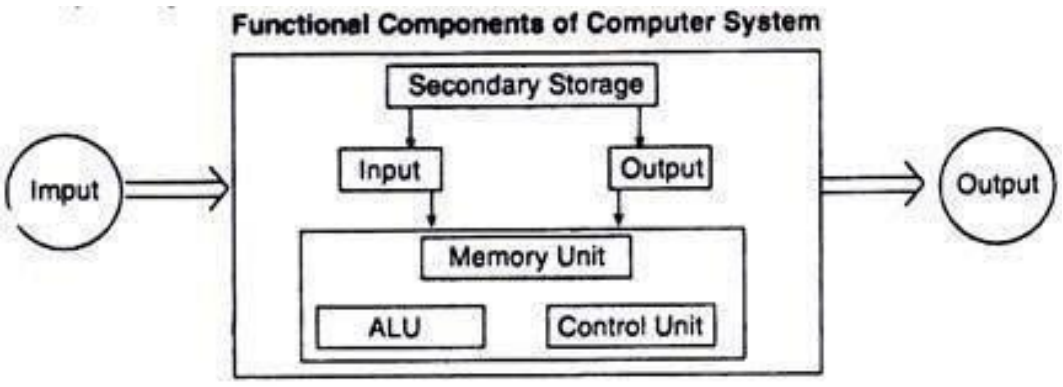
A computer is an electronic machine that converts data into meaningful information by performing various actions on the data. It accepts the raw data from the user, processes them according to the given set of instructions and gives the result as a human understandable form. The processed data is called as information and stored for future references. The basic working principle of a computer has four steps. They are as follows.

Input: During this process, the computer takes data from the user through input devices such as a keyboard, mouse, joystick, scanner etc. and provides them to the computer forprocessing.

Processing: The CPU processes the input data according to the given set of instructions in association with memory, executes a computer program

Output: The computer displays the result of processing. After processing input data, the computer gives meaningful information called result or output through the output devices such as monitor, printer, speaker etc.

Storage: The computer permanently stores the processed result or output on the storage devices like Hard disk, Pen drive, CD/DVD etc.



Types of Computers:

Computers can be generally classified by size and power

- **Personal computer**: A small, single-user computer is based on a microprocessor. In addition to the microprocessor, a personal computer has a keyboard for entering data, a monitor for displaying information, and a storage device for savingdata.
- **Workstation**: A powerful, single-user computer. A workstation is like a personal computer, but it has a more powerful microprocessor and a higher-qualitymonitor.
- **Minicomputer**: A multi-user computer is capable of supporting hundreds of users simultaneously.
- **Mainframe**: A powerful multi-user computer is capable of supporting thousands of users simultaneously.
- **Super Computer**: An extremely fast computer that can perform millions of instructions per second.

Laptop : A laptop is a portable personal computer powered by a battery, or an AC cord plugged into an electrical outlet, which is also used to charge the battery. Laptops have an attached keyboard and a touchpad, trackball, or isometric joystick used for navigation.

Tablet Computers: Like laptops, tablet computers are designed to be portable. However, they provide a very different computing experience. The most obvious difference is that tablet computers don't have keyboards or touchpads. Instead, the entire screen is touch-sensitive, allowing you to type on a virtual keyboard and use your finger as a mouse pointer. Tablet computers are mostly designed for web browsing, watching videos, reading e-books, and playing games.

Netbook and Notebook : Notebooks and Netbooks use the same basic form factor but the main differentiator is size. In general, netbook computers are smaller and lighter than notebook computers, which in turn are smaller and lighter than laptops. Netbooks were primarily designed for internet-related activities and tasks, such as email and social media. On the other hand, notebooks were developed with the ability to handle most desktop operations.

Smartphones:. A smartphone is a handheld powerful computer that is designed to run a variety of applications in addition to phone service. They are basically small tablet computers, having touchscreen interface and function as portable media players, digital cameras, video cameras, GPS navigational device.

PDA: A personal digital assistant, also known as a handheld PC, can function as a cellular phone, fax sender, Web browser and personal organizer. It provides computing and information storage and retrieval capabilities for personal or business use, often for keeping schedules, calendars and address book information handy.

Impact of Computers on society

It is obvious that computers are revolutionizing our daily life.Due to the advancement of science and technology more and more people are using computers in their lives..The following applications are the basic reasons for increased attraction towards the use of computers in homes and offices.

- A Computer is capable of high speed calculation diligently and accurately, which has made it an integrated part in all business organizations. They are mainly used for Payroll calculations, Budgeting, Sales analysis, Financial forecasting, Managing employee database, Maintenance of stocks, etc.
- Today, banking is almost totally dependent on computers. Online accounting facility and ATM machines which are completely automated are making it even easier for customers to deal with banks.
- Insurance companies are keeping all records up-to-date with the help of computers. Insurance companies, finance houses, and stock broking firms are widely using computers for their concerns.

- The computer helps in providing a lot of facilities in the education system.
- In marketing computers are used for Advertising and for Home Shopping
- Computers have become an important part in hospitals, labs, and dispensaries. It is used in scanning and diagnosing different diseases. ECG, EEG, ultrasounds and CT scans, etc. are also done by computerised machines. Nowadays, Surgical robots are alsoused.
- Computers are widely used for Engineering purpose. One of the major areas is CAD (Computer Aided Design) that provides creation and modification of images. They are mainly used in Structural Engineering, Industrial Engineering and Architectural Engineering
- Some military areas where a computer has been used are Missile Control, Military Communication, Military Operation and Planning, Smart Weapons
- Computers play an important role in communication. Some important applications are E-mail, Chatting, Usenet, FTP, Telnet, Video-conferencing
- Computers play an important role in government services like Budgets, Sales tax department, Income tax department, Computerisation of voters lists, Computerisation of PAN card and Weather forecasting etc.
- Entertainment (video games, pretty much every other form of media in electronic form).

Negative impacts of computers on society

Computers have touched every aspect of our technological civilization and have the potential to do even more. Despite the fact that computers have greatly improved our lives and society we must also become aware of the negative impact it can have on individuals if not used responsibly.

- Excessive use of computers and consumerism has led to e-waste generation which causes a lot of environmental problems.
- People leave their computers on non stop resulting in a lot of energy consumption and enormous amounts of paper are being used daily to print out electronically stored data.
- Computer use can result in vision problems called Computer Vision Syndrome (CVS). Symptoms of CVS include eye strain, blurred vision and dry eyes.
- People who use the computers for an extensive period of time may complain of headaches, insomnia, back pain and pains in their wrists, arms and necks.
- Computer hackers and malicious users can hack accounts and steal personal information that could be used for identity theft.

- Social networking sites can also lead to depression as many people tend to compare their lives with others.

Types of Software

Software is a set of instructions that tells a computer exactly what to do. Examples of software are Ms Word, Excel, Power Point, Google Chrome, Photoshop, MySQLetc.

The software can be categorized according to what it is designed to accomplish. There are two main types of software:

- **System software**
- **Application software**

System software

Basically, it is a software to manage computer hardware behavior so as to provide basic functionalities that are required by the user. In simple words, we can say that system software is an inter mediator or a middle layer between the user and the hardware. These computer software sanction a platform or environment for the other software to work in. This is the reason why system software is very important in managing the entire computer system. When you first turn on the computer, it is the system software that gets initialized and gets loaded in the memory of the system.

It consists of programs that startup the computer and perform some utility functions such as checking and getting the computer ready for use. They are usually written to accomplish loading, execution, storage, and retrieval of files from/into the computer. The system software runs in the background and is not used by the end-users. This is the reason why system software is also known as ‘low-level software’.

They are also known as Closed-source software. These types of applications are usually paid and have intellectual property rights or patents over the source code. The use of these are very restricted and usually, the source code is preserved and kept as a secret.

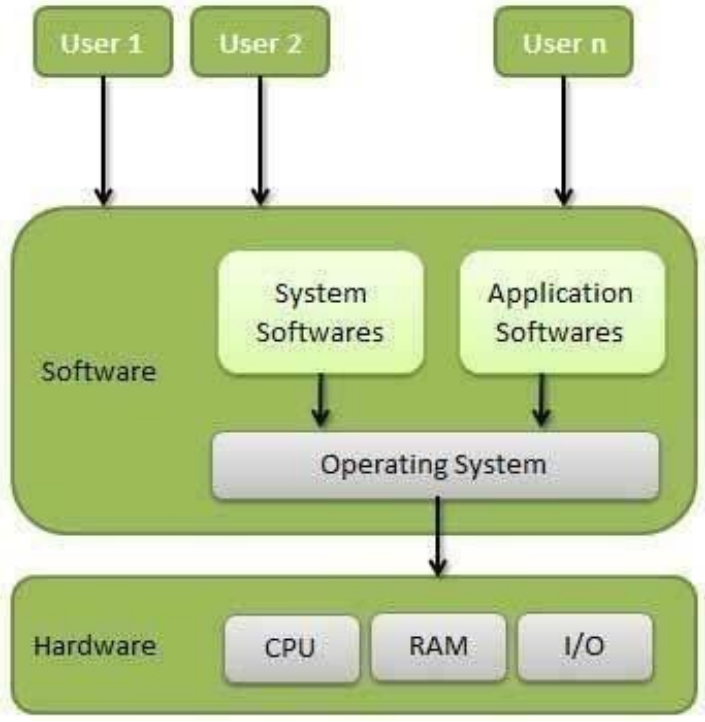
System software includes Operating systems, Device drivers, Programming Language Translators and Utility software.

- **Operating System**

It is the most prominent example of System Software.

An Operating System (OS) is an interface between a computer user (application programs) and computer hardware. An operating system is a software which performs all the basic tasks like file management, memory management, process management, handling input and output, and controlling peripheral devices such as disk drives and printers.

Every device, whether a desktop, laptop or mobile phone requires an operating system to provide the basic functionality to it.



There are various types of operating systems such as real-time, embedded, distributed, multiuser, single-user, internet, mobile, and many more. It is important to consider the hardware specifications before choosing an operating system. Some examples of operating systems are Android, CentOS, iOS, Linux, Mac OS, MS Windows, Ubuntu and Unix.

Mobile OS

A mobile operating system, also called a mobile OS, is an operating system that is specifically designed to run on mobile devices such as mobile phones, smartphones, PDAs, tablet computers and other handheld devices.

A mobile OS is similar to a standard OS (like Windows, Linux, and Mac) but is relatively simple and light and primarily manages the wireless variations of local and broadband connections, mobile multimedia and various input methods.

Popular Mobile Operating Systems

1. Android OS (Google Inc.)

The Android mobile operating system is Google's open and free software stack that includes an operating system, middleware and also key applications for use on mobile devices, including smartphones.

2. Bada (Samsung Electronics)

Bada is a proprietary Samsung mobile OS that was first launched in 2010. The Samsung Wave was the first smartphone to use this mobile OS. Bada provides mobile features such as multipoint-touch, 3D graphics and of course, application downloads and installation.

3. BlackBerry OS (Research In Motion)

The BlackBerry OS is a proprietary mobile operating system developed by Research In Motion for use on the company’s popular BlackBerry handheld devices.

4. iPhone OS / iOS(Apple)

Apple's iPhone OS was originally developed for use on its iPhone devices. Now, the mobile operating system is referred to as iOS and is supported on a number of Apple devices including the iPhone, iPad, iPad 2 and iPod Touch.

5. MeeGo OS (Nokia and Intel)

A joint open source mobile operating system which is the result of merging two products based on open source technologies: Maemo (Nokia) and Moblin (Intel). MeeGo is a mobile OS designed to work on a number of devices including smartphones, netbooks, tablets, in-vehicle information systems and various devices using Intel Atom and ARMv7 architectures.

6. Palm OS (Garnet OS)

The Palm OS is a proprietary mobile operating system (PDA operating system) that was originally released in 1996 on the Pilot 1000 handheld. Palm OS 5 was extended to provide

support for a broad range of screen resolutions, wireless connections and enhanced multimedia capabilities and is called Garnet OS.

7. Symbian OS (Nokia)

Symbian is a mobile operating system (OS) targeted at mobile phones that offers a high-level of integration with communication and personal information management (PIM) functionality. Nokia does not maintain Symbian as an open source development project.

8. WebOS (Palm/HP)

WebOS is a mobile operating system that runs on the Linux Kernel.. It is a proprietary Mobile OS which was eventually acquired by HP and now referred to as webOS (lower-case w) in HP literature.

9. Windows Mobile (Windows Phone)

Windows Mobile is Microsoft's mobile operating system used in smartphones and mobile devices – with or without touchscreens.

- **Device Drivers**

It is a type of software that controls particular hardware which is attached to the system. Hardware devices that need a driver to connect to a system include displays, sound cards, printers, mice and hard disks. Further, there are two types of device drivers: Kernel Device Drivers and User Device Driver. Some examples of device drivers are:

- BIOS Drive
- Display Drivers
- Printer Drivers
- ROM Drivers
- Sound card Driver
- USB Drivers
- VGA Drivers
- Virtual Device Drivers

- **Programming Language Translators**

These are mediator programs on which software programs rely to translate high-level language

code to simpler machine-level code. Besides simplifying the code, the translators also do the following :

- Assign data storage
- Enlist source code as well as program details
- Offer diagnostic reports
- Rectify system errors during the runtime

Interpreter, Compiler and Assemblers are translators.

- **Utility Software**

Utility software improves the function of computers, helps users perform multiple tasks efficiently. Some of the functions performed by various utility software include data compression, data recovery, disk defragmentation, computer resources and files management, system diagnosis, and more. It is designed to aid in analyzing, optimizing, configuring and maintaining a computer system. It supports the computer infrastructure. Some examples of utility tools are:

- Avast Antivirus
- Directory Opus
- McAfee Antivirus
- PiriformCCleaner
- Razer Cortex
- Windows File Explorer
- WinRAR
- WinZip

Application Software

Application Software also known as end-user programs or productivity programs are software that helps the user in completing tasks such as doing online research, jotting down notes, setting an alarm, designing graphics, keeping an account log, doing calculations or even playing games. They lie above the system software. Unlike system software, they are used by the end-user and are specific in their functionality or tasks and do the job for which they are designed.. For example, a browser is an application designed specifically for browsing the internet and MS

PowerPoint is an application used specifically for making presentations. Application Software or simply apps can also be referred to as non-essential software as their requirement is highly subjective and their absence does not affect the functioning of the system. All the apps that we see on our mobile phones are also examples of Application Software. There are certain software that are exclusively designed for app development like Meteor and Flutter. These are also examples of Application software.

Features of application software are as follows -

- Close to the user
- Easy to design
- More interactive
- Slow in speed
- Generally written in high-level language
- Easy to understand
- Easy to manipulate and use
- Bigger in size and requires large storage space

Differences between System Software and Application software

No	System Software	Application Software
1	System Software maintains the system resources and gives the path for application software to run	Application software is built for specific tasks.
2	Low level languages are used to write the system software	high level languages are used to write the application software.
3	It's a general purpose software.	it is a specific purpose software.
4	Without system software, system can't run	Without application software system always runs
5	System software runs when the system is turned on and stops when the system is turned off.	application software runs as per the user's request.
6	Examples of system software are operating system, utility software etc.	Examples of application software are Photoshop, VLC player etc.
7	System Software programming is complex than application software programming	Application software programming is simpler as compared to system software programming.

There are various types of application software

Word Processors:

These applications are used for documentation. Along with that it also helps in storing, formatting and printing of these documents. Some examples of word processors are MS Word, Apple iWork- Pages , Corel WordPerfect and Google Docs

Database Software:

This software is used to create and manage a database. It is also known as the Database Management System or DBMS. They help with the organization of data. Some examples of **DBMS** are dBase, FoxPro, MS Access, MySQL,ORACLE and SQL Server

Multimedia Software:

It is the software that is able to play, create or record images, audio or video files. They are used for video editing, animation, graphics and image editing, Some examples of Multimedia Software are Adobe Photoshop, Inkscape, Media Monkey, Picasa, VLC Media Player, Windows Media Player and Windows Movie Maker

Education and Reference Software:

These types of software are specifically designed to facilitate learning on a particular subject. There are various kinds of tutorial software that fall under this category. They are also termed as academic software. Some examples are Delta Drawing , GCompris, Jumpstart titles, KidPix, MindPlay and Paint.

Graphics Software

As the name suggests, Graphics Software has been devised to work with graphics as it helps the user to edit or make changes in visual data or images. It comprises picture editors and illustration software. Some examples are Adobe Photoshop, Autodesk Maya, Blender, Carrara, CorelDRAW,GIMP, Modo and PaintShop Pro

Web Browsers

These applications are used to browse the internet. They help the user in locating and retrieving information across the web. Some examples of web browsers are Google Chrome, Internet Explorer, Microsoft Edge, Mozilla Firefox, Opera, Safari and UC Browser.

License Based Software Classification

Proprietary Software: Proprietary software is any software that is copyrighted and bears limits against use, distribution and modification that are imposed by its publisher, vendor or developer. Proprietary software remains as the property of its owner/creator and is used by end-users/organizations under predefined conditions. Proprietary software may also be called closed-source software or commercial software. In general, proprietary software doesn't provide end users or subscribers with access to its source code. It can be purchased or licensed for a fee,but relicensing, distribution or copying is prohibited.

Examples of proprietary software include Microsoft Windows, Adobe Flash Player, PS3 OS, iTunes, Adobe Photoshop, Google Earth, macOS (formerly Mac OS X and OS X), Skype, WinRAR, Oracle's version of Java and some versions of Unix.

Free and open-source software (FOSS)

Free and open-source software (FOSS) allows users and programmers to edit, modify or reuse the software's source code. This gives developers the opportunity to improve program functionality by modifying it. The term “free” indicates that the software does not haveconstraints on copyrights. The term “open source” indicates the software is in its project form, enabling easy software development from expert developers collaborating worldwide without any need for reverse engineering. The source code is openly shared so that people can inspect, modify, and enhance and are encouraged to voluntarily improve the design of the software. Some open source software are

LibreOffice,VLC Media Player, PHP,Firefox, Java,Ubuntu, Wordpress,Thunderbird, FileZilla and Linux

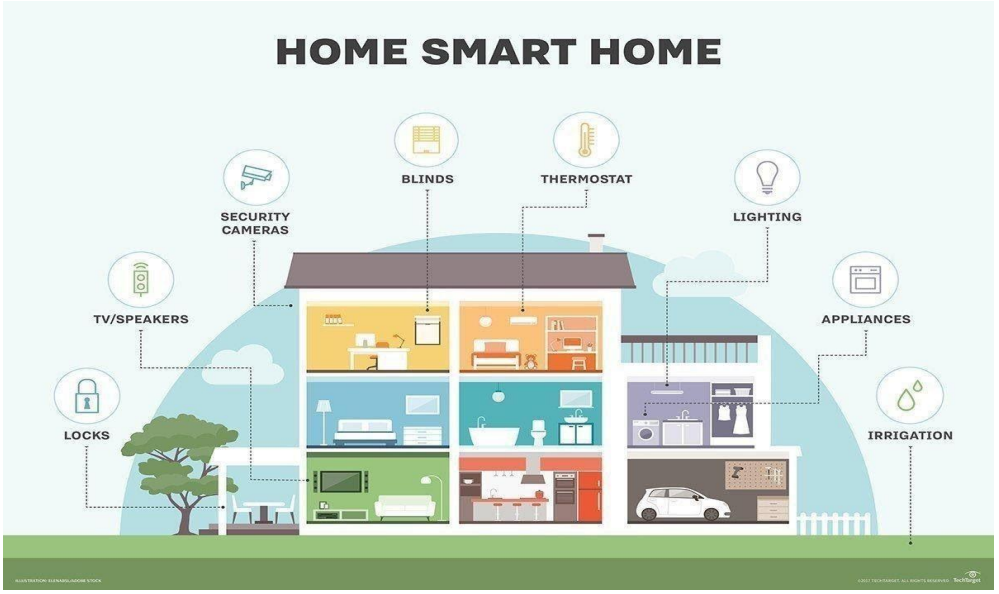
UNIT II: Recent Trends in Computer Science and E-Commerce

The internet of things **(IoT)**, is a system of interrelated computing devices, mechanical and digital machines, objects, animals or people that are provided with unique identifiers (UIDs) and the ability to transfer data over a network without requiring human-to-human or human-to-computer interaction.

IoT Based Applications

Smart home

A smart home is a residence that uses internet-connected devices to enable the remote monitoring and management of appliances and systems, such as lighting, heating and security. Smart home technology is often referred to as home automation or domotics. (from the Latin"domus" meaning home). It provides homeowners security, comfort, convenience and energy efficiency by allowing them to control smart devices, often by a smart home app on their smartphone or other networked device. Internet of things (IoT), smart home systems and devices often operate together, sharing consumer usage data among themselves and automating actions based on the homeowners' preferences.



Wearable

Wearable technology is a category of electronic devices that can be worn as accessories, embedded in clothing, implanted in the user's body, or even tattooed on the skin.

Wearable devices are worn on the wrist (smart watches), put on like a spectacle (Google Glass), smart garments and Skin coloured xatoo/patch like sensors.

Smart City

A Smart city is an urban area that uses different types of electronic Internet of Things (IoT) sensors to collect data and then use insights gained from that data to manage assets, resources and services efficiently. The cities then use this data to improve infrastructure, public utilities and services.

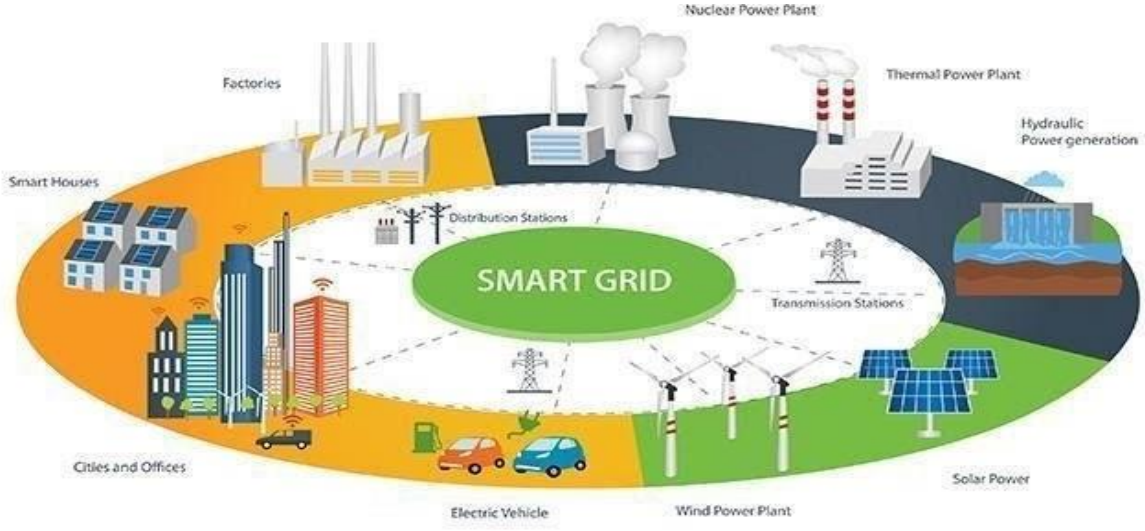
Smart city includes traffic management, water distribution, waste management, urban security and environmental monitoring. IoT solutions in the area of Smart City solve traffic congestion problems, reduce noise and pollution and help make cities safer.

Smart Grid

Smart grid uses information about the behaviours of electricity suppliers and consumers in an automated fashion.

This technology helps in:

1. Deliver power more efficiently
2. Improve operations
3. Reduce emissions and management costs
4. Restore power failures faster.



Smart Agriculture

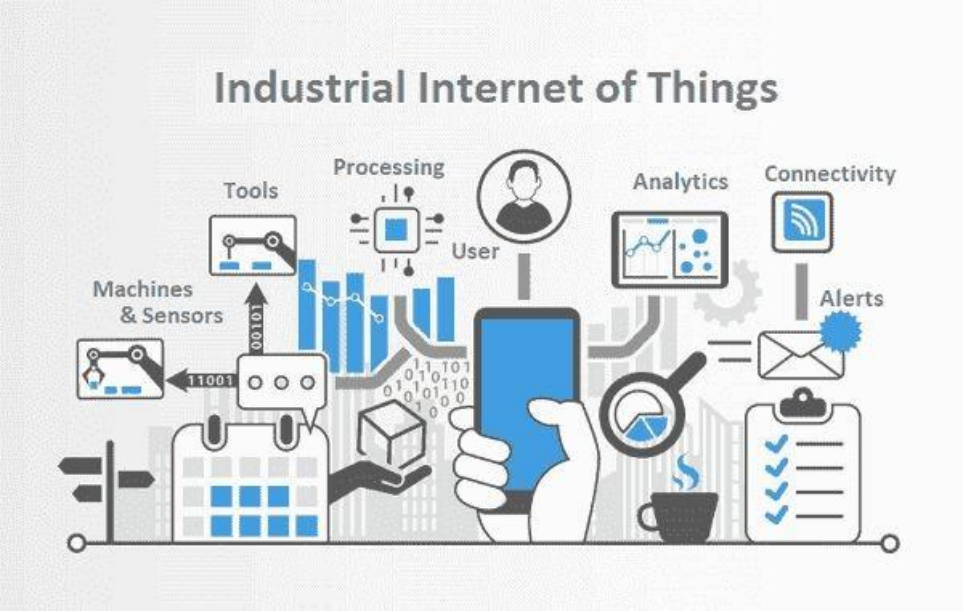
Smart Agriculture is a farming management concept using modern technology to increase the quantity and quality of agricultural products. To feed the ever-growing massive population, agriculture must be tied to technology.. Farmers in the 21st century have access to GPS, soil scanning, data management, and Internet of Things technologies.

The most popular smart agriculture gadgets are weather stations, combining various smart farming sensors. Located across the field, they collect various data from the environment and send it to the cloud. The provided measurements can be used to map the climate conditions, choose the appropriate crops, and take the required measures to improve their capacity (i.e. precision farming).

Industrial IoT

The industrial internet of things (IIoT) refers to the extension and use of the Internet of Things(IoT) in industrial sectors and applications. With a strong focus on machine-to-machine (M2M) communication, big data, and machine learning, the IIoT enables industries and enterprises to have better efficiency and reliability in their operations. The IIoT encompasses industrial applications, including robotics, medical devices, and software-defined production processes.

The convergence of IT(Information Technology) and OT (Operational Technology) provides industries with greater system integration in terms of automation and optimization, as well as better visibility of the supply chain and logistics. The monitoring and control of physical infrastructure in industrial operations are made easier through the use of smart sensors and actuators as well as remote access and control.



Mobile Based Application

A mobile application, most commonly referred to as an app, is a type of application software designed to run on a mobile device, such as a smartphone or tablet computer. A mobile application may also be known as an app, web app, online app, iPhone app or smartphone app.

There are three main types of mobile apps . They are

1. Native mobile apps

2. Web-based mobile apps

3. Hybrid apps.

Native Mobile Apps

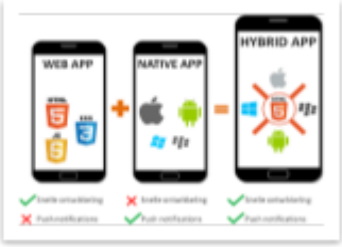
Native apps are developed for a certain mobile device operating system like Windows Phone or Android. Therefore, they are native for a certain device or platform. Apps built for Android, Windows Phone, Blackberry, Symbian cannot be used on any other platform except on their own. Therefore, a mobile app designed for Android can only be used on an Android device. Main advantages of native apps are good user experience and high performance. In addition, an access to a broad range of APIs puts no limits on app usage. Native mobile apps are accessible from app stores of their kind and have very

clear tendency of reaching target customers. Some disadvantages of native mobile apps include higher costs in comparison to other types of mobile apps.

Web-Based Apps

Web-based applications behave in a very similar fashion to those native mobile apps. Web apps use a certain browser in order to run and they are commonly written in CSS, JavaScript or HTML5. The greatest advantage of web apps is that they require a minimum of device memory. Users can access web apps from any device that is connected to the Internet. All personal databases are saved on a certain server, so the use of web applications with poor internet connection commonly results in very bad user experience. Another drawback of web apps is access to not so many APIs, with exception of geolocation and several others.

Hybrid Apps



Hybrid mobile apps are specifically built using different multi-platform web technologies like JavaScript and HTML5. Hybrid apps are website applications created in a native wrapper that means they use elements of both native and web-based apps. Hybrid apps also possess common cons and pros of both web mobile and native mobile applications. Hybrid multi-platform mobile apps are relatively easy to develop, low-cost maintenance and provide smooth updates. On the other hand, hybrid applications lack speed, performance and overall optimization compared to native mobile apps. There are also specific design issues and they do not look the same on different platforms.

E-Learning

E-learning can best be defined as the science of learning without using paper printed instructional material. E-learning is the use of telecommunication technology to deliver information for education and training. With the progress of information and communication technology development, E-learning is emerging as the paradigm of modern education. A number of other terms are also used to

describe this mode of teaching and learning. They include online learning, virtual learning, distributed learning, network and web-based learning. The term E-learning comprises a lot more than online learning, as the letter "e" in E-learning stands for the word "electronic", E-learning would incorporate all educational activities that are carried out by individuals or groups working online or offline

E-learning platforms

People can do an online course via a wide variety of different platforms such as:

- MOOCs (Massive Online Open Courses), e.g. Coursera or Futurelearn.
- Virtual learning environment (VLE), such as Learn or Blackboard.
- Video streaming services, such as YouTube.
- Virtual instructor-led training (VILT), e.g. WebEx for webinars.
- Discussion boards.
- Forums.
- Podcasts.

MOOC stands for Massive Online Open Courses. They are free online programs thatseveral colleges offer. Even some of the world's most famous and prestigious universities use them.

Someprogramsallowyoutopayforthecertificationofthecourse.Thesecoursescancount as college credits, while others have value in the job market.

e-Learning Websites

1. Khan Academy

Khan Academy is a non-profit educational organization created in 2008 by Salman Khan with the goal of creating a set of online tools to educate students. The organization produces short lessons in the form of videos. Its website includes supplementary practice exercises and materials for educators. Khan academy provides an easy path for students to learn about any subject they choose. Khan Academy has youth appeal with its avatars and ability to earn badges.

2. **Coursera**

Coursera is an American online learning platform founded in 2012 by Stanford professors Andrew Ng and Daphne Koller that offers massive open online courses, specializations, and degrees.

Coursera has partnered with museums, universities, and other institutions to offer students free classes on an astounding variety of topics. Students can browse the list of available topics or simply answer the question "What would you like to learn about?", then when they answer that question they are led to a list of available courses on that topic. Coursera provides plenty of information about each class. This includes:

- A course syllabus.
- Course format.
- Recommended background and experience.
- Materials needed.
- Course at a glance.
- Students who finish a course may often receive a statement of accomplishment from the instructor.

3. W3 Schools

W3 Schools is a free eLearning website that is dedicated to teaching various programming languages. For each concept that students wish to master, theygo through a variety of online tutorials, take tests, and ultimately complete each course. Students can take a final test to prove their mastery, and if they pay an extra fee receive a certificate of completion.

4. T**edEd**

Ted-Ed is educational videos on a variety of general education topics that can be accessed for free. Not only are there motivational speakers on Ted, there are also topical videos, often less than ten minutes each that are full of important information.

5. SWAYAM

Swayam is a programme initiated by the Government of India and designed to achieve the three cardinal principles of Education Policy viz., access, equity and quality. The objective of this effort is to take the best teaching learning resources to all, including the most disadvantaged. Categories of courses include - Engineering, Science, Humanities, Management, Language, Mathematics, Arts and Commerce, General, Library, Education. Courses delivered through SWAYAM are available free of cost to the learners.

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This is done through a platform that facilitates hosting of all the courses, taught in classrooms from Class 9 till post-graduation to be accessed by anyone, anywhere at any time. All the courses are interactive, prepared by the best teachers in the country and are available, free of cost to any learner. The courses hosted on SWAYAM are in 4 quadrants – (1) video lecture, (2) specially prepared reading material that can be downloaded/printed (3) self-assessment tests through tests and quizzes and (4) an online discussion forum for clearing the doubts.

In order to ensure that best quality content is produced and delivered, nine National Coordinators have been appointed. They are:

1. AICTE (All India Council for Technical Education) for self-paced and international courses
2. NPTEL (National Programme on Technology Enhanced Learning) for Engineering
3. UGC (University Grants Commission) for non technical post-graduation education
4. CEC (Consortium for Educational Communication) for under-graduate education
5. NCERT (National Council of Educational Research and Training) for school education
6. NIOS (National Institute of Open Schooling) for school education
7. IGNOU (Indira Gandhi National Open University) for out-of-school students
8. IIMB (Indian Institute of Management, Bangalore) for management studies
9. NITTTR (National Institute of Technical Teachers Training and Research) for Teacher Training programme

Courses delivered through SWAYAM are available free of cost to the learners, however learners wanting a SWAYAM certificate should register for the final proctored exams that come at a fee and attend in-person at designated centres on specified dates. Eligibility for the certificate will be announced on the course page and learners will get certificates only if this criteria is matched. Universities/colleges approving credit transfer for these courses can use the marks/certificate obtained in these courses for the same

E-Commerce

E-Commerce (electronic commerce) is the buying and selling of goods and services, or the transmitting of funds or data, over an electronic network, primarily the internet. These business transactions occur either as business-to-business (B2B),business-to-consumer (B2C), consumer-to-consumer or consumer-to-business. The terms E-Commerce and e-business are often used interchangeably. The term e-tail is also sometimes used in reference to the transactional processes for online shopping.

History of E-Commerce

The beginnings of E-Commerce can be traced to the 1960s, when businesses started using Electronic Data Interchange (EDI) to share business documents with other companies. In 1979, the American National Standards Institute developed ASC X12 as a universal standard for businesses to share documents through electronic networks.

After the number of individual users sharing electronic documents with each other grew in the 1980s, the rise of eBay and Amazon in the 1990s revolutionized the E-Commerce industry. Consumers can now purchase endless amounts of items online, from e-tailers, typical brick and mortar stores with E-Commerce capabilities and one another.

Types of E-Commerce

Business-to-business (B2B) E-Commerce refers to the electronic exchange of products, services or information between businesses rather than between businesses and consumers. Examples include online directories and product and supply exchange websites that allow businesses to search for products, services and information and to initiate transactions through e-procurement interface

Business-to-consumer (B2C) is the retail part of E-Commerce on the internet. It is when businesses sell products, services or information directly to consumers. The term was popular during the dot-com boom of the late 1990s, when online retailers and sellers of goods were a n novelty. Today, there are innumerable virtual stores and malls on the internet selling all types of consumer goods. The most recognized example of these sites is Amazon, which dominates the B2C market.

Consumer-to-Consumer (C2C) is a type of E-Commerce in which consumers trade products, services and information with each other online. These transactions are generally conducted through a third party that provides an online platform on which the transactions are carried out.Online auctions and classified advertisements are two examples of C2C platforms, with eBay and Craigslist being two of the most popular of these platforms. Because eBay is a business, this form of E-Commerce could also be called C2B2C -- consumer-to-business-to-consumer.

Consumer-to-Business (C2B) is a type of E-Commerce in which consumers make their products and services available online for companies to bid on and purchase. This is the opposite of the traditional commerce model of B2C.A popular example of a C2B platform is a market that sells royalty-free photographs, images, media and design elements, such as iStock. Another example would be a job board. **Business-to-Administration (B2A)** refers to transactions conducted online between companies and public administration or government bodies.

Consumer-to-administration (C2A) refers to transactions conducted online between individual consumers and public administration or government bodies. The government rarely buys products or services from citizens, but individuals frequently use electronic means in the following areas:

- Education: disseminating information, distance learning/online lectures, etc.
- Social security: distributing information, making payments, etc.
- Taxes: filing tax returns, making payments, etc.
- Health: making appointments, providing information about illnesses, making health services payments, etc.

Benefits of E-Commerce

The benefits of E-Commerce include its around-the-clock availability, the speed of access, the wide availability of goods and services for the consumer, easy accessibility and international reach.

Availability. Aside from outages or scheduled maintenance, E-Commerce sites are available 24x7, allowing visitors to browse and shop at any time.

Speed of access. While shoppers in a physical store can be slowed by crowds, E-Commerce sites run quickly, which is determined by computers and bandwidth considerations.

Wide availability. E-Commerce enables brands to make a wide array of products available, which are then shipped from a warehouse after a purchase is made.

Easy accessibility. In E-Commerce, visitors can browse product category pages and use the site search feature to find the product immediately.

International reach. With E-Commerce, businesses can sell to any customer who can access the web. E-Commerce has the potential to extend a business' customer base globally.

Lower cost. Pure play E-Commerce businesses avoid the cost associated with physical stores, such as rent, inventory and cashiers, although they may incur shipping and warehouse costs.

Personalization and product recommendations. E-Commerce sites can track visitors' browse, search and purchase history. They can leverage this data to present useful and personalized product recommendations. Examples include the sections of Amazon product pages labeled "Frequently bought together" and "Customers who viewed this item also viewed."

Disadvantages of E-Commerce. The disadvantage of E-Commerce is limited customer service since consumers are not being able to see or touch a product prior to purchase and the wait time for product shipping.

Limited customer service. If a customer has a question or issue in a physical store, he or she can see a clerk, cashier or store manager for help. In an E-Commerce store, customer service may be limited: the site may only provide support during certain hours of the day, or a call to a customer service phone number may keep the customer on hold.

Not being able to touch or see. While images on a web page can provide a good sense about a product, it's different from experiencing it "directly."

Wait time. If a customer sees an item that he or she likes in a store, the customer pays for it and then goes home with it. With E-Commerce, there is a wait time for the product to be shipped to the customer's address.

Security. Skilled hackers can create authentic-looking websites that claim to sell well-known products. Instead, the site sends customers forfeit or imitation versions of those products -- or, simply collects customers' credit card information. Bonafide E-Commerce sites also carry risk, especially when customers store their credit card information with the retailer to make future purchases easier. If the retailer's site is hacked, hackers may come into the possession of customers' credit

Digital Payments

A Digital Payment occurs when goods or services are purchased through the use of various electronic mediums. There is no use of cash or cheques in this type of payment method. The Government of India has been taking several measures to promote and encourage digital payments in the country. As part of the 'Digital India' campaign, the government aims to create a 'digitally empowered' economy that is 'Faceless, Paperless, Cashless'. There are various types and modes of digital payments. Some of these include the use of debit/credit cards, internet banking, mobile wallets, digital payment apps, Unified Payments Interface (UPI) service, Unstructured Supplementary Service Data (USSD), Bank prepaid cards, mobile banking, etc.

Digital payment methods are often easy to make more convenient and provide customers the flexibility to make payments from anywhere and at any time. These are good alternatives to traditional methods of payment and speed up the transactions. Post demonetization, people slowly started embracing digital payments and even small time merchants and shop owners started accepting payments through the digital mode.

Types of Digital Payment Methods in India

1. Banking cards
2. USSD
3. Aadhaar Enabled Payment System (AEPS)
4. UPI
5. Mobile Wallets
6. Bank pre-paid cards
7. Point of Sale (PoS)
8. Internet Banking
9. Mobile Banking
10. Bharat Interface for Money (BHIM) app

1. **Banking cards**: Cards are among the most widely used payment methods and come with various features and benefits such as security of payments, convenience, etc. The main advantage of debit/credit or prepaid banking cards is that they can be used to make other types of digital payments. For example, customers can store card information in digital payment apps or mobile wallets to make a cashless payment. Some of the most reputed and well-known card payment systems are Visa, Rupay and MasterCard, among others. Banking cards can be used for online purchases, in digital payment apps, PoS machines, online transactions, etc.

2. **USSD:** Another type of digital payment method, *99#, can be used to carry out mobile transactions without downloading any app. These types of payments can also be made with no mobile data facility. This facility is backed by the USSD along with the National Payments Corporation of India (NPCI). The main aim of this type of digital payment service is to create an environment of inclusion among the underserved sections of society and integrate them into mainstream banking. This service can be used to initiate fund transfers, get a look at bank statements and make balance queries. Another advantage of this type of payment system is that it is also available in Hindi.

3. **AEPS:** Expanded as Aadhaar Enabled Payment System, AEPS, can be used for all banking transactions such as balance enquiry, cash withdrawal, cash deposit, payment transactions, Aadhaar to Aadhaar fund transfers, etc. All transactions are carried out through a banking correspondent based on Aadhaar verification. There is no need to physically visit a branch, provide debit or credit cards, or even make a signature on a document. This service can only be availed if your Aadhaar number is registered with the bank where you hold an account. This is another initiative taken by the NPCI to promote digital payments in the country.

4. **UPI:** UPI is a type of interoperable payment system through which any customer holding any bank account can send and receive money through a UPI-based app. The service allows a user to link more than one bank account on a UPI app on their smartphone to seamlessly initiate fund transfers and make collect requests on a 24/7 basis and on all 365 days a year. The main advantage of UPI is that it enables users to transfer money without a bank account or IFSC code. All you need is a Virtual Payment Address (VPA). There are many UPI apps in the market and it is available on both Android and iOS platforms. To use the service one should have a valid bank account and a registered mobile number, which is linked to the same bank account. There are no transaction charges for using UPI. Through this, a customer can send and receive money and make balance enquiries.

5. **Mobile Wallets:** A mobile wallet is a type of virtual wallet service that can be used by downloading an app. The digital or mobile wallet stores bank account or debit/credit card information or bank account information in an encoded format to allow secure payments. One can also add money to a mobile wallet and use the same to make payments and purchase goods and services. This eliminated the need to use credit/debit cards or remember the CVV or 4-digit pin. Many banks in the country have launched e-wallet services and apart from banks, there are also many private players. Some of the mobile wallet apps in the market are Paytm, Mobikwik, Freecharge, etc. The various services offered by mobile wallets include sending and receiving money, making payments to merchants, online purchases, etc. Some mobile wallets may charge a certain transaction fee for the services offered.

6. **Bank pre-paid cards:** A prepaid card is a type of payment instrument on to which you load money to make purchases. The type of card may not be linked to the bank account of the customer. However, a debit card issued by the bank is linked with the bank account of the customer.

7. **PoS terminals:** Traditionally, PoS terminals referred to those that were installed at all stores where purchases were made by customers using credit/debit cards. It is usually a hand held device that reads banking cards. However, with digitization the scope of PoS is expanding and this service is also available on mobile platforms and through internet browsers. There are different types of PoS terminals such as Physical PoS, Mobile PoS and Virtual PoS. Physical PoS terminals are the ones that are kept at shops and stores. On the other hand, mobile PoS terminals work through a tablet or smartphone. This is advantageous for small time business owners as they do not have to invest in expensive electronic registers. Virtual PoS systems use web-based applications to process payments.

8. **Internet Banking:** Internet banking refers to the process of carrying out banking transactions online. These may include many services such as transferring funds, opening a new fixed or recurring deposit, closing an account, etc. Internet banking is also referred to as e-banking or virtual banking. Internet banking is usually used to make online fund transfers via NEFT, RTGS or IMPS. Banks offer customers all types of banking services through their website and a customer can log into his/her account by using a username and password. Unlike visiting a physical bank, there are no time restrictions for internet banking services and they can be availed at any time and on all 365 days in a year. There is a wide scope for internet banking services.

9. **Mobile Banking:** Mobile banking is referred to the process of carrying out financial transactions/banking transactions through a smartphone. The scope of mobile banking is only expanding with the introduction of many mobile wallets, digital payment apps and other services like the UPI. Many banks have their own apps and customers can download the same to carry out banking transactions at the click of a button. Mobile banking is a wide term used for the extensive range or umbrella of services that can be availed under this.

10. **Bharat Interface for Money (BHIM) app**: The BHIM app allows users to make payments using the UPI application. This also works in collaboration with UPI and transactions can be carried out using a VPA. One can link his/her bank account with the BHIM interface easily. It is also possible to link multiple bank accounts. The BHIM app can be used by anyone who has a mobile number,debitcard and avalid bank account. Money can be sent to different bank accounts, virtual addresses or to an Aadhaar number. There are also many banks that have collaborated with the NPCI and BHIM to allow customers to use this interface.

Benefits of Digital Payments

- Faster, easier, more convenient: Perhaps, one of the biggest advantages of cashless payments is that it speeds up the payment process and there is no need to fill in lengthy information. There is no need to stand in a line to withdraw money from an ATM or carry cards in the wallet. Also, with the move to digital, banking services will be available to customers on a 24/7 basis and on all days of a year, including bank holidays. Many services like digital wallets, UPI, etc, work on this basis.

- Economical and less transaction fee: There are many payment apps and mobile wallets that do not charge any kind of service fee or processing fee for the service provided. The UPI interface is one such example, where services can be utilized by the customer free of cost. Various digital payments systems are bringing down costs.

- Waivers, discounts and cashbacks: There are many rewards and discounts offered to customers using digital payment apps and mobile wallets. There are attractive cash back offers given by many digital payment banks. This comes as a boon to customers and also acts as motivational factor to go cashless.
- Digital record of transactions: One of the other benefits of going digital is that all transaction records can be maintained. Customers can track each and every transaction that is made, no matter how small the transaction amount is.
- One stop solution for paying bills: Many digital wallets and payment apps have become a convenient platform for paying utility bills. Be it mobile phone bills, internet or electricity bills, all such utility bills can be paid through a single app without any hassle.
- Helps keep black money under control: Digital transactions will help the government keep track of things and it will help eliminate the circulation of black money and counterfeit notes in the long run. Apart from this, this may also give a boost to the economy as the cost of minting currency also goes down.

UNIT III: Social Media

Introduction

Social media are web-based communication tools that enable people to interact with each other by sharing and consuming information. Social media facilitates the sharing of ideas, thoughts, and information through the building of virtual networks and communities. Social media typically features user-generated content and personalized profiles.

Forms of Social Media

Social media may take the form of a variety of tech-enabled activities. These activities include photo sharing, blogging, social gaming, social networks, video sharing, business networks, virtual worlds, reviews and much more. Even governments and politicians utilize social media to engage with constituents and voters.

For individuals, social media is used to keep in touch with friends and extended family. Some people will use various social media applications to network career opportunities, find people across the globe with like interests, and share their thoughts, feelings, insight, and emotions. Those who engage in these activities are part of a virtual social network.

For businesses, social media is an indispensable tool. Companies use the platform to find and engage with customers, drive sales through advertising and promotion, understand consumer trends, and offer customer service or support.

Facebook

Facebook is a popular free social networking website that allows registered users to create profiles, upload photos and video, send messages and keep in touch with friends, family and anyone. Some of the advantages of Facebook business pages include: Brand awareness: Facebook is one of the largest social media platforms in the world.

Facebook is user-friendly and open to everyone. Even the least technical-minded people can sign up and begin posting on Facebook. Although it started out as a way to keep in touch or reconnect with long-lost friends, it rapidly became the darling of businesses that were able to closely target an audience and deliver ads directly to the people most likely to want their products or services. Facebook makes it simple to share photos, text messages, videos, status posts and feelings on Facebook. The site is entertaining and a regular daily stop for many users.

Twitter

Twitter is a microblogging and social networking service on which users post and interact with messages known as "tweets". Registered users can post, like, and retweet tweets, but unregistered users can only read them. Twitter allows users to communicate with one another through short messages containing 280 characters known as tweets, which makes the platform super simple and easy to share social updates. Twitter is a great way of getting consumers' attention and awareness, and it is free.

LinkedIn

LinkedIn is a social networking site designed specifically for the business community. The goal of the site is to allow registered members to establish and document networks of people they know and trust professionally.

LinkedIn connects the world's professionals to make them more productive and successful. The site, which was launched in May 2003, currently has over 300 million members from 200 countries, representing 170 industries. According to Reid Hoffman, the co-founder, 27 percent of LinkedIn subscribers are recruiters.

A LinkedIn member’s profile page, emphasizes skills, employment history and education, has professional network news feeds and a limited number of customizable modules. Basic membership for LinkedIn is free. Network members are called “connections.” Unlike other free social networking sites like Facebook or Twitter, LinkedIn requires connections to have a pre-existing relationship. With basic membership, a member can only establish connections with

someone he has worked with, knows professionally. Premium subscriptions can be purchased to provide members with better access to contacts in the LinkedIn database.

Instagram

Instagram is a social media app that allows users to share photos and videos from their lives, add captions, edit filters, tweak settings, engage with others and explore. This photo and video-sharing social networking service is owned by Facebook, Inc.

Similar to Facebook or Twitter, everyone who creates an Instagram account has a profile and a news feed. When you post a photo or video on Instagram, it will be displayed on your profile. Other users who follow you will see your posts in their own feed. Likewise, you'll see posts from other users whom you choose to follow. It's like a simplified version of Facebook, with an emphasis on mobile use and visual sharing. Just like other social networks, you can interact with other users on Instagram by following them, being followed by them, commenting, liking, tagging and private messaging. You can even save the photos you see on Instagram.

Importance of Social Media in Business Communication

Originally, Social Media was a way to interact with friends and family but later on, Business Organizations have taken interest in this popular communication method to reach out to customers. Social Media plays a significant role in helping to grow businesses. Social Media platforms are becoming a natural place to reach targeted potential customers as 50% of World's population use social media nowadays. Here are some of the advantages of using social media to build a brand or to run an existing business

- By using social media, a business organization can create a real human connection to the customers
- Social Media plays an important role in Lead Generation by offering an easy way for the customers to express interest in their business.
- Social Media is becoming the most important part of a sales funnel of any business as a number of people using social media is growing day by day.
- Social Media is an excellent platform to promote one's well-researched content in front of new people to grow the audience base.
- Social Media gives business owners the opportunity to connect with their fans and followers every time they log in to their account.

Disadvantages of Social Media

There are lots of common problems that most major social media platforms haven't totally solved, despite their effort to do so.

Spam: Social media makes it easy for spammers both real people and bots to bombard other people with content

Cyberbullying/Cyberstalking: Children and teenagers are especially susceptible to cyberbullying because they take more risks when it comes to posting on social media. And now that we all interact on social media via our mobile devices, most major platforms make it possible to share our locations, opening up the doors for cyberstalkers to targetus.

Self-image manipulation: What a user posts about themselves on social media only represents a small portion of their life. Users have the power to completely control what parts they do and don't want to broadcast on social media to manipulate their own self-image.

Hacking – Personal data and privacy can easily be hacked and shared on the Internet, which can make financial losses and loss to personal life. Similarly, identity theft is another issue by hacking their personal accounts.

Addiction – The addictive part of the social media is very bad and can disturb personal lives as well. The teenagers are the most affected by the addiction of social media. They get involved very extensively and are eventually cut off from the society. It can also waste individual time that could have been utilized by productive tasks and activities.

Information overload: It's not unusual to have over 200 Facebook friends or follow over 1,000 Twitter accounts. With so many accounts to follow and so many people posting new content, it's almost impossible to keep up.

Fake news: Fake news websites promote links to their own totally false news stories on social media in order to drive traffic to them. Many users have no idea that they're fake in the first place.

Privacy/Security: Many social media platforms still get hacked from time to time despite having good security measures in place. Some don't offer all the privacy options that users needto keep their information as private as they want them to be.

Protecting ourselves from Social Networking problems

To protect yourself from being phished on social media, there are a number of steps you should take:

- Never accept friend requests from someone you don't know – Social media platforms are all about keeping in touch with friends and building connections, but with so many fakeaccounts, users should always err on the side of caution when accepting a friend request from someone they're not familiar with.
- Never click on links requesting personal information – Reputable social media platforms willnever ask users to click on a link to update their personal details. These links will nearly always be created to steal sensitive information or deliver malware. If you're unsure if the request is legitimate or not, go directly to the support pages on your social media network and double-check.
- Use unique login details for each account– When phishing scams are so common across social media, it's always best to use a unique username and password for each site so that in the unfortunate event of being phished, the attackers won't have access to your other online accounts.
- Only enter personal information on a secure website – The URL on a secure site will always begin with a 'https'. The 's' stands for secure and ensures that all communication between your browser and the website you are visiting is encrypted.
- Install Anti-Virus Software – The installation of antivirus software will help detect threats on your computer and block unauthorised users from gaining access.
- Keeping operating systems up to date – It's important to ensure that your software is regularly updated to prevent hackers from gaining access to your device through vulnerabilities in older and outdated systems.
- Use enhanced privacy settings – Regularly check and adjust your privacy settings to restrict what people can and can't see on your profile.

Cybercrime

Cybercrime is defined as a crime where a computer is the object of the crime or is used as a toolto commit an offense. A cybercriminal may use a device to access a user's personal information, confidential business information, government information, or disable a device. Crime and cybercrime have become an increasingly large problem in our society, even with the criminal justice system in place. Both in the public web space and dark web, cybercriminals are highly skilled and are not easy to find. Cybercrime has created a major threat to those who use the internet, with millions of users' information stolen within the past few years. It has also made a major dent in many nations' economies.

Types of Cybercrime

DDoS Attacks

These are used to make an online service unavailable and take the network down by overwhelming the site with traffic from a variety of sources. Large networks of infected devices known as Botnets are created by depositing malware on users' computers. The hacker then hacks into the system once the network is down.

Botnets

Botnets are networks from compromised computers that are controlled externally by remote hackers. The remote hackers then send spam or attack other computers through these botnets. Botnets can also be used to act as malware and perform malicious tasks.

IdentityTheft

This cybercrime occurs when a criminal gains access to a user's personal information to steal funds, access confidential information, or participate in tax or health insurance fraud. They canalso open a phone/internet account in your name, use your name to plan a criminal activity and claim government benefits in your name. They may do this by finding out user's passwords through hacking, retrieving personal information from social media, or sending phishing emails.

Cyberstalking

This kind of cybercrime involves online harassment where the user is subjected to a plethora of online messages and emails. Typically cyberstalkers use social media, websites and search engines to intimidate a user and instill fear. Usually, the cyberstalker knows their victim and makes the person feel afraid or concerned for their safety.

Social Engineering

Social engineering involves criminals making direct contact with you usually by phone or email. They want to gain your confidence and usually pose as a customer service agent so you'll givethe necessary information needed. This is typically a password, the company you work for, or bank information. Cybercriminals will find out what they can about you on the internet and then attempt to add you as a friend on social accounts. Once they gain access to an account, they can sell your information or secure accounts in your name.

PUPs

PUPS or Potentially Unwanted Programs are less threatening than other cybercrimes, but are a type of malware. They uninstall necessary software in your system including search engines and pre-downloaded apps. They can include spyware or adware, so it's a good idea to install an antivirus software to avoid the malicious download.

Prohibited/Illegal Content

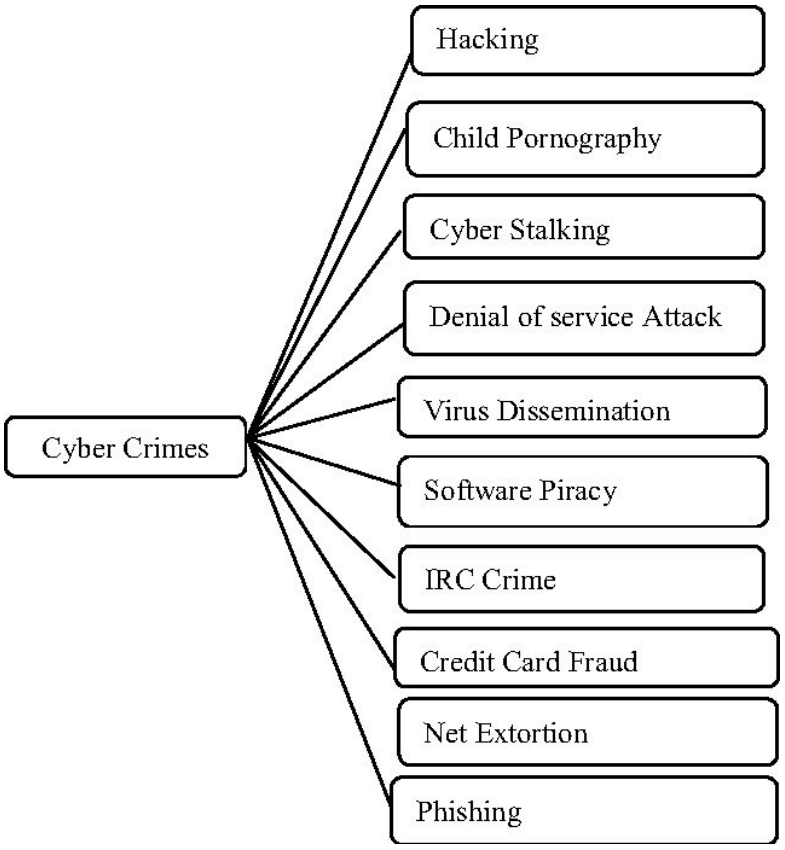
This cybercrime involves criminals sharing and distributing inappropriate content that can be considered highly distressing and offensive. Offensive content can include, but is not limited to, sexual activity between adults, videos with intense violent and videos of criminal activity. Illegal content includes materials advocating terrorism-related acts and child exploitation material. This type of content exists both on the everyday internet and on the dark web, an anonymous network.

Online Scams

These are usually in the form of ads or spam emails that include promises of rewards or offers of unrealistic amounts of money. Online scams include enticing offers that are "too good to be true" and when clicked on can cause malware to interfere and compromise information.

Exploit Kits

Exploit kits need a vulnerability (bug in the code of a software) in order to gain control of a user's computer. They are readymade tools criminals can buy online and use against anyone with a computer. The exploit kits are upgraded regularly similar to normal software and are available on dark web hacking forums.



Phishing

Phishing is the fraudulent use of electronic communications to deceive and take advantage of users. Phishing attacks attempt to gain sensitive, confidential information such as usernames, passwords, credit card information, network credentials, and more. By posing as a legitimate individual or institution via phone or email, cyber attackers use social engineering to manipulate victims into performing specific actions—like clicking on a malicious link or attachment—or willfully divulging confidential information.

Phishing Methods

Phishing attempts most often begin with an email attempting to obtain sensitive information through some user interaction, such as clicking on a malicious link or downloading an infected attachment.

- Through link manipulation, an email may present with links that spoof legitimate URLs; manipulated links may feature subtle misspellings or use of a subdomain.
- Phishing scams may use website forgery, which employs JavaScript commands to make a website URL look legitimate.

- Using covert redirection, attackers can corrupt legitimate websites with malicious pop-up dialogue boxes that redirect users to a phishing website.
- Infected attachments, such as .exe files, Microsoft Office files, and PDF documents can install ransomware or other malware.

Phishing scams can also employ phone calls, text messages, and social media tools to trickvictims into providing sensitive information.

Types of Phishing Attacks

Some specific types of phishing scams use more targeted methods to attack certain individuals or organizations.

Spear Fishing

Spear phishing email messages won't look as random as more general phishing attempts. Attackers will often gather information about their targets to fill emails with more authentic context. Some attackers even hijack business email communications and create highly customized messages.

Clone Phishing

Attackers are able to view legitimate, previously delivered email messages, make a nearly identical copy of it—or "clone"—and then change an attachment or link to something malicious.

Whaling

Whaling specifically targets high profile and/or senior executives in an organization. The content of a whaling attempt will often present as a legal communication or other high-level executive business

How to Prevent Phishing Attacks

Organizations should educate employees to prevent phishing attacks, particularly how to recognize suspicious emails, links, and attachments. Cyber attackers are always refining theirtechniques, so continued education is imperative.

Some tell-tale signs of a phishing email include:

- 'Too good to be true' offers
- Unusual sender

- Poor spelling and grammar
- Threats of account shutdown, etc., particularly conveying a sense of urgency
- Links, especially when the destination URL is different than it appears in the email content
- Unexpected attachments, especially .exe files

Security measures can include:

- **Two Factor Authentication** incorporating two methods of identity confirmation—something you know (i.e., password) and something you have (i.e., smartphone)
- **Email filters** that use machine learning and natural language processing to flag high-risk email messages.
- **Augmented password logins** using personal images, identity cues, security skins,etc.

Hacking

Hacking is the process of gaining unauthorized access into a computer system, or group of computer systems. This is done through cracking of passwords and codes which gives access to the systems. Cracking is the term which specifies the method by which the password or code is obtained.

Types of Hackers

White, black, and grey refer to the relationship between the hacker and the systems they are attacking.

Black Hat Hackers

A black-hat hacker is an individual who attempts to gain unauthorized entry into a system or network to exploit them for malicious reasons. The black-hat hacker does not have any permission or authority to compromise their targets. They try to inflict damage by compromising security systems, altering functions of websites and networks, or shutting down systems. They often do so to steal or gain access to passwords, financial information, and other personal data.

White Hat Hackers

White-hat hackers, on the other hand, are deemed to be the good guys, working with organizations to strengthen the security of a system. A white hat has permission to engage the targets and to compromise them within the prescribed rules of engagement. White-hat hackers are often referred to as ethical hackers. This individual specializes in ethical hacking tools, techniques, and methodologies to secure an organization’s information systems.

Grey Hat Hackers

Grey hats exploit networks and computer systems in the way that black hats do, but do so without any malicious intent, disclosing all loopholes and vulnerabilities to law enforcement agencies or intelligence agencies. Usually, grey-hat hackers surf the net and hack into computer systems to notify the administrator or the owner that their system/network contains one or more vulnerabilities that must be fixed immediately. Grey hats may also extort the hacked, offering to correct the defect for a nominal fee.

Common Hacking Tools

To accomplish a perfect hack, hackers implement a wide variety of techniques such as Rootkits,Keyloggers and Vulnerability Scanner

Cyber Security

Cyber security refers to the technologies, processes, and practices designed to protect networks, devices, programs, and data from attack, damage, or unauthorized access. Cyber security may also be referred to as information technology security.

Cyber Security Techniques

There are many cyber security techniques to combat the cyber security attacks. The next section discusses some of the popular techniques to counter the cyber attacks.

Authentication

It is a process of identifying an individual and ensuring that the individual is the same who he/she claims to be. A typical method for authentication over internet is via username and password. With the increase in the reported cases of cyber crime by identity theft over internet, the organizations have made some additional arrangements for authentication like One Time Password (OTP), as the name suggest it is a password which can be used one time only and is sent to the user as an SMS or an email at the mobile number/email address that is specified during the registration process. It is known as a two-factor authentication method and requires two types of evidence to authenticate an individual to provide an extra layer of security for authentication. Some other popular techniques for two-way authentication are: biometric data, physical token, etc. which are used in conjunction with username and password.

Encryption

It is a technique to convert the data in unreadable form before transmitting it over the internet. Only the person who have the access to the key can convert it in the readable form and read it. Formally encryption can be defined as a technique to lock the data by converting it to complex codes using mathematical algorithms. The code is so complex that even the most powerful computer will take several years to break the code. This secure code can safely be transmitted over the internet to the destination. The receiver, after receiving the data can decode it using the key. The decoding of the complex code to original text using key is known as decryption. If the same key is used to lock and unlock the data, it is known as symmetric key encryption.

In symmetric key encryption, the after coding of data, the key is sent to the destination user via some other medium like postal service, telephone, etc. because if the key obtained by the hacker, the security of the data is compromised. Key distribution is a complex task because the security of key while transmission is itself an issue. To avoid the transfer of key a method called asymmetric key encryption, also known as public key encryption, is used. In asymmetric key encryption, the key used to encrypt and decrypt data are different. Every user posses two keys viz. public key and private key. As the name suggests, the public key of every user is known to everyone but the private key is known to the particular user, who owns the key. Suppose sender A wants to send a secret message to receiver B through the internet. A will encrypt the message

using B"s public key, as the public key is known to everyone. Once the message is encrypted, the message can safely be sent to B over the internet. As soon as the message is received by B, he will use his private key to decrypt the message and regenerate the original message.

Digital Signatures

It is a technique for validation of data. Validation is a process of certifying the content of a document. The digital signatures not only validate the data but also used for authentication. The digital signature is created by encrypting the data with the private key of the sender. The encrypted data is attached along with the original message and sent over the internet to the destination. The receiver can decrypt the signature with the public key of the sender. Now the decrypted message is compared with the original message. If both are same, it signifies that the data is not tempered and also the authenticity of the sender is verified as someone with the private key(which is known to the owner only) can encrypt the data which was then decrypted by his public key. If the data is tempered while transmission, it is easily detected by the receiver as the data will not be verified. Moreover, the message cannot be re-encrypted after tempering as the private key, which is possessed only by the original sender, is required for this purpose.

As more and more documents are transmitted over the internet, digital signatures are an essential part of the legal as well as the financial transition. It not only provides the authentication of a person and the validation of the document, it also prevents the denial or agreement at a later stage. Suppose a shareholder instructs the broker via email to sell the share at the current price. After the completion of the transaction, by any chance, the shareholder reclaims the shares by claiming the email to be forge or bogus. To prevent these unpleasant situations, the digital signatures are used.

Antivirus

There are varieties of malicious programs like virus, worms, trojan horse, etc that are spread over the internet to compromise the security of a computer either to destroy data stored into the computer or gain financial benefits by sniffing passwords etc. To prevent these malicious codes

from entering your system, a special program called an anti-virus is used which is designed to protect the system against virus. It not only prevents the malicious code from entering the system but also detects and destroys the malicious code that is already installed into the system. The antivirus program regularly updates its database and provides immunity to the system against the new viruses, worms which keep emerging.

Firewall

It is a hardware/software which acts as a shield between an organization's network and the internet and protects it from threats like virus, malware, hackers, etc. It can be used to limit the persons who can have access to your network and send information to you.

There are two types of traffic in an organization viz. inbound traffic and outbound traffic. Using a firewall, it is possible to configure and monitor the traffic of the ports. Only the packets from trusted source addresses can enter the organization's network and the sources which are blacklisted and unauthorized addresses are denied access to the network. It is important to have firewalls to prevent the network from unauthorized access, but firewall does not guarantee this until and unless it is configured correctly. A firewall can be implemented using hardware as well as software or the combination of both.

Hardware Firewalls: Hardware firewalls are routers through which the network is connected to the network outside the organization i.e. Internet.

Software Firewalls: These firewalls are installed on the server and client machines and it acts as a gateway to the organization's network.

In the operating system like Windows 2003, Windows 2008 etc. it comes embedded with the operating system. The only thing a user needs to do is to optimally configure the firewall according to their own requirement. The firewalls can be configured to follow “rules” and “policies” and based on these defined rules the firewalls can follow the following filtering mechanisms.

Proxy- All the outbound traffic is routed through proxies for monitoring and controlling the packet that are routed out of the organization.

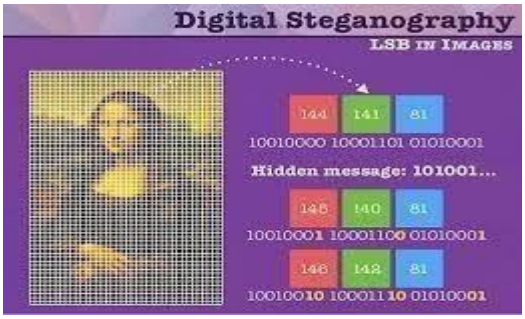
Packet Filtering- Based on the rules defined in the policies each packet is filtered by their type, port information, and source & destination information. The example of such characteristics is IP address, Domain names, port numbers, protocols etc. Basic packet filtering can be performed by routers.

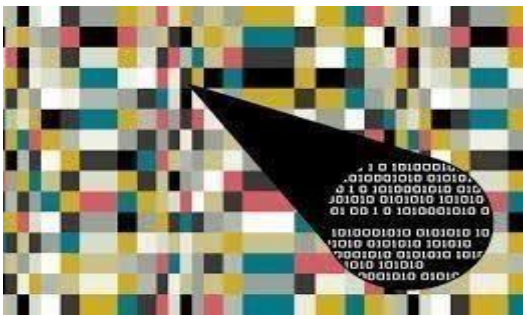
Stateful Inspection -Rather than going through all the fields of a packet, key features are defined. The outgoing/incoming packets are judged based on those defined characteristics only.

The firewalls are an essential component of the organization's network. They not only protect the organization against the virus and other malicious code but also prevent the hackers from using your network infrastructure to launch DOS (Denial of Service) attacks.

Steganography

It is a technique of hiding secret messages in a document file, image file, and program or protocol etc. such that the embedded message is invisible and can be retrieved using special software. Only the sender and the receiver know about the existence of the secret message in the image. The advantage of this technique is that these files are not easily suspected.





There are many applications of steganography which includes sending secret messages without ringing the alarms, preventing secret files from unauthorized and accidental access and theft , digital watermarks for IPR issues, etc.

Let us discuss how the data is secretly hidden inside the cover file (the medium like image, video, audio, etc which is used to embed secret data) without being noticed. Let us take an example of an image file which is used as a cover medium. Each pixel of a high resolution image is represented by 3 bytes (24 bits). If the 3 least significant bits of this 24 bits are altered and used for hiding the data, the resultant image, after embedding the data into it, will have an unnoticeable change in the image quality and only a very experienced and trained eye can detect this change. In this way, every pixel can be used to hide 3 bits of information. Similarly, introducing a white noise in an audio file at regular or random intervals can be used to hide data in audio or video files. There are various free softwares available for Steganography. Some of the popular ones are QuickStego, Xiao, Tucows, OpenStego, etc.There are many applications of Steganography which includes sending secret messages without ringing the alarms, preventing secret files from unauthorized and accidental access and theft , digital watermarks for IPR issues, etc

UNIT-IV Introduction to SoftSkills

The Learning Objectives:

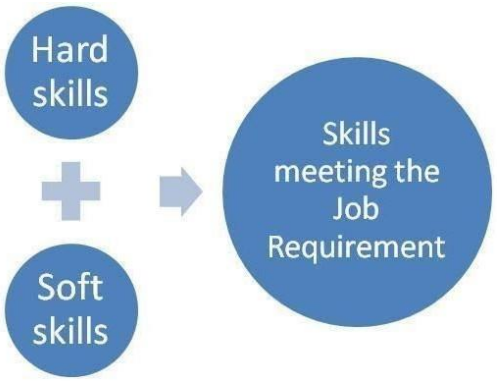
The Main tasks of the Soft Skills module are to develop and enhance:

- Critical and reflective thinking
- Self-management and self awareness skills
- Communication skills, including interpretation and use of feedback
- Team working and peer support strategies

"Soft Skills" correlates with some terms of a very close meaning: "Life Skills", "Emotional Intelligence Quotients", "Social Skills", and "Interpersonal Skills".

Soft skills is a term often associated with a person's Emotional Intelligence Quotient, the cluster of personality traits, social graces, communication, language, personal habits, friendliness, managing people, leadership, etc. that characterize relationships with other people. Soft skills, also known as people skills, complement hard skills to enhance an individual's relationships, job performance and career prospects. It's often said that hard skills will get you an interview butyou need soft skills to get – and keep – the job.

Hard skills are part of the skill set that is required for a job. Hard skills include the expertise necessary for an individual to successfully do the job.



What are Soft Skills?

Soft skills could be defined as **life skills** which are behaviors used appropriately and responsibly in the management of personal affairs. Practicing life skills leads to qualities such as self-esteem, sociability, tolerance, competencies to take action, and capabilities to have the freedom to decide what to do..

Social skills are any skills facilitating interaction and communication with others. Social rulesand relations are created, communicated, and changed in verbal and nonverbal ways. The process of learning these skills is called socialization.

Interpersonal skills are also referred to as people skills or communication skills. Interpersonal skills are the skills a person uses to communicate and interact with others. They include persuasion, active listening, delegation, and leadership. Interpersonal skills reveal how people relate to one another.

Why Soft Skills?

Most interactions with other people require some level of soft skills. For example, in a company you might be negotiating to win a new contract, presenting your new idea to colleagues, networking for a new job, and so on. We use soft skills everyday at work and developing these soft skills will help you win more business and accelerate your career progression.

On the other hand, lack of soft skills can limit your potential, or even be the downfall of your business. By developing strong leadership, delegation, teamwork, and communication abilities, you can run projects more smoothly, deliver results that please everyone, and even positively influence your personal life by improving how you interact with others.

Outside office, soft skills such as communication are used to build friendship groups and meet potential partners. Soft skills are useful both in our professional and personal lives.

Soft Skills will aid in the following:

Self

An awareness of the characteristics that define the person one is and wants to become.

Opportunity

An awareness of the possibilities that exist, the demands they make and the rewards and satisfactions they offer.

Aspirations

The ability to make realistic choices and plans based on sound information and on self - opportunity alignment.

Results

The ability to review outcomes, plan and take action to implement decisions and aspirations, especially at points of transition.

In order to **SOAR** students need two things:

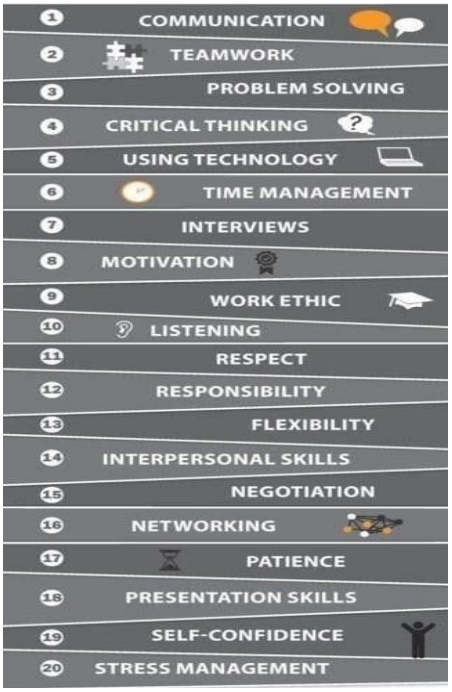
Academic Roots : Discipline based knowledge and understanding

Academic Wings : The ability to enhance that knowledge and understanding with awareness (self and others), critical thinking, reflective practice

Soft skills focus more on people than processes.

Soft skills = People skills=Street Smarts

Personal character traits and interpersonal skills for working with others



Categories of Soft skills

Generally, soft skills may be subdivided into three basic categories

1. Personal skills
2. Interpersonal skills
3. Methodical skills

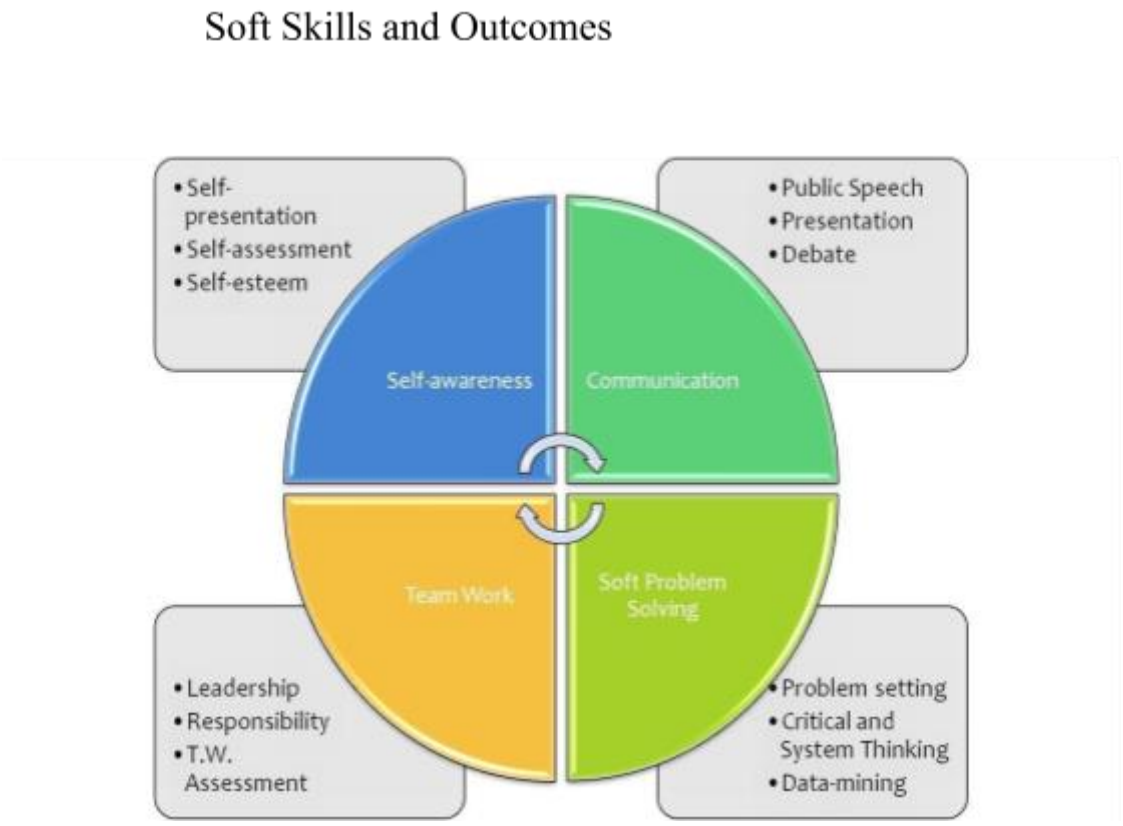
Essentially, there are three kinds of skills – those related to thinking called ‘thinking skills’, skills related to dealing with others called ‘social skills’ and those related to knowledge acquisition and using it for problem solving. While thinking skills relate to the personal skills, social skills include interpersonal skills and methodical skills relate to professional skills. Thesethree types of skills are needed for a successful life.

one way of categorizing social skills can be found in the table below:

Skill Set	**Used for**	**Examples**
Foundation Skills	Basic social interaction	Ability to maintain eye contact, maintain appropriate personal space, understand gestures and facial expressions
Interaction Skills	Skills needed to interact with others	Resolving conflicts, taking turns, learning how to begin and end conversations, determining appropriate topics for conversation, interacting with authority figures

Affective Skills	Skills needed for understanding oneself and others	Identifying one's feelings, recognizing the feelings of others, demonstrating empathy, decoding body language and facial expressions, determining whether someone is trustworthy.
Cognitive Skills	Skills needed to maintain more complex social interactions	Social perception, making choices, self-monitoring, understanding community norms, determining appropriate behavior for different social situations.

Soft skills and its outcomes are described in the diagram below.



Integral Parts of Soft Skills

i. Self-Management System

Self-Management System consists of Self-motivation, taking responsibility, task setting/prioritizing, time-management. The structure of Self-Management System is detected in the table below.

Self-Management Structure

ii. Critical Thinking

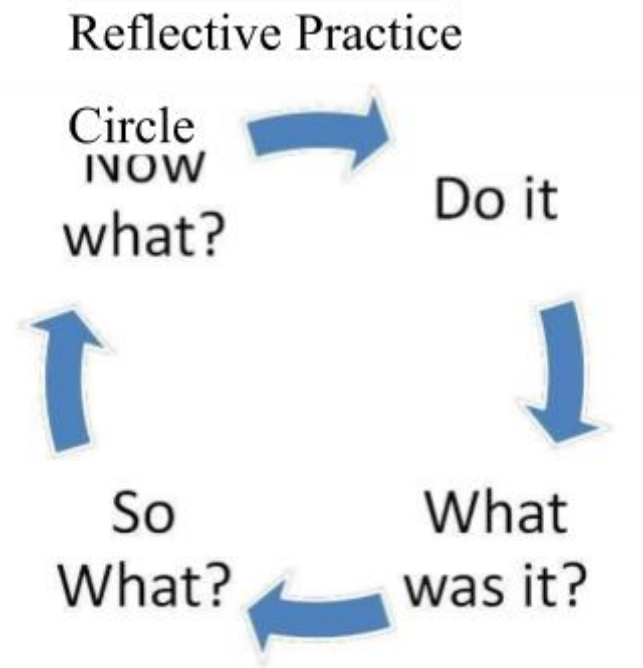
Critical thinking is the ability to think clearly and rationally about what to do or what to believe. It includes the ability to engage in reflective and independent thinking. Anyone with critical thinking skills is able to do the following:

- understand the logical connections between ideas
- identify, construct and evaluate arguments
- detect inconsistencies and common mistakes in reasoning
- solve problems systematically
- identify the relevance and importance of ideas
- reflect on the justification of one's own beliefs and values

A critical thinker is able to deduce consequences from what he knows, and he knows how to make use of information to solve problems and to seek relevant sources of information to inform himself.

iii. Reflection

Reflection is a form of thinking used to fulfill a purpose or to achieve some anticipated outcome and is largely based on the further processing of knowledge and understanding that we already possess.



Reflective Practice is triggered with the help of Self assessment questions:

- What am I trying to do exactly?
- Why am I doing it?
- What went well and why?
- What went less well and why?
- How could I do better next time?

iv. Communication and Interaction

Effective communication provides for high level of

Presentation skills

- to increase both skills and confidence levels
- to improve research, design and communication skills
- to develop team working and project management skills
- to strengthen learning and enthusiasm for further knowledge
- to promote critical and analytical thinking

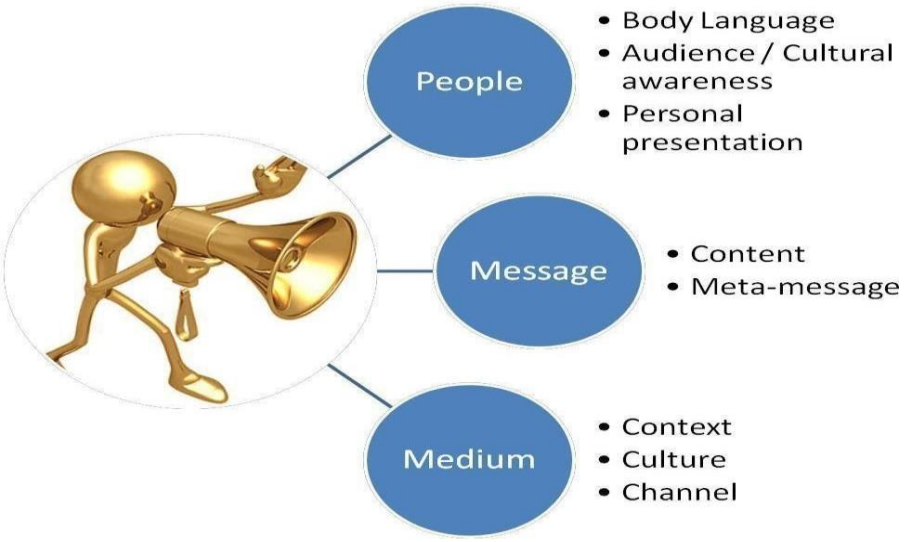
Academic debates

- Content and formats of academic debate
- Listening skills
- Giving and receivingfeedback
- Reacting to groundedcriticism

Effective listening and writing

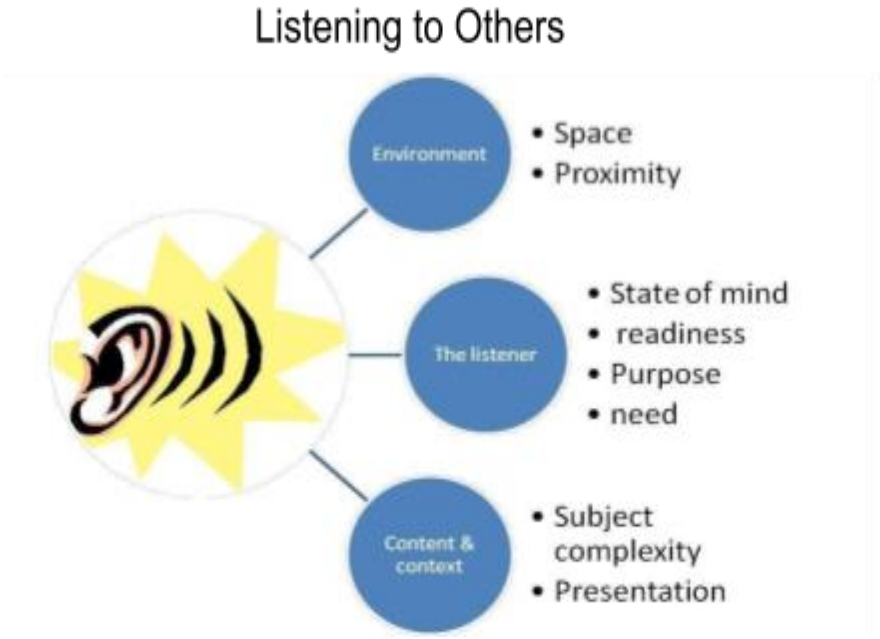
Listening means attentiveness and interest perceptible in the posture as well as expressions. The ability to communicate clearly, concisely, and concretely in writing ensures that everyone you work with understands what you're telling them. Because written communication skills are so important in business, it's worth taking the time to improve yours. The following diagram explains more about the skillful writing

Skillful Writing



Skillful writing examples:

- Technical Writing
- Script writing / audience analysis / performance / reflection
- Observation (self and others)
- Press release



v. Group work

Group work is one of the most useful ways of learning about cooperation, shared responsibility, project planning, and time management. Learning how to work successfully in a group has a close association with how we participate in the workplace and includes:

- Social responsibility
- Using logical and rational arguments to persuade others
- Identifying the needs of others and building positive relationships
- Understanding group dynamic
- Understanding yourself in relation to others and how they might perceive you
- Reflection on the image

vi. Assertiveness

Assertiveness means “confident behaviour” and “self-confidence”. It is an individual ability to advance on their aims, needs, wishes, claims, interest and feelings. Phenomenon of assertiveness presupposes an existence of subjective attitude toward Self (self-allowance to have the own claims), social readiness and ability to realize it in adequate manner (to have the own claims and achieve their realization), freedom from social fear and inhibition (ability to register and reveal own claims).

Assertive Behavior

vii. Peer-to-Peer

Peer – to- Peer is an interaction and learning method (technology) when the source of knowledge is not a professor but a peer student (peer instructor). It promotes participation and interaction. Peer-to-Peer activity includes both trainers and trainees into campus life and promotes a sense of belonging that combats the anonymity and isolation many students experience at large universities during the first year of study.

Why Peer to Peer?

Based on research, we found out those seniors:

- Will seek out peers when they have difficulties or challenges
- Want to help themselves
- Want to be helpful.

What are the benefits of receiving Peer Support?

- Gain social and emotional support/encouragement
- Learn about resources
- Build self-help skills.

What are the benefits of providing Peer Support?

- Reaffirm that helping others helps the self
- Enjoy the opportunity to share existing skills and information
- Gain new skills, knowledge, and experience

UNIT-V Understanding Self and TeamBuilding

Introduction

Self can be called the combination of our knowledge, intellect, values and attitudes – the conscious and unconscious. There are a number of frameworks for understanding the self. Over the last hundred years or so a process of scientific enquiry into the self has begun, but even then our knowledge is far from complete. Since our action or behaviour is the only observable part of our self, scientists have focussed largely on this and on personality as manifestation of self.

It is generally agreed that the self has three broad aspects. These three constantly interact with each other causing congruence or confusion depending on whether they are in harmony or not.

The cognitive self: This refers to our mental or intellectual capacities, our ability to store and process information, our memory and logical abilities. In some individuals this aspect is highly developed and in others it is not. A culture with its emphasis on formal schooling encourages the development of the cognitive aspects.

The affective self: This refers to our emotional side, our capacity to feel and express emotions. The development of our affective self has its roots in our childhood experiences of being loved, held or hugged. The immediate family people who lay more emphasis on their cognitive selftend to look down upon those who are more impulsive, emotional, and possess artistic talent. Our dominant culture dictates that a good memory for facts is more important thancreativity.

The behavioural self: A common assumption is that knowing intellectually about something will automatically lead to appropriate change in behaviour. In reality, most of our behaviour is moulded by our emotions and beliefs. Learning which occurs on the cognitive level is not as effective in changing behaviour as learning which occurs on the emotional or affective level. Ultimately the belief dictates our behaviour.

Transactional Analysis (TA)

Eric Berne, an American psychiatrist, in 1958 formulated his own theory based on his clinical experience and coined the word Transactional Analysis - popularly known as TA.

- Transactional Analysis is a theory of personality and behaviour and a systematic tool for personal growth and personal change.
-

• Gives us a picture of how people are structured psychologically.

• Provides a theory of communication.

• Offers a theory of child development – the concept of life script explains how our present life patterns originated in childhood.

Philosophy of Transactional Analysis

- All individuals are born OK, as princes and princesses.
- All individuals have the capacity to think except the severely damaged brain.
- All individuals decide their own destiny and these decisions can bechanged.

Structural analysis of Ego states

Ego state is a consistent pattern of feeling and experience directly related to a corresponding consistent pattern of behaviour.

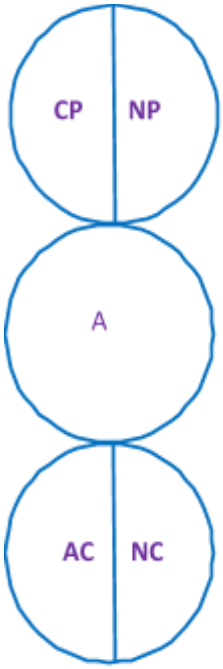
The functional model of Ego states

Functionally the Parent (P) ego state manifests as Critical Parent (CP) and Nurturing Parent (NP); Child (C) ego state manifests as Adapted Child (AC), and Natural Child(NC)

The **Parent** ego state is the set of feelings, attitudes,values and prejudices and behaviours introjected from parents and significant parental figures.

The **Adult** ego state is those feelings, attitudes, behaviours related to current here-and-now reality.

The **Child** ego state is the archaic feelings, emotions, attitudes,
and behaviours, which are remnants of the person's past.



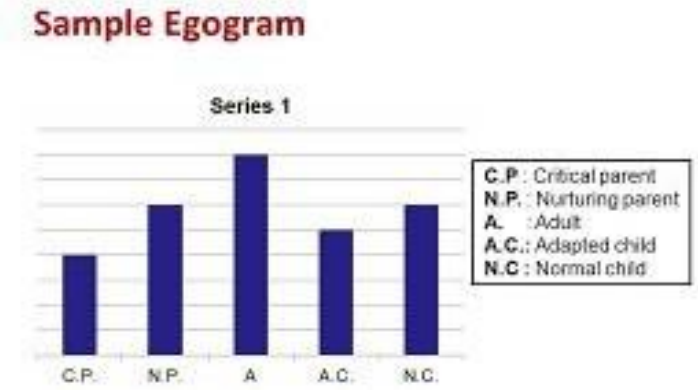
While in Critical Parent people manifest themselves as disappointed, aggrieved, feeling always right, patronising, controlling, critical, putting down others, as nurturing parent, people act loving, caring, concerned, understanding etc.

As an Adult, we function as a computer, process data, organise information, estimate probabilities, make logical statements, and provide non-judgmental feedback.

When we are in our Natural Child, we tend to laugh, share fun, feel excited and enthusiastic, and express our anger, sadness and fear freely without any inhibition. In Adapted Child, we exhibit behaviour of rebellion or compliance.

Egogram

The Egogram is a relationship diagram, depicting the amount of energy a person uses externally, or actively, as one relates to others. It is a bar chart representing the person's entire personality.It is drawn as a way of providing feedback to someone regarding how others experience him or her. Egogram is based on constancy hypothesis, which states that the amount of psychic energy within a person remains constant. For example, if a person starts to increase the energy in his Natural Child, there will be lesser energy available for his other egostates.



Strokes

A stroke is defined as a unit of recognition.

Types of strokes: Strokes are of different kinds.

- Verbal or Non-verbal
- Positive or Negative
- Conditional or Unconditional.

Any transaction is an exchange of strokes. No communication is possible without non-verbal strokes. Positive strokes invite us to feel OK about others, and ourselves while Negative strokes invite us to feel not OK about ourselves, about others or both. A negative stroke is better than no stroke.

Conditional strokes are for something the person does (for "doing"), whereas Unconditional strokes are for what the person is (the "being"). There can be positive conditional (e.g. "This is a good piece of embroidery") or negative conditional (e.g. "I don't like the way you stitch"). Or it could be positive unconditional (e.g."You are great") or negative unconditional (e.g."I hate you"). Stroking reinforces behaviour, be it positive or negative.

Stimulation and recognition hunger: There are various hungers, which human beings have. Physiological hungers are satisfied by food, water, etc. Psychological hunger could be for stimulus or structure. One such psychological hunger is the need for physical and mental stimulation. While young we need physical touch. As we grow this is substituted by other forms of recognition. This is defined as recognition hunger. Strokes help us satisfy this hunger for stimulus and recognition.

Life Position

Depending on the experience and messages the child encounters with, each child takes one of the four life positions. This is called the basic life position. Life positions are psychological senses regarding self, others and life, which the person takes. These also determine the person's attitudes and perceptions.

1. **I'm Ok, You're OK:** All are born in this position. This is the potentially healthy position. Persons in this position are realistic; not threatened of their shortcomings. Even when they have reverses they get up and go ahead with the business of life. Their basic operation is "Get-On-With".

2. **I'm OK, You're not OK:** People in this position feel they are victims of circumstances. Predominant feeling is anger. They are blamers. Such people are stroked only conditionally as children. Their basic operation is "Get-Rid- Of".
3. **I'mnotOK,You'reOK:**Peopleinthispositionfeelinferiorandpowerlesswhenthey compare themselves with others. They are shy and withdrawn and quite often pessimistic. Predominant feeling is sadness. Basic operation is "Get-Away-From"
4. **I'm not OK, You're not OK:** Futile position. People in this lose interest in living.A hopeless position. Their parents were never pleased with them for anything. Basic operation is "Get-Nowhere-With".

Egogram And Life Positions Questionnaire:

This questionnaire has been developed to help you understand your behaviour and personal patterns – the hallmark for personal growth and developing soft skills. Please declare which statement fits best at this present moment. It will be most helpful to you the more open and honest you can be with yourself. Please use the following numbers to describe youranswer:

4 = this statement fitsextremely well *3 = this statement fitswell*

2 = this statementfits sometimes *1 = this statement fits hardly ever*

0 = this statement doesn't fit at all

1.	I find it easy to assert myself.	
2.	I'm very sympathetic when people come to me with theirproblems.	
3.	My course of action when solving problems is more logical than intuitive.	
4.	I see myself as an impulsive human being.	
5.	I feel inhibited more often than I would like to.	
6.	I think it is important to respect traditions.	

7.	It gives me a huge satisfaction to consider other people's needs.	
8.	I usually keep my cool and stay business like when confronted with atypical situations.	
9.	I tend to fulfil my desires as quickly as possible.	
10.	I rather agree with somebody else than to get into an endless discussion.	
11.	I get angry about people who challenge recognised and accepted ways of thinking and behaviour.	
12.	I'm a forgiving person.	
13.	In all I do, I try to do it as perfectly as possible.	
14.	I'm a fun-loving person.	
15.	I often try to find out what other people expect of me so that I can comply accordingly.	
16.	I take leadership in critical situations because I know from experience what will work in such circumstances.	
17.	I strongly believe that all human beings are basically OK.	
18.	I analyse facts before I make a decision.	
19.	I have more interests and hobbies than the average person.	
20.	I believe that at the end of the day it's best to obey the authority.	
21.	I believe that society would benefit from a harsher punishment for any violations.	
22.	I find it very satisfying to help others to develop their potential and growth.	

23.	It seems that I developed the ability to think and act independently rather than to conform to other people's views.	
24.	I'm imaginative and I have lots of good ideas.	
25.	It seems that I pity myself more than others.	
26.	I have fairly clear ideas about what is right and what is wrong.	
27.	I often find myself in the role of consoling others.	
28.	I keep cool when others would either feel agitated or would switch off.	
29.	I'm rather spontaneous and I don't hesitate.	
30.	I try not to show feelings even when I'm very hurt inside.	
31.	I don't like to show my weaknesses to others.	
32.	I often get asked for advice.	
33.	My aim is to be objective.	
34.	I'm a curious person and I like to try out new things.	
35.	I find it hard to ask for something I need.	
36.	Once I've made up my mind I don't like to change it.	
37.	When I see that somebody has trouble with their tasks then I'm happyto ease their workload.	
38.	Whenever I work I'd like to do it thoroughly.	
39.	I'm frank with people. I say what I think and feel.	
40.	I feel that I can't cope on my own in many situations.	

41.	I am often surprised to see how stupid people are.	
42.	I enjoy helping others to get out of difficult situations.	
43.	I have a very good ability to explain things to people in a clear and accurate manner.	
44.	I find it hard to understand why so many people take life so seriously.	
45.	I continuously try to comply with what other people expect of me.	
46.	I do my day-to-day work in a routine rather than to try out new and inventive ways.	
47.	I feel exploited by others.	
48.	When having discussions my point of views rank amongst the best.	
49.	Patience is not my biggest strength.	
50.	If somebody is angry with me I try to conciliate with him/her.	
51.	I see myself as being confident.	
52.	I'm able to solve problems/tasks as well as other people would do.	
53.	I'd like to describe myself as being a fairly optimistic human being.	
54.	I know that I have many good qualities and skills.	
55.	I feel comfortable with myself.	
56.	I have little self-confidence.	
57.	I haven't achieved much in my life that I feel really proud of.	

58.	At times I think that I’m not good enough.	
59.	At times I feel useless.	
60.	In many situations I feel inferior to other people.	
61.	I really get on with everybody.	
62.	I especially feel comfortable with people who have other views than mine.	
63.	I consider it to be especially important what other people feel and think.	
64.	I believe it to be worthwhile to be open and honest towards others.	
65.	I sincerely believe that human beings are well able to lead and control themselves so that they can develop further.	
66.	I noticed that in conflict situations I tend to be right after all.	
67.	If I’m honest to myself I can see that I do criticise others more than that I praise them.	
68.	I quickly discover other people’s weak points.	
69.	My experience is that when I give someone an inch they will take a mile.	
70.	I believe that human beings really need a strong and leading hand.	

EGOGRAM:

YOUR PERSONALITY PROFILE

	CP		NP		A		NC		AC
1		2		3		4		5	
6		7		8		9		10	
11		12		13		14		15	
16		17		18		19		20	
21		22		23		24		25	
26		27		28		29		30	
31		32		33		34		35	
36		37		38		39		40	
41		42		43		44		45	
46		47		48		49		50	
Total		Total		Total		Total		Total	

LIFE POSITIONS

HOW YOU APPRECIATE YOURSELF AND OTHERS

	I am OK		**I'm not OK**		**You're OK**		**You're not OK**
51		**56**		**61**		**66**	
52		**57**		**62**		**67**	
53		**58**		**63**		**68**	
54		**59**		**64**		**69**	
55		**60**		**65**		**70**	
Total		**Total**		**Total**		**Total**	

After completing the Egogram and Life Positions Questionnaire, ask the participants to transfer the scores to the tables above.

Explain that Egogram depicts the score for five aspects of one's personality:

CP = Critical Parent, NP = Nurturing Parent, A = Adult, NC = Natural Child, and AC = Adapted Child. The highest possible score for each of these five aspects is 40 (maximum score for each statement – 4 x 10(no. of questions)). Higher the score, stronger the particular Ego state.

Explain that Life Positions depicts how you appreciate yourself and others.

The highest possible score for each of these basic four Life Positions is 20 (maximum score for each statement – 4 x 5 (number of questions). Higher the score, stronger the particular Life Position.

Team and Team Building

A team is a set of interpersonal relationships structured to achieve established goals. As such, successful team performance requires use of interpersonal competencies among the team members. The productivity of teams is not a simple function of team member's technical competencies and task abilities. Small groups of motivated individuals are the secret to team productivity.

A team is two or more individuals, including duly constituted leadership, working in concert, engaged in clearly understood interdependent roles, towards achievement of mutually shared tasks. "Leadership", "Task", and "Role" are, thus the core ingredients of a "Team". These concepts – among others – need to be understood and operationalised effectively in order for a team to come into being, and stay as a tool for the organisation.

Human beings are born individuals with strong survival instincts. Physiological survival takes the form of a level of personal comfort, freedom from pain and disease, and of course avoidance of death. Psychological survival, in due course, may begin to mean things like respect from others, recognition, and self esteem. These motives would seem familiar in terms of our ability to compete and achieve, repel potential danger, and build a social system conducive to ourindividual safety, security and support needs; but it rarely becomes a source of sustained mutuality.

Team work therefore, has often to be learnt: Learning team-work may not necessarily mean learning any new skills. It may, though mean learning how to apply the skills we possess to team situations such that rather than threatening, the team is seen as an instrument to preserve our individuality and survival. Unfortunately, in most cases, our normal learning process is not conducive to developing this attitude. The learning, therefore, has to be attitudinal and motivational rather than knowledge and skills oriented. Most of our early learning takes place in the family and in formal education. Neither of these adequately stresses the need for us to become good team players.

Most organisational tasks are accomplished by teams:

Organisations use teams for specific missions and the more effective leaders like to turn their people into teams. Teams are created to pool the talent, energy, and initiative of several persons so that this group of persons can achieve what may be very difficult for any individual to achieve alone. Creative team work is important in any organisation not only because it helps to achieve organisational objectives but also because it is a powerful means of developing and motivating human resources.

A team which works smoothly or harmoniously does not necessarily contribute to organisational effectiveness and innovation. The following may act as useful indicators of danger in team functioning:

- A hazard of cohesive team working is "groupthink" – a situation in which the ideology of the team clouds the capacity of team members to think and act rationally about the task, particularly when the team is under pressure.
- Another hazard is that individuals may consciously conform to the norms as dictates of the group even when these violate their own notion of right and wrong. For instance, members of a management team may collectively decide to launch a programme which is known to be unproven or risky simply because no one wants to spoil the teamspirit.

Features of effective creative teams

Creative functional teams that contribute to organisational effectiveness are known to manifest some of the following features.

Participatory leadership: Leadership is not just the style, skills and competencies of the leader, but also the systems and processes through which leadership is institutionalised in the organisation, and experienced by the followers. Some examples of the institutionalising mechanisms can be the organisational values, and the systems devised for organisational processes such as, leader-follower mutuality and thrust, motivation, communication, influencing, decision-making, goal-setting, control, performance management.

The leader need not necessarily be personally very creative; but it is important that she/he

respects and understands creativity and regards the nurturance and evocation of creativity in her/his team members as one of his/her main tasks. She/he must delegate sizable powers to team members, encourage initiative, and provide a sense of security to creative members using innovative approaches that may or may not eventually work. The leader may also help the team by encouraging a system, in which relatively young or inexperienced members (progenies) are informally attached to mature innovators (mentors) to learn the ropes. Role modelling within limits seems to play a major role in creativity. Participatory leadership means creating an interdependency by empowering, freeing up, and serving others. The atmosphere tends to be informal, comfortable, relaxed. The leadership shifts from time to time, depending on the circumstances. There is little evidence of a struggle for power as the team operates.

Shared responsibility: Shared responsibility must accompany participatory leadership. This involves establishing an environment in which all team members feel as responsible as the leader for the performance of the work unit.

Aligned on purpose: All members have a sense of common purpose about why the team exists and the function it serves. The task or the objective of the team is well understood and accepted by the members. There would have been free discussion of the objective at some point, until it was formulated in such a way that the members of the group could commit themselves to it.

High communication: This involves the existence of a climate of trust, and open honest communication. The team has lots of discussion, in which almost everyone participates, but it remains pertinent to the task. If the discussion gets off the subject, someone will bring it back. Face-to-face interaction has considerable impact on morale, and effectiveness. To obtain meaningful face-to- face interaction, team size needs to be small. A perception that one's participation and efforts are needed increases as the size of the team decreases.

Future focussed: Creative effective teams concern themselves not only with the present, but also with the future of the organisation. Accordingly, they see change as an opportunity for growth. Rapid response to the environment, and identifying and acting on opportunities is an essential characteristic of creative teams.

Focussed on task: Task includes the mission, the strategy, the programmes etc. of the organisation, in ways and forms entrusted to a team. Unless this task is appropriately determined and defined to clear and identical understanding of all members, including the expected quantity, quality, time-schedule, resources available – financial, human, and others, there can be no team.

Focused on task means keeping operations focused on results, and sharing responsibility for the operations. The team is self-conscious about its own operations.

Structures, roles and rules: A team's work consists of routine or repetitive tasks, as well as non-routine and emergency or urgent tasks. It makes sense to develop some sort of a structure – it needs not be a tight one – to take care of both sorts of tasks. Thus, some division of work, specialisation, and delineation of responsibilities helps ensure that all the needed work gets done. Some rules are also useful to have in order to avoid needless conflicts; and some procedures for repetitive tasks may be usefully laid down to minimise members "rediscovering the wheel". A number of roles may need to be played for a team to perform well.

However, the roles of team members are not defined only in aid of the routine tasks; these are only minimum elements of their roles. Some role ambiguity to accommodate the situational compulsions helps interaction. Periodic interchange of roles within the team can help develop this versatility.

In an effective team, clear assignments are made and accepted. Individual accountability is an important aspect of role clarity. It becomes visible when the performance of each individual team member is assessed to (a) inform the team which members need more assistance or encouragement in completing their assigned work; and (b) increase members' awareness that their contributions to the team effort are identifiable.

Shared values: Teams work well when members share in ideology. An ideology consists of connected values. The point is that when members share certain core values, teamwork is facilitated, and members are likely to put their heart and soul into the team's work. Some shared ideologies help a team operate more innovatively: curiosity, sensitivity, entrepreneurship, complexity, independence, reality contact, experimentation, persistent striving for distant goals. These values are consistent with a democratic idealistic value system.

Creative talents, skills and resources: Since teams often pursue tasks that are bigger than what any one person acting alone can pursue, it stands to reason that a team must have a fairly wide spectrum of expertise, skills and abilities, and also an access to other types of resources – money, equipment, technical skills, authority to act, and so forth.

Creative talents refer to those personal attributes of individual members that help remove barriers to creativity, and enable creative application of individual talents and skills.

Collaborative skills need a special mention in this context. Teams function effectively if members do have and use the needed collaborative skills. Placing socially unskilled individuals in a team and telling them to collaborate does not guarantee that they required to work as part of teams and, therefore, lack proficiency in the needed collaborative skills for doing so. Persons must be helped in acquiring proficiency in social skills for high-quality collaboration and be motivated to use them. All the small-group skills are relevant to team effectiveness.

Tolerance or dissent: The team is comfortable with disagreement and shows no signs of having to avoid conflict or to keep everything on a plane of sweetness and light. Disagreements are not suppressed or overridden by premature team action. The reasons are carefully examined, and the team seeks to resolve them rather than to dominate the dissenter.

Decision by consensus: Most decisions are reached by a kind of consensus in which it is clear that everybody is in general agreement and willing to go along. However, there is little tendency for individuals who oppose the action to keep their opposition private and thus let and apparent consensus mask real disagreement. Formal voting is at a minimum; the team does not accept a simple majority as a proper basis for action.

Constructive criticism: Criticism is frequent, frank, and relatively comfortable. There is little evidence of personal attack, either openly or in a hidden fashion. The criticism has a constructive flavour in that it is oriented toward removing an obstacle that faces the team and prevents it from getting the job done.

Respect for feelings: People are free in expressing their feelings as well as their ideas both on the problem and on the team's operation. There is little pussyfooting, there are few "hidden agendas". Everybody appears to know quite well how everybody else feels about any matter under discussion.

Positive interdependence: Positive interdependence is the perception that the performance of the team is mutually caused by all members; that one is linked with others in a way that one cannot succeed unless these others do. A belief that "you one cannot succeed unless these others do. A belief that "you sink or swim together". Positive interdependence promotes working together to maximise joint benefits, sharing resources, providing mutual support, and celebrating joint success.

This positive interdependence may include positive goal interdependence, positive reward interdependence where joint reward is given for successful team work, positive role

interdependence where team members are assigned complementary and interconnected roles, positive task interdependence where division of labour is such that the actions of one member have to be completed if the next member is to complete his/her responsibilities, positive resource interdependence where each member has only a portion of the resources (information or material) necessary for the task to be completed.

Group processing: Teams that reflect on their internal processes, instead of concentrating only on task achievement, are likely to be more effective in the long run. Thus, a team that periodically reviews the processes of communication, conformity and deviance, leadership and followership, and value and norm formation is likely to remain healthy and energetic. To make the team function innovative, the process of problem solving and decision making also needs to be reviewed periodically.

Concluding words on team development

In addition to the other essential elements, effective teams need to be cohesive, to maintain trust among members, and to have norms that promote productivity. And, this requirement calls for special attention. Cohesion is the extent to which the influence on members to remain in the team is greater than the influence on members to leave the team. It is the sum of all the factors influencing members to stay in the team. Cohesion is determined by the assessment of team members of the desirable and undesirable consequences of team membership. Highly cohesive teams are a source of security for members. They serve to reduce anxiety and to heighten self-esteem. There are several ways in which a team can increase its cohesion:

1. Structuring cooperation among members: One of the most predictable outcomes of cooperative interaction is that team members will like each other and value their membership in the team.
2. Successfully meeting the personal needs of members: For a team to be cohesive, the members' needs for mutual inclusion, mutual influence, and mutual affection among themselves must be met.
3. Maintaining a high level of trust among members: without a high level of trust, a team cannot be cohesive.


Soft Skills
Collaborative
Warm
Humble
Self-Aware
Respectful
Accountable
Approachable
Integrity
Positive
Team Player
Tactful

BIOCHEMISTRY

Volume - 1

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BIOCHEMISTRY

Volume - I

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PREFACE

We are happy to publish "Biochemistry" Volume -I to the Undergraduate and Postgraduate students of Science. This book covers basic concepts and theory of biochemistry. The basic concepts in life processes can be understand with the knowledge of biochemistry. Glossary and index is given at the end of the book.

Comments and suggestions for this book from faculty and students for the improvement of this book are always welcome. We request the readers to send your valuable suggestions and comments for the upcoming editions to smcjasbooks@gmail.com

Features of our Publications

- Standard definitions.
- Explanations from references.
- Fulfill the needs of all students.

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We thank almighty for giving abundant blessings to fulfill the complete vision to bring this Biochemistry book.

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UNIT I

BASICS OF BIOCHEMISTRY

Biochemistry: Introduction, Applications.

Biomolecules: Elements of Life, Atoms, Molecules.

Bonds: Definition, Types - Primary Bonds (Ionic Bond, Covalent Bond), Secondary Bonds (Hydrogen Bond, Van der Waals Bond).

pH: Definition, Measurement - Values of pH and regulation, Henderson and Hasselbalch's equation.

Buffer: Definition, Buffer action, Importance of Buffers, Biological buffer system

Water: Different forms of water, Sources, Water content of organisms, Structure and Properties of water

1 BASICS OF BIOCHEMISTRY

1.1 BIOCHEMISTRY

Biochemistry usually involve the nature of the chemical constituents of living matter, their transformation from one form to another form in the living systems and the energy changes associated with these transformations. Such studies have been conducted both in plant and animal tissues and also in microorganisms like bacteria and viruses.

In earlier, Biochemistry was called as Chemical Physiology since it was originated as an offshoot from human physiology. But, physiology deals with the study of normal functional phenomena of living beings, while biochemistry is concerned with the chemical aspects of those functional phenomena. Under the topic of biochemistry all the biological phenomena are analyzed in terms of chemistry. For this reason, this field is variously named as Biological Chemistry or Chemical Biology. In short, biochemistry is defined as the "Chemistry of living things".

As the knowledge of biochemistry increasing day-by-day, newer disciplines such as Enzymology (study of enzymes), Endocrinology (study of endocrine secretions or hormones), Clinical biochemistry (study of clinical diagnosis of various diseases), Molecular biochemistry (study of genetic materials), Agricultural biochemistry, Drug biochemistry etc., are emerging from the parent biochemistry.

Biochemistry deals with the chemical aspects of the plants and animals and their functions. Biochemistry has two branches

1. **Descriptive biochemistry**: Qualitative and quantitative characterization of various cell compounds.
2. **Dynamic biochemistry**: Elucidation of the nature and mechanism of the reactions involving the cell components.

The term biochemistry was coined by Carl Neuberg in 1903. He was the father of biochemistry. He developed the concept of oxidation and also animal respiration. He equated respiration with combustion.

The characteristic feature of the living organisms are mainly due to the presence of certain organic molecules. Since these organic molecules are present in the living system, they are usually called as **Biomolecules**. These biomolecules generally contain carbon, hydrogen, oxygen etc. Biomolecules are of two types: macro molecules and micro molecules.

Macromolecules are very large, complex, organic molecules with high molecular weights. Examples are carbohydrates, lipids, nucleic acids, proteins etc. All these macromolecules are made up of many simple, smaller building block molecules which are usually called as monomers. Since the macromolecules are formed by the repeating units of monomers they are also named as **Biopolymers**.

Micromolecules are simpler and smaller organic molecules with low molecular weights. They are generally acting as building blocks of macromolecules and are called as monomers or subunits. They can show their activities either individually or in the form of macromolecules by joining together themselves by characteristic linkages.

Examples are

1. Simple sugars which are acting as submits of higher carbohydrates like disaccharides, oligosaccharides and polysaccharides.
2. Amino acids are acting as monomeric units of proteins.
3. Fats are nothing but the esters of fatty acid.
4. Nucleotides are the subunits of the macromolecules – DNA and RNA.

1.2 APPLICATIONS OF BIOCHEMISTRY

Biochemistry is found to have large-scale applications in various areas of industry, agriculture, medicine and pharmacy.

Physiology

Biochemistry helps us to identify the physiological alteration in the body. Any disease can be known by understanding the biochemical changes.

Nutrition Deficiency

In today's scenario, maintaining nutrition in our body is essential. The biochemical changes identified in our body, helps to maintain the essential vitamins and minerals in our body.

Genetic Engineering

By applying biochemical techniques, improved domestic animals and cultivated plants are produced through genetic engineering

Hormonal Deficiency

Hormonal balance is very essential insert - for our body. Deficiency of hormones leads to several major problems.

Agriculture

Biochemistry helps us to enhance the growth of plants. It also helps to get the increased yield. To protect cultivated plants from pests, biological preparations of superior quality that are not harmful to human or animals are currently being manufactured.

Industry

Biological methods are available for the disposal of industrial and domestic wastes. Biochemical processes are widely used in the food industry (in the preparation of bread, cheese, wine etc) and in the leather industry.

Medicine

Biochemical estimations are very essential. Various test such as kidney function test, blood test, liver function test, serum cholesterol test helps to maintain the body requirements. Drug composition, its metabolism, drug transferring reactions in our body can be understood only with the knowledge of biochemistry.

1.3 Biomolecules

Biomolecule refers to a molecule produced by living organisms such as plants and animals. The four major classes of biomolecules are carbohydrates, proteins, lipids and nucleic acids. The chemicals are combined in a particular ratio to form life.

Chemicals present in the environment are nitrogen, hydrogen, carbon and oxygen in the form of gases. They combined together to form ammonia, methane and water.

$N_2 + 3H_2 \longrightarrow 2NH_3$ Ammonia

$C + 2H_2 \longrightarrow CH_4$ Methane

$½\ O_2 + H_2 \longrightarrow H_2O$ Water

Micro molecules like amino acids, fatty acids, monosaccharides, purines, pyrimidines etc are formed from these essential elements. The micro molecules combined together to form macro molecules such as proteins, polysaccharides, lipids, nucleic acids, nucleoprotein etc.

The plants and animals are made up of cells which is composed full of chemicals. The chemicals present in our body constitute inorganic substances like water, minerals, salts, gases etc and organic substances like carbohydrates, proteins, fats, nucleic acids, nucleoproteins etc.

1.4 CHEMICAL COMPOSITION OF LIFE

Cell is the fundamental unit of life. It is the store house of biomolecules which are responsible for various bioactions. The various macro molecules associate together in different proportions to form organelles such as ribosomes, golgi bodies, mitochondria, nucleus etc.

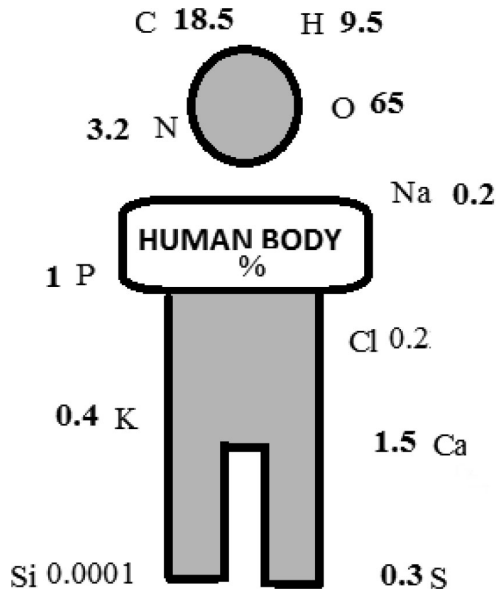


Fig 1.1 Elemental composition of human body

The chemicals making a cell are inorganic compounds and organic compounds. The inorganic substances do not contain C-H groups and they comprise 81% of a cell. The organic compounds constitute 19% of a cell and they contain C-H groups.

Table 1.1 Chemical composition of a cell

Compounds	Percentage
Inorganic Compounds	81.0%
Water	80.0%
Inorganic Salts	1.0%
Organic Compounds	19.0%
Carbohydrates	1.0%
Lipids	3.5%
Proteins	12.0%
Nucleotides	2.0%
Other Compounds	0.5%

Macro biogenic elements are the major elements which make up the living organisms. They constitute 1%. These include 6 elements namely oxygen, carbon, nitrogen, hydrogen, calcium and phosphorous.

Chemical composition of earth's crust and living organisms

Table 1.2 Relative contents in % of certain chemical elements on earth's crust and man

Elements	Earth's Crust %	Man %	Elements	Earth's Crust %	Man %
H	1.9	9.5	P	2.6	1
O	47	65	S	0.05	0.3
C	0.08	18.5	K	2.5	0.4
N	0.0001	3.2	Cl	0.017	18.5
Na	2.8	0.2	Si	27.7	0.0001
Ca	3.6	1.5	Al	8.1	0.0001

- Certain elements present in large quantity on earth are represented in higher concentration in the living organisms. Example: Hydrogen.
- Certain elements present in large quantity on earth are represented in very small quantity in living organisms. Example: Silicon.

1.5 ELEMENTS OF LIFE

- All substances in the universe are made up of elements. An element is formed of many similar units called atoms. An atom consists of proton, electron and neutron.

1.5.1 ATOMS

Definition

An atom is defined as the smallest unit of an element. It combines with one another by chemical bonding to produce molecules. Atoms are formed of electrons and a nucleus. The nucleus contains protons and neutrons.

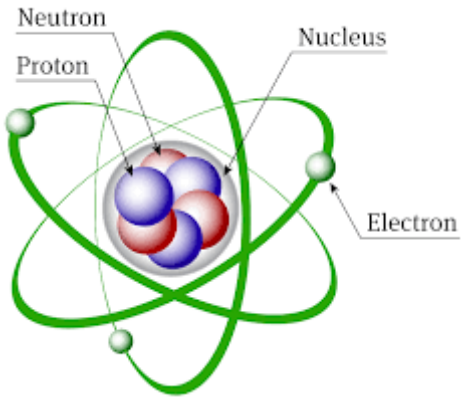


Fig.1.2 Structure of an Atom

1.5.2 Molecules

Definition

Two or more atoms unite together to form a molecule. A molecule may be formed by the union of similar or dissimilar atoms.

Examples

- Oxygen molecule (O_2) is formed by the combination of 2 oxygen atoms.
- Hydrogen (H_2) molecule is formed by the combination of 2 hydrogen atoms.
- Water (H_2O) is a molecule. It is formed by the combination of 2 hydrogen atoms and one oxygen atom.

1.5.3 Stable and Unstable Atoms

Stable atoms: In stable atoms the outer shell is complete by the presence of full number of electrons. It has enough binding energy to hold the nucleus together permanently. They are called stable atoms.

E.g. Helium - In helium the outer shell has complete two set of electrons.

Unstable atoms: The atoms which does not have the complete set of electrons in the outer shell are called unstable atoms. An unstable atom does not have enough binding energy to hold the nucleus together permanently.

E.g. Hydrogen has only one electron instead of two in the outer shell.

Oxygen atom has 6 electrons instead of 8 in the outer shell.

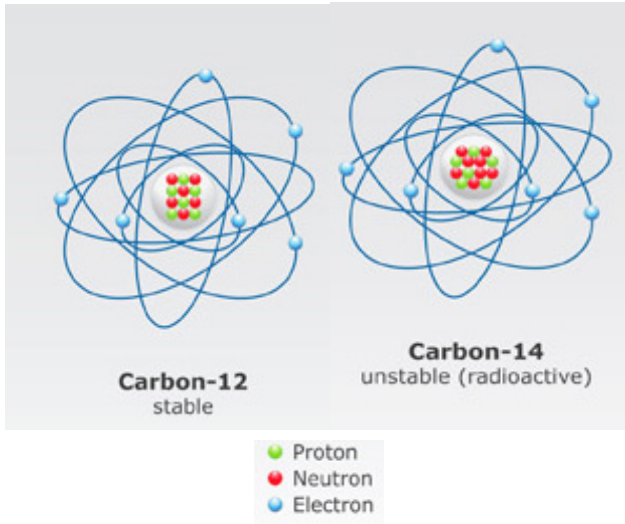


Fig 1.3 Stable and Unstable atoms

1.6 ATOMIC THEORY

Dalton proposed a theory on atoms called atomic theoy. The atomic theory states that each matter is made up of tiny particles called atoms. The main principles of atomic theory are the following

- Each matter is formed of tiny particles called atoms
- Atoms cannot be created or destroyed or divided
- Atoms of the same element are similar and equal in weight
- Atoms of different elements are dissimilar and different in weight and properties

Atoms of different elements have different numbers of electrons, protons and neutrons. For example hydrogen has one electron, one proton and no neutron. Hence the outer shell contains one electron.

Helium atom contains two protons, two neutrons and two electrons. Hence in the helium atom, the outer shell contains two electrons.

Carbon atom has 6 electrons, 6 protons and 6 neutrons. The electrons are arranged in two shells. The first shell contains two electrons and the outer shell contains four electrons.

Oxygen atom contains 8 electrons, 8 protons, 8 neutrons. The electrons are arranged in two shells. The first shell contains two electrons and the outer shell contains 6 electrons.

1.7 ATOMIC WEIGHT

The sum of weights of protons, neutrons and electrons of an atom is called atomic weight. The atomic weight of the atom is approximately equal to the number of its protons and neutrons. The atomic weights range from 1 to 256.

Example

- Hydrogen atom has one proton and no neutron. Hence the atomic weight of hydrogen atom is 1+0 = 1.
- Oxygen atom has 8 protons and 8 neutrons. Hence the atomic weight of oxygen atom is 8 +8 = 16.

1.8 ATOMIC NUMBER

The atomic number or proton number (symbol Z) is the number of protons found in the nucleus of every atom of element. The atomic number uniquely identifies an element. It is similar to the charge number of the nucleus.

Example

Hydrogen atom one proton then the atomic number is 1.
Carbon atom 6 protons and the atomic number of carbon is 6.

1.9 BONDS

Atoms of two or more elements are combined by mutual attraction is called chemical bonds.

The *bond* may result from the electrostatic force of attraction between oppositely charged ions as in ionic *bonds* or through the sharing of electrons as in covalent *bonds*.

1.10 TYPES OF BONDS

The bonds are classified into two types, namely

- **Primary bonds**

  They are strong bonds and hold the atoms together in molecules.

- **Secondary bonds**
  They are weak bonds and they are frequently found in biological molecules. They are found in other organic molecules too.

1.10.1 PRIMARY BONDS

Primary bonds are the strong bonds. They need more energy for the formation of breaking of primary bonds. They bind one atom with another.

The primary bonds are of two types, namely

- Ionic bonds
- Covalent bonds

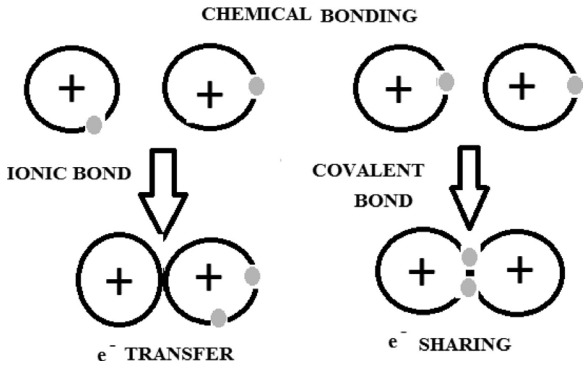


Fig 1.4 Primary Bonds

Ionic Bonds or Electrovalent Bonds

- The bond formed by the transfer of electrons from one atom to another is called ionic bonds.
- The term 'electrovalent bond' was proposed by Kossel in 1961.
- Electrovalent bond links two or more atoms in a molecule or compound. The bond is formed by the process of ionization. Hence the bond is called ionic bond and the compound is called ionic compound or electrovalent compound or polar compound.
- Ionic bonds are formed in crystalline inorganic salts.

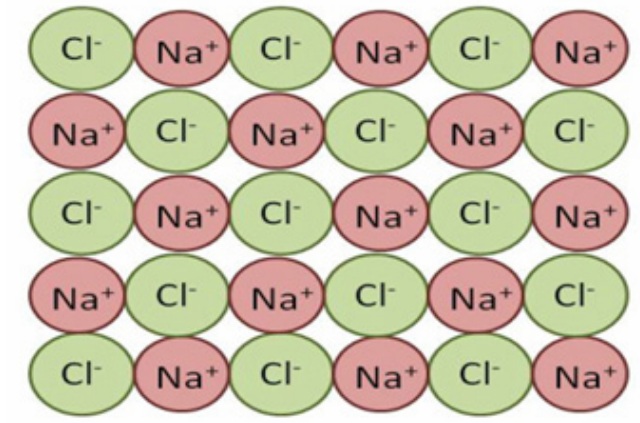


Fig 1.5 Ionic bond of table salt, NaCl

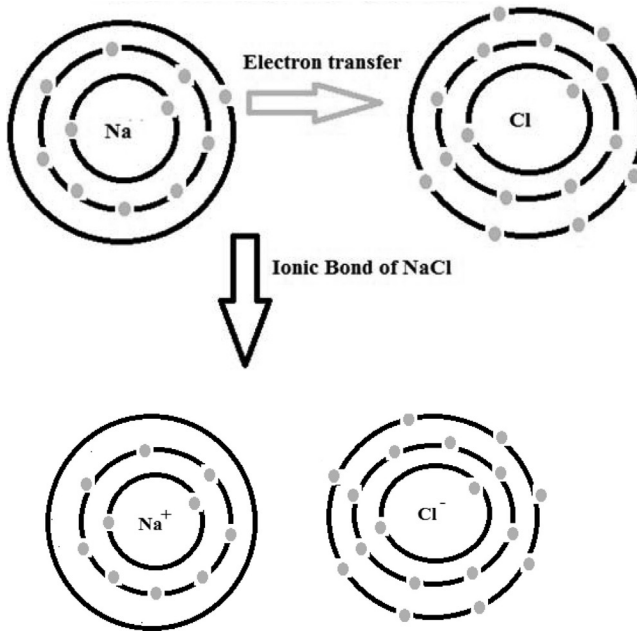


Fig 1.6 Electrovalent bond in Sodium Chloride

Example

Sodium Chloride is an ionic compound. Sodium atom (2, 8, and 1) has one electron in the outermost orbit. Chlorine atom (2, 8 and 7) has 7 electrons in the outermost orbit. The sodium atom transfers one electron to the chlorine atom. Since sodium losses a negatively charged electron, it becomes positively charged

or electropositive. As chlorine gains a negatively charged electron, it becomes negatively charged or electronegative. The Na^+ ion and Cl^- ion are held together in NaCl by the electrostatic force of attraction. The valency of an element is the number of electrons an atom gains or losses in order to become stable. So the valency of Na is 1 and that of Chlorine is also 1.

Covalent Bonds

- The term 'covalent bond' was proposed by Lewis in 1966. This bond is very common in organic compound. The atoms also neither lose nor gain electrons. These compounds are called molecular compounds.
- In covalent bonds the atoms are linked together by the sharing of electrons between the atoms.
- The covalent bonds are also called homopolar bonds. They are strong bonds. Covalent bonds are endergonic. i.e., their formation needs energy. 95% of the chemicals in the cells are bounded covalently.

Example

Methane (CH_4)

Carbon combines with 4 hydrogen atoms to form methane. The carbon atom has 4 electrons in the outer shell. It requires 4 electrons to complete its outer shell. It completes its outer shell by sharing electrons with 4 hydrogen atoms.

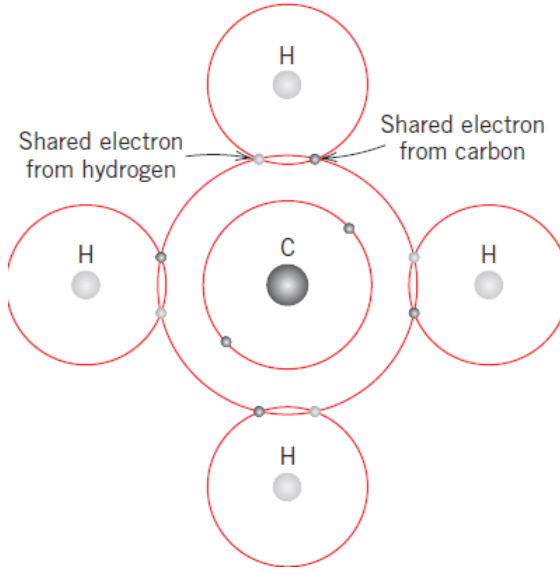


Fig 1.7 Bonding in Methane

1.10.2 Secondary Bonds

Secondary bonds are weaker bonds. They are formed between molecules or within a molecule. They are very important in joining the biochemical compounds. The secondary bonds are easily formed and broken. They need only very little energy for formation or breaking. The important secondary bonds are

- Hydrogen bonds and
- Van der Waals bonds

Hydrogen Bonds

- The term hydrogen bond was proposed by Latimer and Rodenbash.
- The attractive force that binds the hydrogen atom of one molecule with the electronegative atom of another molecule is called hydrogen bond.
- The hydrogen bond is also called proton bonds.
- Hydrogen bonding does not involve transfer or sharing of electrons.
- When a hydrogen atom carrying positive charge, approaches an atom with negative charge an association is formed. This bonding is called hydrogen bond.

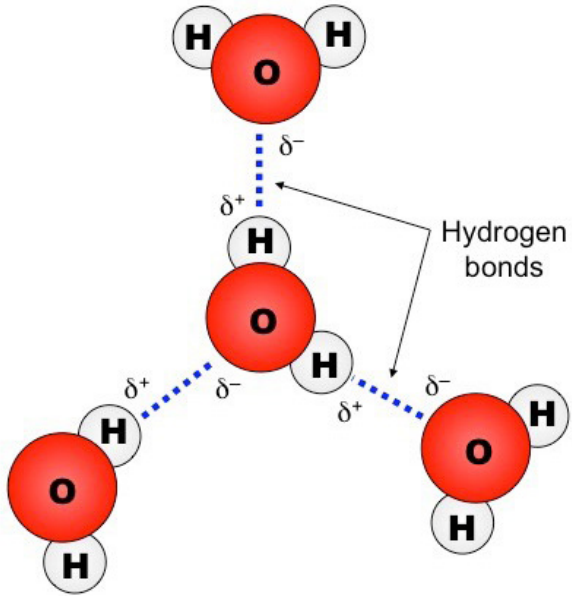


Fig 1.8 Hydrogen Bond

Van der Waals Bond

- Van der Waals bonds are the weakest bonds. They are secondary bonds.
- They act between molecules which are brought close together. These bonds result from the fluctuating charges caused by the nearness of the molecules.
- However, when the molecules are too close, repulsive forces operate because of the overlapping of the outer shells of the atoms involved.
- These bonds are formed between all types of molecules, polar as well as non-polar.

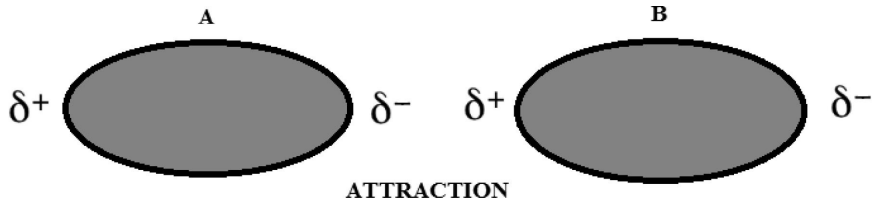


Fig 1.9 Van der Waals bond

1.11 pH

pH is the measure of acidity or alkalinity of a solution.

- The term pH is defined as the negative logarithm of the hydrogen ion concentration in a solution.
- The term pH was introduced by Sorensen in 1909.

$$pH = -\log 10 (H^+)$$

In the expression 'pH',

- ♦ p stands for power and
- ♦ H stands for the hydrogen ion

- The pH of a neutral solution is said to be 7.
- If the pH is less than 7, the solution is said to be acidic.
- If the pH is greater than 7, the solution is said to be basic or alkaline.

It is also clear that lower the pH, the more acidic is the solution. Similarly, higher the pH the more basic is the solution. A solution having pH = 0 is acidic. pH of a solution decreases when it is heated.

Example

In a litre of pure water, 1/10,000,000 gram of hydrogen ions can be detected.

It can be written in terms of a power, 10^{-7}. In terms of pH scale, it is simply referred to as pH 7.

At pH 7, the concentration of free H^+ and OH^- are exactly the same and thus pure water is neutral.

Water is slightly ionized into H^+ ions and OH^- ions.

$$H_2O \longrightarrow H^+ + OH^-$$

1.11.1 pH Measurement

- pH can be measured for an aqueous solution that can be performed by using indicator dyes such as phenolphthalein, phenol red, litmus etc.
- pH can be measured with the help of pH paper and pH meter.
- In pH meter, pH can be measured with the use of glass electrodes.

Values of pH and regulation

- For the normal human blood, the pH is close to 7.4. When the pH level decreases below 7.3, it leads to acidosis. If the pH level falls below 7 it is lethal to life.
- The pH of the fluid of the prostatic cells is lower than 5.
- The osteoblastic cells is about 8.0 or even higher.
- In most cells, the pH is found to be nearer to 7.
- The metabolic reactions and enzyme activities require an optimum pH. At the optimum pH, the reaction rate is maximum. When the pH level is altered, the reaction rate also decreases.

1.12 BUFFER

A solution that resists change in pH by addition of a small amount of an acid or a base is called a buffer solution. The capacity of a solution to resist alteration in its pH value is known as buffer capacity. The capacity to resist changes in pH depends upon

i. The actual concentrations of salt and acid present in the buffer and
ii. The salt acid concentration ratio

When a drop of HCl is added to a litre of pure water, the pH of the water changes immediately from 7 to about 2.2. A buffer can be represented by placing the acid or base as the numerator and its salt as the denominator.

$$\text{Acid buffer} = \frac{\text{Acid}}{\text{Salt}}$$

$$\text{Basic buffer} = \frac{\text{Base}}{\text{Salt}}$$

Example

A solution of acetic acid and sodium acetate ($CH_3COOH + CH_3COONa$) is an example of a buffer that consists of a weak acid and its salt.

1.12.1 Buffer Action

The ability of the buffer solution to resist the changes in pH value on the addition of small amount of an acid or a base is known as buffer action.

The buffer action of an acid buffer can be explained by taking an acetate buffer. It is formed of acetic acid (CH_3COOH) and its salt sodium acetate (CH_3COONa). It is represented as follows:

MECHANISM OF BUFFER ACTION

Acetate buffer

$CH_3COONa \longrightarrow CH_3COO^- + Na^+$

$CH_3COOH \longrightarrow CH_3COO^- + H^+$

+ 1 MOLE NaOH

$CH_3COOH + OH^- \rightleftharpoons CH_3COO^- + H_2O$ (WEAK ELECTROLYTE)

+1 MOLE HCl

$CH_3COO^- + H^+ \rightleftharpoons CH_3COOH$ (WEAK ELECTROLYTE)

1.12.2 Importance of Buffers

1. It protects the cells and tissues from sudden change in pH.
2. It helps the organisms to carry out the biochemical reactions in a narrow range of pH.

3. It is helpful in many biological experiments.
4. It is able to neutralize small amounts of added acid or base, thus maintaining the pH of the solution relatively stable.

1.13 WATER

Water is the basic molecule of life. It is important for life. It is known that none of the organisms can survive without water. About 70% of our earth is covered with water. Human body also constitutes 70% water. Life originated in the water medium. Water is the most significant molecule which connects the physical world with the biological processes. Therefore, water is described as the mother of life or soul of life.

1.13.1 Different Forms of Water

Water exists in three forms namely,

- **Vapour or steam -** Above 100^{o}C, it occurs in the form of vapour or steam.
- **Solid** - Below 0^{o}C, it becomes solid called ice.
- **Liquid -** Between 0^{o}C and 100^{o}C, it remains in the liquid form.

1.13.2 Sources of Water

Water is available to the biological system from a number of sources such as sea, ponds, pools, lakes, rivers, streams, springs, wells, precipitation etc.

1.13.3 Water Content of Organisms

Plants - The water content of plants is 90% of the total weight.

Animals - In animals, the average water content is more than 50% of their body weight.

Human beings - In human beings, the water content increases and decreases based on age and body. The body water ranges between 45% and 75%. With an increase in age, the total water decreases as if the organism become drier.

Why the water content decreases as age increases?

- **New-borns** - A higher content of total water in new-borns is due to the extracellular water.
- **Infants** - In normal infants the water content is 75% of the total body weight.

- **Adults** - The water content of the body gradually decreases as age increases and it reaches the minimum of 45% in adult (above 55 years).

With increasing age, the total water decreases since the organisms has become drier.

Why a fatty man has less water than lean man?

The average % of total body water ranges around 67%. The variation in body water between normal individuals is caused chiefly by the variable fat content since adipose tissue contains less water. Hence a fatty-man has less water than lean-man. The extracellular water makes up 20% to 26% of the body weight. Of these intercellular fluid 12 to 16%, blood plasma 5%, lymph 2% and intracavitary water 1 to 3%.

In human body, water occurs in two phases, namely

- Intracellular water and
- Extracellular water

Intracellular Water

Water present inside the cell is called intracellular water. It constitutes 35% to 45% of the total body weight. It occurs in two forms, namely free water and bound water. Free water constitutes 95% of the water content of the cell. The bound water constitutes 5% and it occurs in chemical combination with proteins and other constituents of cytoplasm.

Extracellular Water

Extracellular water exists outside the cell. It includes the **intercellular fluid, plasma, lymph** and **intracavitary water** such as cerebrospinal fluid, intraocular fluid, pericardial fluid, synovial fluid and the secretions of salivary, sweat and tear glands.

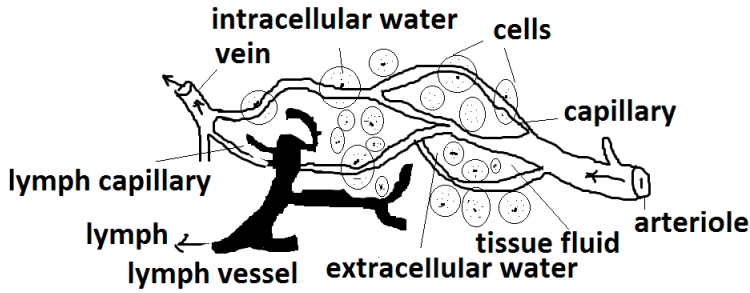


Fig 1.10 Intra and Extracellular water

1.13.4 Structure of Water Molecule

- Water is an inorganic compound.
- The molecular formula of water is H_2O. It contains two hydrogen atoms and one oxygen atom.
- The hydrogen and oxygen atoms are held together by covalent bonds (bonds formed by the sharing of two electrons).
- The three atoms in the water molecule (two hydrogen atoms and one oxygen atom) are not in a line. They are arranged in the form of the letter V, with oxygen atom at the tip and the hydrogen atoms at the ends of the two limbs.
- The bond angle between hydrogen and oxygen atoms is 105^o.
- Central Property - Electrical polarity is the central property of water molecule. The oxygen atom is negatively charged and the hydrogen atoms are positively charged. The water molecule is a dipole, as this has two different poles. Water is also called as a polar compound.
- The polar molecules have the property of attracting each other. Owing to this attractive force, water molecules aggregate together. As a result of this force, a water molecule can link with 4 adjacent water molecules. The linking between two water molecules is effected by the formation of a hydrogen bond (O....H) between the oxygen atom of one water molecule and the hydrogen atom of another water molecule. The oxygen atom forms a tetrahedron with the four hydrogen atoms of the neighbouring 4 water molecules.

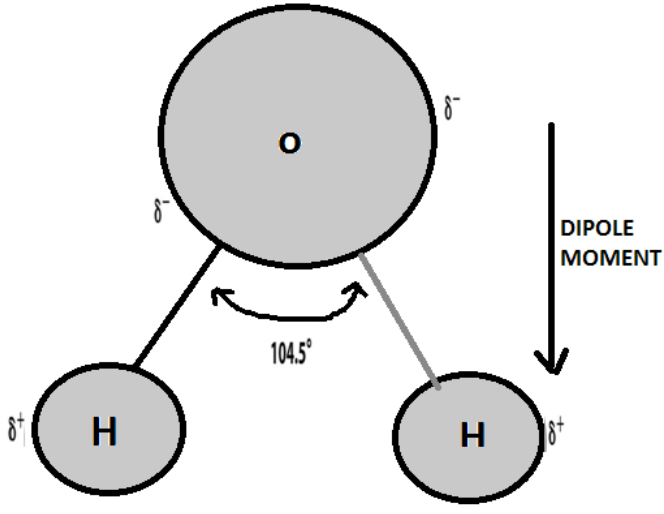


Fig 1.11 Structure of Water Molecule

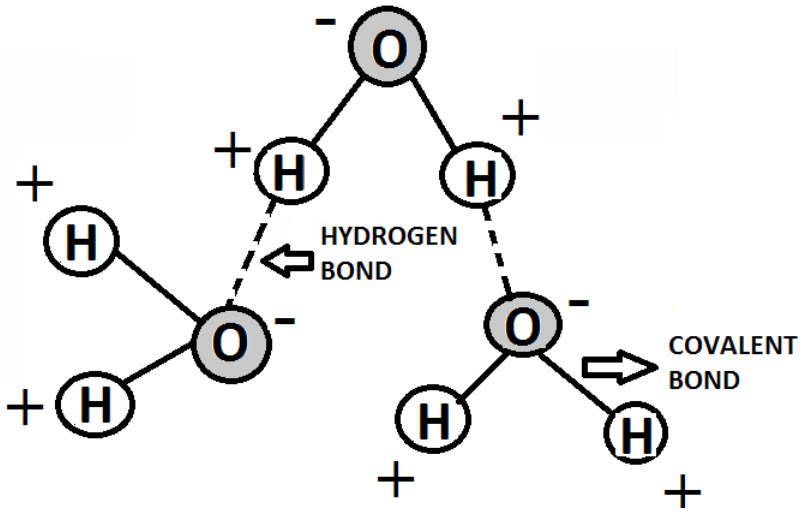


Fig 1.12 Different Bonds in Water Molecule

1.13.5 Properties of Water

The important properties of water are:

- It has the highest boiling point. It is due to the association of water molecules with one another.
- It has the highest melting temperature. This is due to the presence of strong inter molecular forces between multiple water molecules**.**
- It has the highest specific heat known of any substance. The specific heat of a substance is the amount of heat required to raise its temperature by 1°C. The amount of heat energy (calories) required to raise the temperature of one gram of water from 15°C to 16°C is known as specific heat of water.
- It has a high heat of vaporization. It is defined as the number of calories required to change one gram of liquid into vapour. Vaporization is commonly called evaporation. Vaporization is the change from a liquid to a gas. Water takes more than 500 calories to change a gram of water into vapour.
- Water has a very high latent heat of fusion or melting. It is expressed as the number of calories required to convert one gram of solid at freezing point into liquid at the same temperature. This has important inference because a large amount of heat is removed from water before it freezes.
- Water has a maximum density of 4°C. For this reason, water rarely freezes solid in the sea or in deep lakes even during the coldest weather. When the temperature of the deeper water decreases below 4°C, the water rises up

and ice forms on the surface. This tends to prevent the water below from being cooled down to the freezing point. As a result, the living organisms can continue living below the frozen surface of lakes and oceans. It also facilitates easier melting of the ice exposed to the atmosphere when the environmental temperature raises.

- Water has a very high surface tension. It is the force operating on the surface of water. Water will rise due to capillary action in a glass tube of about 0.03mm in diameter to a height of 120cm. This property enables water to move extensively through narrow cavities in the soil and in plant cell walls.
- Water has high viscosity. It helps the water to move through plants.
- Water as a solvent opposes the electrostatic attraction between positive and negative ions that would prevent ionic substances from dissolving. The dielectric constant denotes the ability to oppose attraction of unlike charges.
- Water allows light to penetrate deep into the tissues of leaves.
- Water is a universal solvent. It can dissolve more number of substances. Many substances present within living system occur in solutions of water.
- The polarity of the water molecules is responsible for its use as a solvent. The water molecules cluster around other molecules and so segregate the charged ions. Water molecules have a polar arrangement of oxygen and hydrogen atoms. On one side, (hydrogen) has a positive electrical charge and the other side (oxygen) has a negative charge. This allows the water molecule to become attracted to many other different types of molecules.

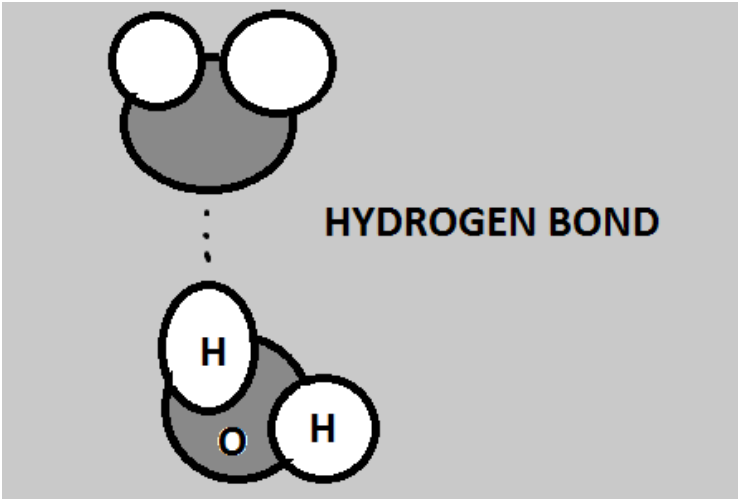


Fig.1.13 Water as a Solvent

1.14 HENDERSON-HASSELBALCH EQUATION

- The Henderson–Hasselbalch equation relates the pH of a solution containing a mixture of the two components to the acid dissociation constant, K_a and the concentrations of the ionic before species in solution.
- The Henderson–Hasselbalch equation can be used to estimate the pH of a buffer solution.
- Henderson-Hasselbalch Equation is important for understanding buffer action and acid-base balance in the blood and tissues of the mammalian system.
- The quantitative relationship among pH, buffering action of a mixture of weak and conjugate base and the pK_a of the weak acid is given by a simple expression called Henderson-Hasselbalch equation.
- This equation is simply a useful way of restating the expression dissociation constant of an acid. To derive the equation, a number of simplifying assumptions have to be made.
- We can write the equilibrium reactions for the dissociation of HA in the buffer solution as follows:

$$HA \longrightarrow H^+ + A^-$$

For the dissociation of a weak acid HA into H^+ and A^-, the Henderson-Hasselbalch equation is derived in the following way

$$K_a = \frac{[H^+]\ [A^-]}{[HA]}$$

1. Rearrange the K_a equation to solve for $[H^+]$

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

2. Convert to logarithmic functions

$$\log\ [H^+] = \log K_a + \log \frac{[HA]}{[A^-]}$$

3. Make the expression negative (or multiply by -1)

$$-\log\ [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

4. Substitute pH for $-\log\ [H^+]$ and pK_a for $-\log K_a$

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

5. Now, to remove the minus sign, invert the last term, i.e., - log $\frac{[HA]}{[A^-]}$ to obtain Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

The equation is expressed more generally as :

$$pH = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

This equation fits the titration curve of all weak acids and enables one to deduce a number of important quantitative relationships. Henderson-Hasselbalch equation is of great predictive value in protonic equilibria as illustrated below:

A. When $[A^-] = [HA]$ or when an acid is exactly half neutralized: Under these conditions,

$$pH = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{1} = pK_a + 0 = pK_a$$

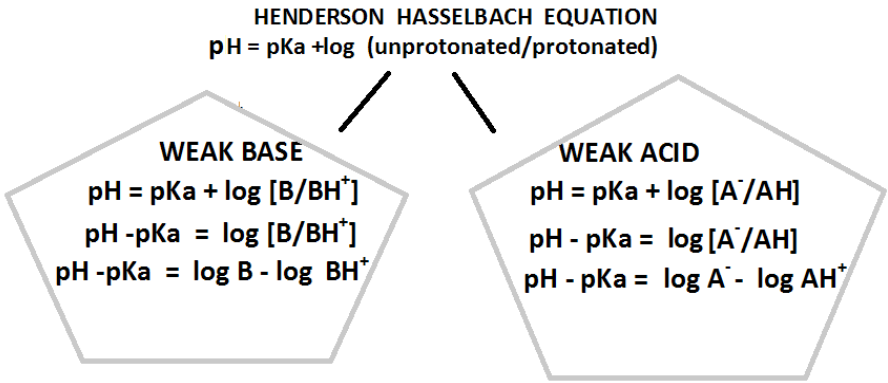
Therefore, at half neutralization $pH = pK_a$. The equation, thus, shows why the pK_a of a weak acid is equal to the pH of the solution at the midpoint of its titration.

B. When the ratio $[A^-]/[HA] = 100$ to 1:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{100}{1} = pK_a + 2$$

C. When the ratio $[A^-]/[HA] = 1$ to 10 :

$$pH = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{10} = pK_a + (-1)$$



1.15 BIOLOGICAL BUFFER SYSTEMS

Biological buffers are organic substances that maintain a constant pH over a given range by neutralizing the effects of hydrogen ions. In the body, buffers provide a pH environment conducive to critical biochemical processes.

- Almost every biological process is pH dependent. A small change in pH produces large changes in the rate of the process. Cells and organisms maintain a specific and constant cytosolic pH, keeping biomolecules in the optimal ionic state, usually near pH 7.
- In multicellular organisms, the pH of the extracellular fluid is also tightly regulated. Constancy of pH is achieved primarily by biological buffers: mixtures of weak acids and their conjugate bases. There are some important buffer systems of body fluids which helps for maintaining pH. A certain amount of many of these is usually present in the body and cellular fluids and so the maintenance of a constant pH depends on a system.

Body fluids	**Principal Buffers**
Extracellular fluids	Bicarbonate buffer Protein buffer
Intracellular fluids	Phosphate buffer Protein buffer
Erythrocytes	Haemoglobin buffer

1.15.1 The phosphate buffer system

- This system which acts in the cytoplasm of all cells, consists of $H_2PO_4^-$ as a proton donor and HPO_4^{2-} as proton acceptor
- $H_2PO_4^- \longrightarrow H^+ + HPO_4^{2-}$
- The phosphate buffer system works exactly like the acetate buffer system, except for the pH range in which it functions.
- The phosphate buffer system is maximally effective at a pH close to its pKa of 6.86 and thus tends to resist pH changes in the ranges between 6.4 and 7.4.
- It is therefore effective in providing buffering power in intracellular fluids.
- In case the ratio of $[HPO_4^{2-}] / [HPO_4^-]$ tends to be changed by the formation of more $H_2PO_4^-$, there occurs the renal elimination of $H_2PO_4^-$ for which the ratio ultimately remains unaltered.

1.15.2 The bicarbonate buffer system

- This is the main extracellular buffer system which also provide a means for the necessary removal of the CO_2 produced by tissue metabolism.
- The bicarbonate buffer system is the main buffer in blood plasma and consists of carbonic acid as proton donor and bicarbonate as proton acceptor and function as a buffer in the same way as other conjugate acid base pairs.

$$H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

This system has equilibrium constant

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

- It is unique, however, in that one of its components, carbonic acid, is formed from dissolved carbon dioxide and water, according to the reversible reaction.

$$CO_2 \ (d) + H_2O \longleftrightarrow H_2CO_3$$

which has an equilibrium constant given by the expression

$$K_2 = \frac{H_2CO_3^-}{[CO_2\ (d)]\ [H_2O]}$$

Carbon dioxide is a gas under natural conditions and the concentration of dissolved CO_2 is the result of equilibration with CO_2 of the gas phase(g)

$$CO_2\,(g) \longleftrightarrow CO_2\ (d)$$

This process has an equilibrium constant given by:

$$K_3 = \frac{[CO_2\ (d)]}{[CO_2\ (g)]}$$

- The pH of a bicarbonate buffer system depends on the concentration of H_2CO_3 and HCO_3 the proton donor and acceptor components.
- The concentration of H_2CO_3 in turn depends on the concentration of dissolved CO_2 which in turn depends on the concentration or partial pressure of CO_2 in the gas phase.
- With respect to the bicarbonate system a $[HCO_3^-] / [H_2CO_3]$ ratio of 20 to 1 required for the pH of blood plasma to remain 7.40. The concentration of dissolved CO_2 is included in the $[H_2CO_3]$ value i.e.,

$$[H_2CO_3] = [H_2CO_3] + [CO_2 \text{ (dissolved)}]$$

- If there is a change in the ratio in favour of H_2CO_3 acidosis results. This change can result from decrease in $[HCO_3^-]$ or from an increase in $[H_2CO_3]$. Most common forms of acidosis are metabolic or respiratory.
- Metabolic acidosis is caused by a decrease in $[HCO_3^-]$. For example uncontrolled diabetes with ketosis are a result of starvation.
- Respiratory acidosis is brought about when there is an obstruction to respiration or depression in respiration. If acidosis is not treated promptly the patient may go into comma.
- Alkalosis results when $[HCO_3^-]$ becomes favoured in the bicarbonate / carbonic acid rate. Metabolic alkalosis occurs when the HCO_3^- fraction increase with little or no concomitant change in H_2CO_3. Severe vomiting or ingestion of excessive amounts of sodium bicarbonate can produce this condition.
- Respiratory alkalosis is induced by hyperventilation because an excessive removal of CO_2 from the blood result in decrease in H_2CO_3.
- Alkalosis can produce convulsive seizures in children and tetany in adults.
- The pH of blood is maintained at 7.4 when the buffer ratio $[HCO_3^-]/[H_2CO_3]$ becomes 20. If bicarbonate neutralizes any acid or base, there may be change in buffer ratio and the blood value. But the buffer ratio remains by the respiratory elimination of H_2CO_3 as CO_2 or the urine elimination of HCO_3^-.
- Since cells contain much lower amount of HCO_3^- the importance of bicarbonate buffer inside the cell is negligible.

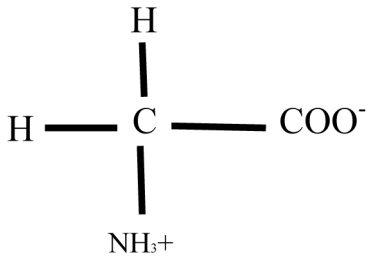
1.15.3 The protein buffer system

The protein buffers are very important in the plasma and the intracellular fluids but their concentration is very low in cerebrospinal fluid, lymph and interstitial fluids. The proteins exist as anions serving conjugate base (Pr) at the blood pH 7.4 and form conjugate acids (HPr) accepting H^+. They have the capacity to buffer some H_2CO_3 in the blood.

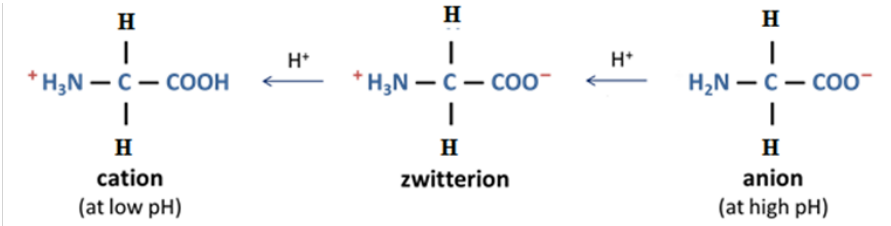
$$H_2CO_3 + Pr^- \longleftrightarrow HCO_3^- + HPr$$

1.15.4 The amino acids buffer system

- Amino acids contain both an acidic (COOH) and a basic ($-NH_2$) group.
- They can be visualized in the form of a neutralize zwitterions in which a hydrogen atom can pass between the carboxyl and amino groups. They may be represented as



- By the addition or subtraction of a hydrogen ion to form the zwitterions, either the cation or atom form will be produced.



- Thus, when OH ions are added to the solution of amino acid, they take up H^+ from it to form anion. If H^+ ions are added, they are taken up by the zwitterions to produce cation form.
- In practice, if NaOH is added, the salt NH_2 ---- CHR --- COONa would form and the addition of HCl would result in the formation of amino acid hydrochloride, ClH---NH_3---CHR---COOH but these substances would ionize in solution to some extent to form their corresponding ions, haemoglobin and plasma protein act as buffers in a similar way.
- Amino acids differ in the degree to which they will produce the cation or anion form. In other words, a solution of an amino acid is not neutral but is either predominantly acidic or basic, depending on which form is present in greater quantity. For this reason, different amino acids may be used as buffers for different pH values and a mixture of them possesses a wide buffer range.

1.15.5 The haemoglobin buffer systems

- These buffer systems are involved in buffering CO_2 inside erythrocytes. The buffering capacity of haemoglobin depends on its oxygenation and deoxygenation.
- Inside the erythrocytes CO_2 combines with H_2O to form a carbonic acid (H_2CO_3) under the action of carbonic anhydrase. At the blood pH 7.4, H_2CO_3 dissociates into H^+ and HCO_3^- and needs immediate buffering. Oxyhaemoglobin on the other side, losses O_2 to form deoxyhaemoglobin (Hb^-) which remains undissociated (HHb) by accepting H^+ from the ionization of H_2CO_3. Thus, Hb^- buffers H_2CO_3 in erythrocytes.

$$HbO_2^- \longleftrightarrow Hb^- + O_2$$

$$Hb^- + H_2CO_3 \longleftrightarrow HHb + HCO_3^-$$

- Some of the HCO_3^- diffuse out into the plasma to maintain the balance between intracellular and plasma bicarbonates. This causes influx of some Cl^- into erythrocytes along the electrical gradient produced by the HCO_3^- outflow.
- $HHbO_2$, produced in lungs by oxygenation of HHb, immediately ionizes into H^+ and HbO_2^-. The released hydrogen ions (H^+) are buffered by HCO_3^- inside erythrocyte to form H_2CO_3 which is dissociated into H_2O and CO_2 by carbonic anhydrase.
- CO_2 diffuse out of erythrocytes and escapes in the alveolar air. Some HCO_3 return from the plasma to erythrocytes in exchange of Cl and are changed to CO_2

$$HHb + O_2 \longleftrightarrow HHbO_2 \longleftrightarrow HbO_2^- + H^+$$

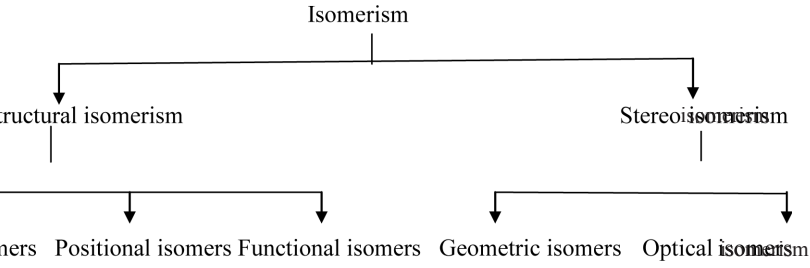
$$HCO_3^- + H^+ \longleftrightarrow H_2CO_3 \longleftrightarrow H_2O + CO_2$$

UNIT II

CARBOHYDRATES

Isomerism: Definition, Types – Structural isomerism and Stereoisomerism.

Carbohydrates: Introduction - Classification - Monosaccharides: Structure and Properties - Glucose and Fructose. Disaccharides: Structure and Properties – Sucrose, Maltose and Lactose. Polysaccharides: Structure and Properties – Homopolysaccharide: Starch, Cellulose, Glycogen and Chitin. Heteropolysaccharide: Hyaluronic acid, Chondroitin, Agar Agar, Heparin.


Isomerism
tructural isomerism
Stereoisomerism
ners
Positional isomers
Functional isomers
Geometric isomers
Optical isomers

2 CARBOHYDRATES

2.1 ISOMERISM

- Isomers are non-identical compounds with the same molecular formula but different arrangements of their atoms.
- Isomers have different physical and chemical properties but the differences may be great or small depending on the type of isomerism.

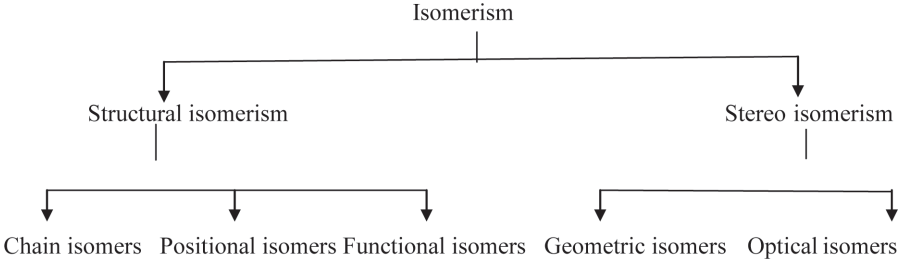


Fig 2.1 Types of isomerism

2.1.1 Structural Isomerism

Structural isomers have the same molecular formula with different structural arrangement of the atoms.

a. Chain isomers- have different arrangements of the carbon chain.

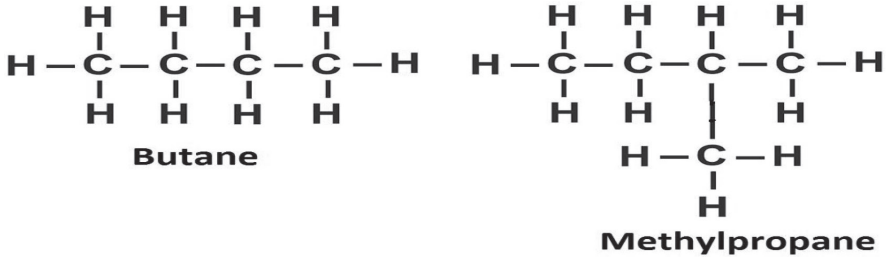


Fig 2.2 Chain isomers - C_4H_{10}

b. Positional isomers- have the same molecular formula but the functional group is in different positions.

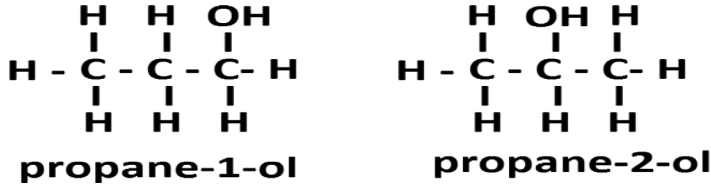


Fig 2.3 Positional isomers - C_3H_8

c. Functional isomers- have same molecular formula but different functional groups.

$H_3C - CH_2 - CH_2OH$ $H_3C - CH_2 - O - CH_3$

n- Propanal **Methyl-Ethyl-Ether**

Fig 2.4 Functional isomers - C_3H_8O

2.1.2 Stereo Isomerism

Stereo isomers have same molecular formula and the same structure but they differ in the arrangement of their atoms in space.

a. Optical isomers

This type of isomerism occurs when a molecule has one or more asymmetric carbon atoms (i.e) a carbon atom linked to four different atoms or groups. The optical isomers have a chiral centre.

i Aldose and Ketose isomers

In this type of isomerism, the two isomers have same molecular formula, same structure but differ in the carbonyl carbon. One contain aldehyde (CHO) group and the other contain keto (C=O) group.

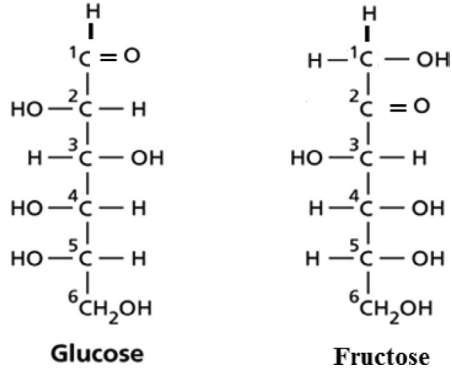


Fig 2.5 Aldose and Ketose isomers

ii D and L isomers

- A monosaccharide can be formed either in D form or L form, In D form. the hydroxyl group at the penultimate carbon (the carbon before the last carbon) is on the right side while in L form, the hydroxyl group at penultimate carbon is on the left side.
- The D and L forms are the mirror images of each other and they are known as **enantiomers.**

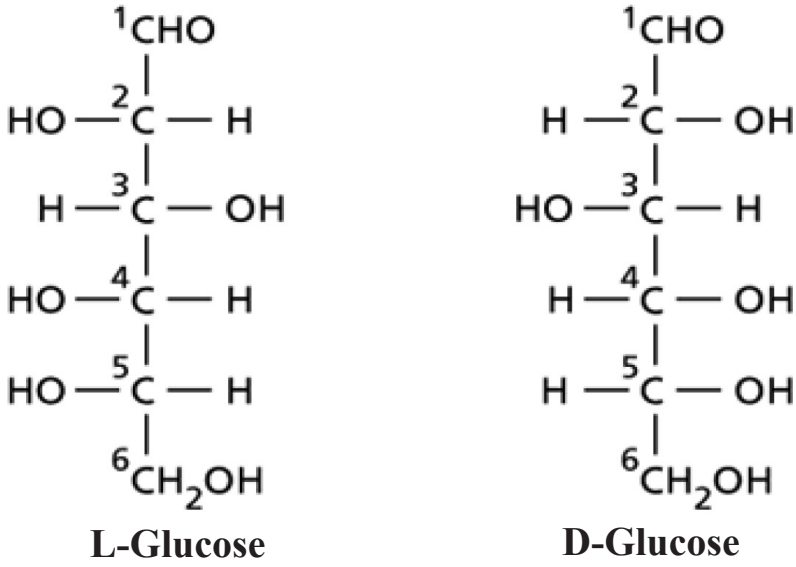


Fig 2.6 Enantiomers - $C_6H_{12}O_6$

iii Epimers

- Epimers differ in configuration around 1 carbon atom other than the carbonyl carbon and the penultimate carbon.
- Glucose and galactose have the same molecular formula $C_6H_{12}O_6$ but differ in the position of the hydroxyl group at C_4. Glucose has OH group at C_4 on the right side while galactose has OH group at C_4 on the left side. So glucose and galactose are C_4 epimers.
- Glucose and mannose have the same molecular formula $C_6H_{12}O_6$ but differ in the position of the hydroxyl group at C_2. Glucose has OH group at C_2 on the right side while mannose has OH group at C_2 on the left side. So glucose and mannose are C_2 epimers.

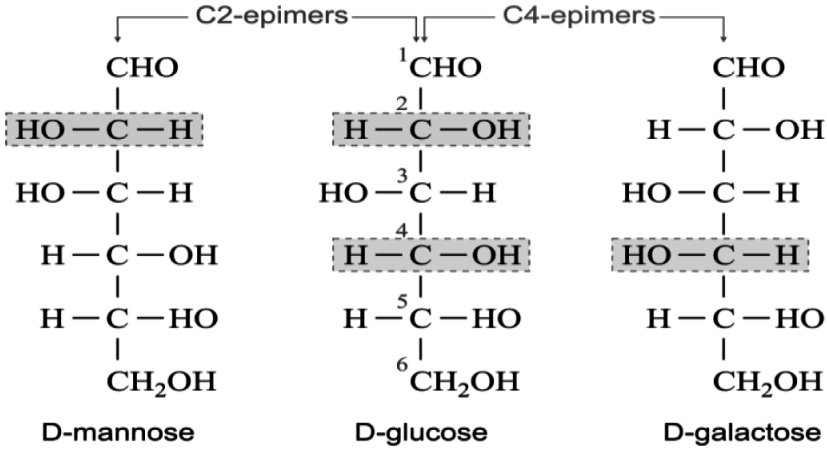


Fig 2.7 Epimers - $C_6H_{12}O_6$

iv α and β isomers

Monosaccharide that differs in configuration only around the carbonyl carbon in cyclic structure is known as **α and β isomers.** In α form, the hydroxyl group attached to the carbonyl carbon is on the right side. In β form, the hydroxyl group attached to the carbonyl carbon is on the left side.

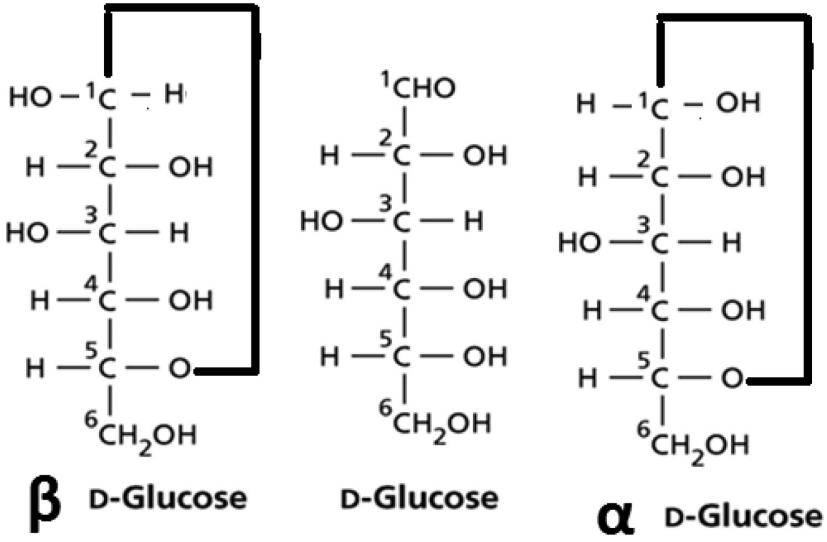


Fig 2.8 α and β isomers - $C_6H_{12}O_6$

b. Geometric isomers

Geometric or cis-trans isomers exist because of the π band of the C=C bond prevents free rotation.

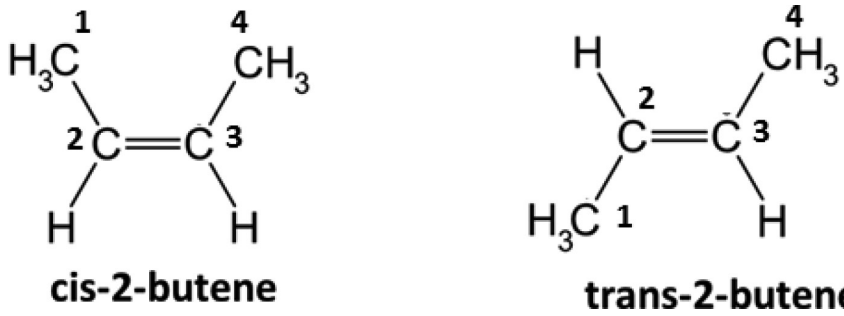


Fig 2.9 Geometric isomers - C_4H_8

2.2 CARBOHYDRATES

- Carbohydrates are the most abundant organic molecules in nature.
- They are composed of the elements carbon, hydrogen and oxygen.
- The name carbohydrate literally means "hydrates of carbon".
- There are several non-carbohydrate compounds (acetic acid, $C_2H_4O_2$; lactic acid, $C_3H_6O_3$) which also appear as hydrates of carbon.
- The general formula for carbohydrate is $(CH_2O)_n$ but, some of the carbohydrate (rhamnohexose, $C_6H_{12}O_5$; deoxyribose $C_5H_{10}O_4$) do not satisfy the general formula.
- Hence, carbohydrates cannot be always considered as hydrates of carbon. The usage of the term carbohydrates is for convenience rather than exactness.
- They are commonly known as saccharides or sugars and are widely distributed in both plants and animals.
- The carbohydrates are defined as polyhydroxy aldehydes or ketones and their derivatives or as substances that yield one of these compounds on hydrolysis.

2.3 CLASSIFICATION OF CARBOHYDRATES

- Carbohydrates are classified into three major groups namely monosaccharides, oligosaccharides and polysaccharides.

2.4 MONOSACCHARIDE

- Monosaccharide is the simplest group of carbohydrates and referred to as simple sugars.
- They have the general formula $C_n(H_2O)_n$.

- They cannot be further hydrolyzed.
- Most of the monosaccharide have the names with suffix as -ose (e.g.) glucose, fructose, mannose etc.

2.4.1 Some Important Monosaccharide

D Glyceraldehyde	-	Simplest sugar
D Glucose	-	Most important in diet
D Fructose	-	Sweetest of all sugar
D Galactose	-	Part of milk sugar
D Ribose	-	Used in RNA

2.4.2 Functional Group

- Monosaccharide contains either a free aldehyde or keto group in their structure. The backbone of monosaccharide is made up of linear single bonded carbon chain. One of the carbon atoms in this backbone contains double bond and an oxygen atom is called as carbonyl carbon atom.
- If the carbonyl carbon is placed at any one end of the backbone, it becomes an aldehyde group.
- If the carbonyl carbon is placed at any other position of the backbone, it becomes a keto group.
- This carbonyl group is acting as functional group of all carbohydrates.

2.4.3 Classification of Monosaccharide

- Based on the functional group and the number of carbon atoms, the monosaccharide are divided into different categories.

Table 2.1 CLASSIFICATION OF MONOSACCHARIDES

Monosaccharides	No. of carbon atoms	Molecular formula	Examples	
			based on aldehyde group	based on ketone group
Trioses	3	$C_3(H_2O)_3$	Glycerose	Dihydroxyacetone
Tetroses	4	$C_4(H_2O)_4$	Erythrose	Erythrulose
Pentoses	5	$C_5(H_2O)_5$	Ribose	Ribulose
Hexoses	6	$C_6(H_2O)_6$	Glucose	Fructose
Heptoses	7	$C_7(H_2O)_7$	Glucoheptulose	Pseudoheptulose

Trioses

- Trioses are monosaccharides containing three carbon atoms.
- The molecular formula of triose is $C_3H_6O_3$.
- The triose containing an aldehyde group is called aldotriose.

  E.g. Glycerose or Glyceraldehyde.
- The triose containing a ketone group is called ketotriose or triulose.

  E.g. Dihydroxyacetone.

Tetroses

- Tetroses are monosaccharides containing 4 carbon atoms.
- The molecular formula of tetrose is $C_4H_8O_4$.
- The tetrose containing an aldehyde group is called aldotetrose and the tetrose containing a keto group is called ketotetrose.

  E.g. Aldotetrose – Erythrose

  Ketotetrose – Erythulose.

Pentoses

- Pentoses are monosaccharides containing 5 carbon atoms.
- The molecular formula of pentose is $C_5H_{10}O_5$.
- The pentose containing an aldo group is called aldopentose. Eg. Ribose.
- The pentose containing a keto group is called ketopentose or pentulose. Eg. Ribulose.

Hexoses

- Hexoses are monosaccharides containing 6 carbon atoms.
- The molecular formula of hexose is $C_6H_{12}O_6$.
- The hexose containing an aldehyde group is called an aldohexose and hexose containing a ketone group is called a ketohexose or hexulose.

  E.g. Aldohexose – Glucose
  Ketohexose – Fructose

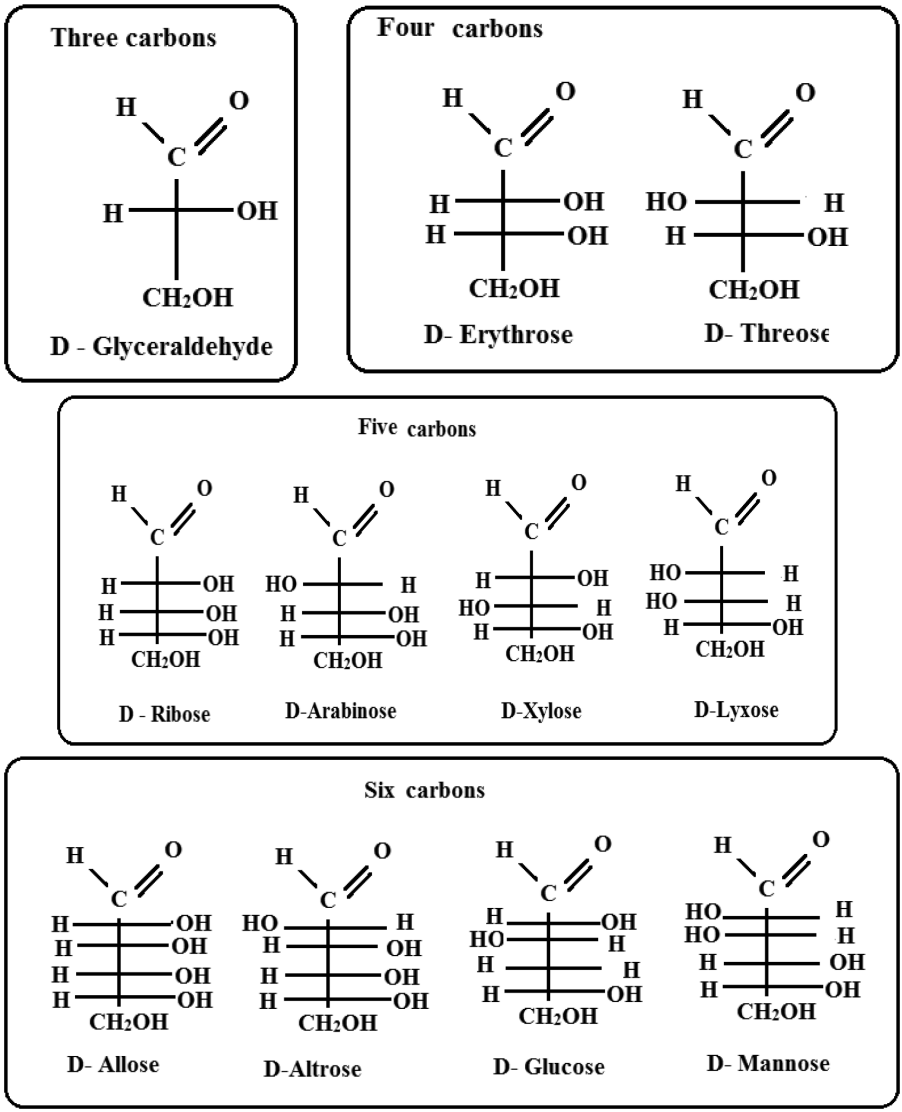


Fig 2.10 Example of D-Aldoses

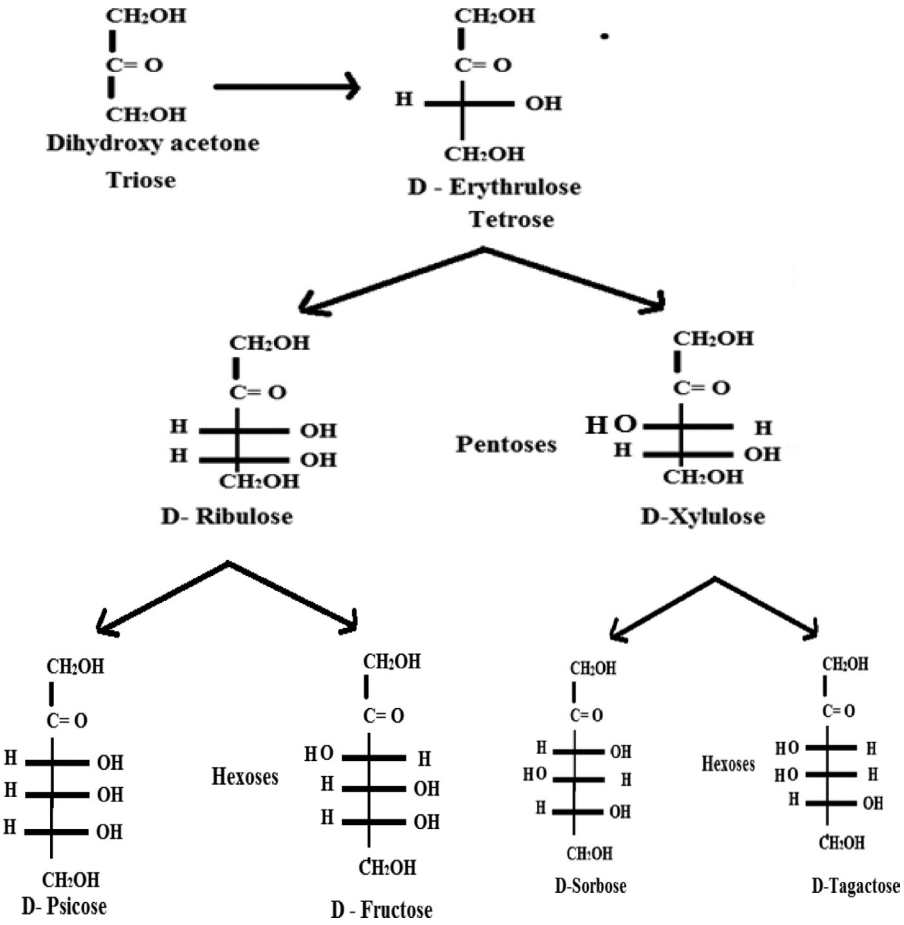


Fig 2.11 Ketoses containing three, four, five, six carbon atoms

2.5 STRUCTURE OF GLUCOSE

Of all the monosaccharide, glucose is an important one. It is the only sugar present in the blood and acts as the source of energy in all living organisms. Structurally glucose exists in two forms.

1. Straight chain structure
2. Ring structure
   a) Hemiacetal ring structure
   b) Haworth projection formula (Pyranose and Furanose Structure)
   c) Chair and boat configurations.

2.5.1 Straight Chain Structure of Glucose

- The molecular formula of glucose ($C_6H_{12}O_6$) was first written by Fitting and Baeyer in 1886.
- This linear form of glucose tells us the presence of an aldehyde group and 5 hydroxyl groups. Later, straight chain structure was demonstrated by Kiliani.
- After 10 years, Emil Fischer succeeded in assigning the spatial relationship of C atoms in the glucose molecule and proved that certain C atoms in glucose molecules are asymmetric.
- Since glucose is a hexose sugar, its structural back bone is made up of 6 carbon atoms which are linked with each other by single bonds.
- In its structure, the first C atom represents aldehyde group and hence glucose is aldohexose. The rest of the C atoms have H and OH groups attached to each.

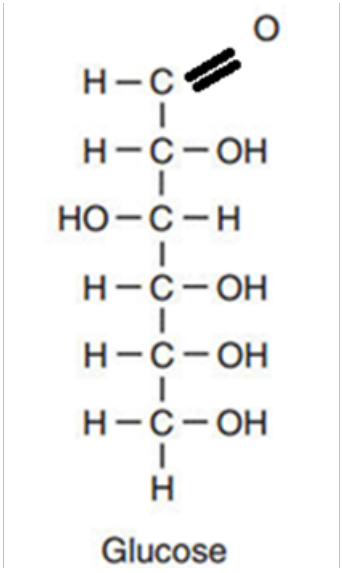


Fig 2.12 Ketoses containing three, four, five, six carbon atoms

- Of the 6 carbon atoms in the structure of glucose four are asymmetric in nature. C atoms present in positions 2, 3, 4 and 5 are attached with 4 different atoms or groups of atoms at their valency bonds.
- We have already seen that, compounds having asymmetric C atoms are optically active and exist in more than one forms called isomers and the possible number of isomers can be calculated by applying Van 't Hoff rule formula : 2^n=I.

- In glucose, 4 asymmetric C atoms are present at positions 2, 3, 4 and 5. So 16 isomers (2^4=16) are possible for the glucose and of these 16 isomers, 8 belonging to the D series and the rest of the 8 belong to the L series.
- These isomers are called as stereoisomer or space isomers since they differ only in the spatial arrangement of H and OH groups.
- In the D series isomers, the OH group at the fifth C atom is on the right side whereas in the L series isomers, the OH group at the fifth C atom is on the left side. In the glucose structure, fifth C atom is the most distant or farthest C atom from carbonyl group (i.e.) CHO group.

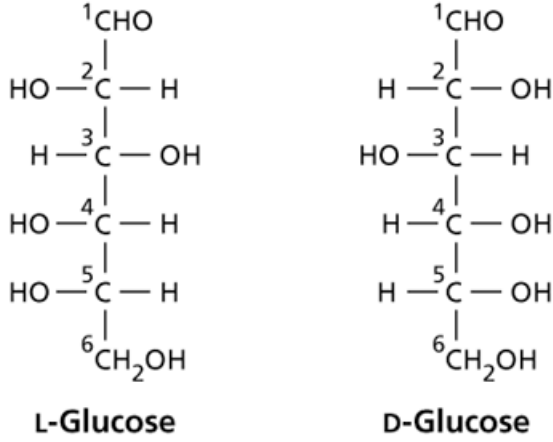


Fig 2.13 Straight Chain Structure of Glucose

2.5.2 Ring Structure of Glucose

- When the straight chain structure of glucose is failed to explain certain other properties Fischer again proposed ring structure of glucose.
- According to him in the solid form glucose is in straight chain structure. But when it is dissolved in solution, it attains ring structure.
- In the natural state, the aldehyde or ketone groups of the monosaccharides with more than five carbon atoms exist in a condensed form such as a hemiacetal or hemiketal.
- This is formed by the condensation of aldehyde group or keto group with any one of the alcoholic groups in the same molecule. This intra molecular hemiacetal formation results in a ring structure called as hemiacetal or hemiketal ring structure.
- Hemiacetal ring structure is either a five membered ring with 4-C atoms and one oxygen atom or a six membered ring with 5-C atom and one oxygen atom.

- There are 2 types of hemi acetal ring structures

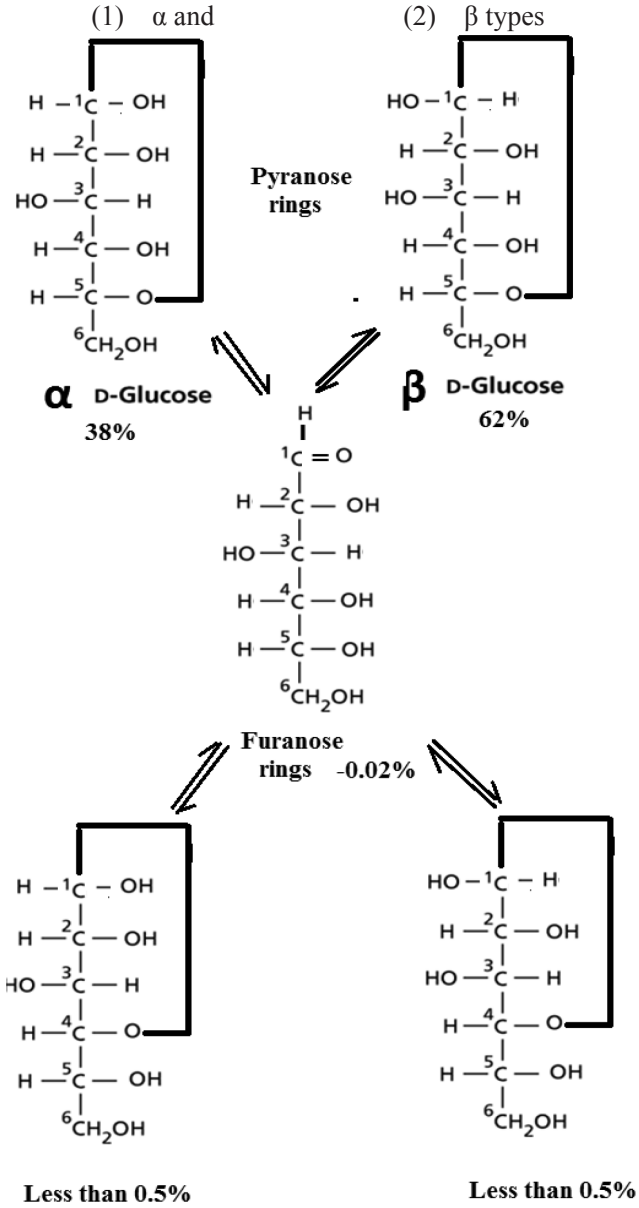


Fig 2.14 Hexoses

- When the H atom of the participating OH group at fifth carbon atom catches the carbonyl oxygen of the rotating aldehyde group and forms the OH group at C-1 at the same side of the ring formation, then it is said to be α form of hemi acetal ring structure of glucose.

- β glucose is formed when the H atom of participating OH at C-5 catches the carbonyl oxygen of the rotating aldehyde group at the opposite side of the ring.
- During this process C-1 of the carbonyl group becomes asymmetric, because now it has four different groups of atoms attached to it. Now the aldehyde group H-C=O is changed to H-C-OH.
- The α and β forms of the sugars are called as "anomers". The α form is called as α anomer and the β form is called as β anomer. The C-1atom which has become asymmetric during the formation of α and β anomers is called as anomeric carbon atom.

2.6 HAWORTH PROJECTION FORMULA OR PYRANOSE AND FURANOSE

- Although Fischer formula are useful in indicating configuration differences among sugars they are difficult to write so, Haworth in 1929 proposed a scheme in which all sugars forming 6-membered rings are called pyranose from their relation to pyran, a chemical compound which possess same 6-membered ring structure composed of 5-carbons and one oxygen.
- The sugars those forming 5 membered rings are called as furanose after furan a chemical compound which possesses similar 5-membered ring with 4-C atoms and one oxygen atom.

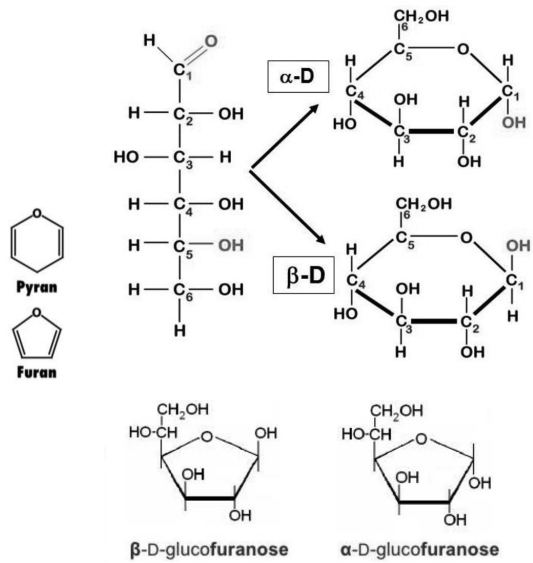


Fig 2.15 Ring Structure of Glucose

- Sugars in pyranose form appear to be most stable than furanose form which is transitory and unstable but the furanose forms are chemically more active than corresponding pyranose forms.
- A pyranose sugar is usually called as hexagon and furanose sugar as pentagon. The ring is considered to be at right angles to the plane of the paper.
- Usually, individual sugars are described by prefixing the main part of their common name to pyranose or furanose.
- For E.g. Glucose in pyranose form is called as glucopyranose and glucose in furanose form is called as glucofuranose.

BOAT AND CHAIR CONFIGURATIONS OF GLUCOSE

- It is otherwise called as "Conformational structures". Even Haworth ring formation does not fully satisfy the requirements for the stability of such molecules.
- The molecules will be more stable if the maximum number of hydroxyl groups is in the equatorial plane, directed outwards, but almost in the same plane as the ring structure.
- This gives the molecule, a structure more resembling "chair" or "boat" configuration. Of these two forms, chair form is more stable.

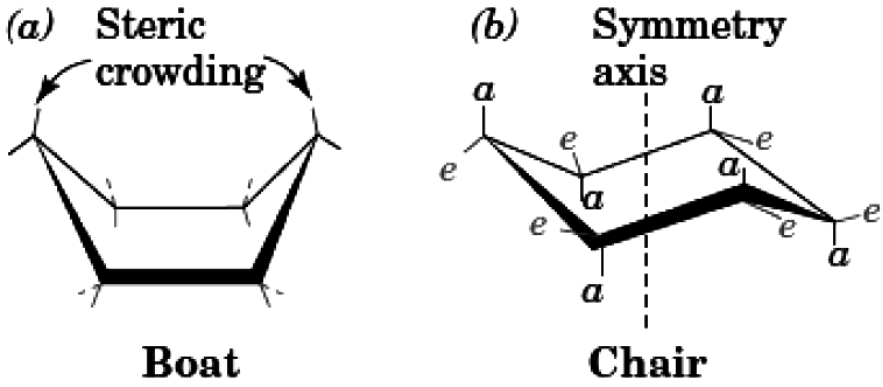


Fig 2.16 Boat and Chair Configurations of Glucose

2.7 PROPERTIES OF MONOSACCHARIDES

2.7.1 Physical Properties

1. **Colour:** Monosaccharides are colourless.
2. **Shape:** They are crystalline compounds.
3. **Solubility:** They are readily soluble in water.
4. **Taste:** They have sweet taste.
5. **Optical Activity:** When an ordinary light beam (which consists of a bundle of electromagnetic waves vibrating in all directions perpendicular to the axis of the beam) is passed through a specially cut crystal of Iceland spar (transparent calcite, $CaCO_3$) or through a special plastic sheet called Polaroid, all vibrations except those in one plane are eliminated. This is called plane polarized light. When such a beam of plane polarized light is passed through a solution exhibiting optical activity, the plane polarized light will be rotated to the right or to the left in accordance with the type of optical isomer present in the solution. This phenomenon is called as optical isomerism. A compound which causes the rotation of plane polarized light to the right is said to be dextrorotatory (d) and a plus (+) sign is used to designate the fact. Rotation of the beam to the left side is said to be levorotatory (l) and is designated by the sign minus (-).When equal amounts of dextrorotatory and levorotatory isomers are present in the solution, the resulting mixture does not show any optical activity, since the activity of each isomer cancel one another. Such a mixture is said to be "racemic" or dl – mixture" or "± mixture".
6. **Mutarotation:** A **change in optical rotation** is called "**Mutarotation**". This is due to the change of one ring form to another ring form (i.e.) α to β form and vice versa. The optical rotation of freshly prepared α - D - glucose is + 112^0. After some time the rotation decreases and finally reaches a steady value of + 52.5^0. Like that, optical rotation of freshly prepared β - D glucose is + 19^0. After some time the optical rotation gradually increases and finally reaches a steady value of + 52.5^0.

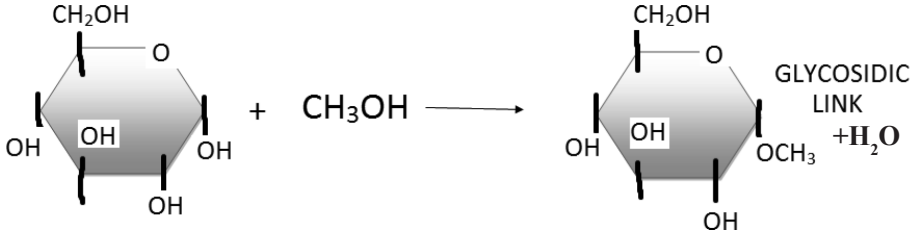
$$+ 112^0 \rightarrow + 52.5^0 \leftarrow + 19^0$$

α D glucose steady value at equilibrium stage β - D - glucose.

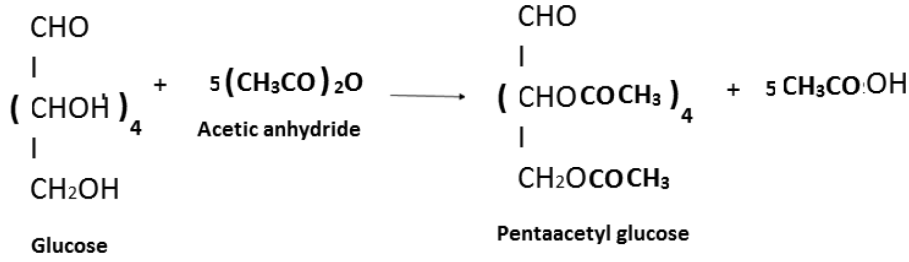
This change in the optical rotation is called as mutarotation and is mainly due to the formation of an equilibrium mixture at 52.5^0 which consists of about one - third of α- D glucose, 2/3 of β - D glucose and very small amount of open chain compound. This mechanism shows that all monosaccharides exist in three forms in nature. Those are α - form, β - D form and aldehyde form. Of these forms, aldehyde form is unstable. Mutarotation is mainly catalyzed by the addition of hydroxyl or hydrogen ions.

2.7.2 Chemical Properties

7. **Glucoside formation:** Glucose reacts with methyl alcohol in the presence of hydrogen chloride gas to give glucosides. Glucoside formation is due to the reaction of alcohol with the glycosidic OH of monosaccharides. In the same way, fructose forms fructosides.



8. **Acetylation:** Glucose reacts with 5 molecules of acetic anhydride to form penta acetyl derivative. As glucose yields a penta acetate derivative on acetylation, it obviously contains five OH groups.



9. **Reduction:** Monosaccharides can be reduced by various reducing agents. The reduction is due to the presence of CHO or CO group. On reduction, they yield alcohols. When sodium amalgam is used, glucose yields sorbitol (glucitol), mannose yields mannitol, galactose yields dulcitol and fructose yields a mixture of sorbitol and mannitol.

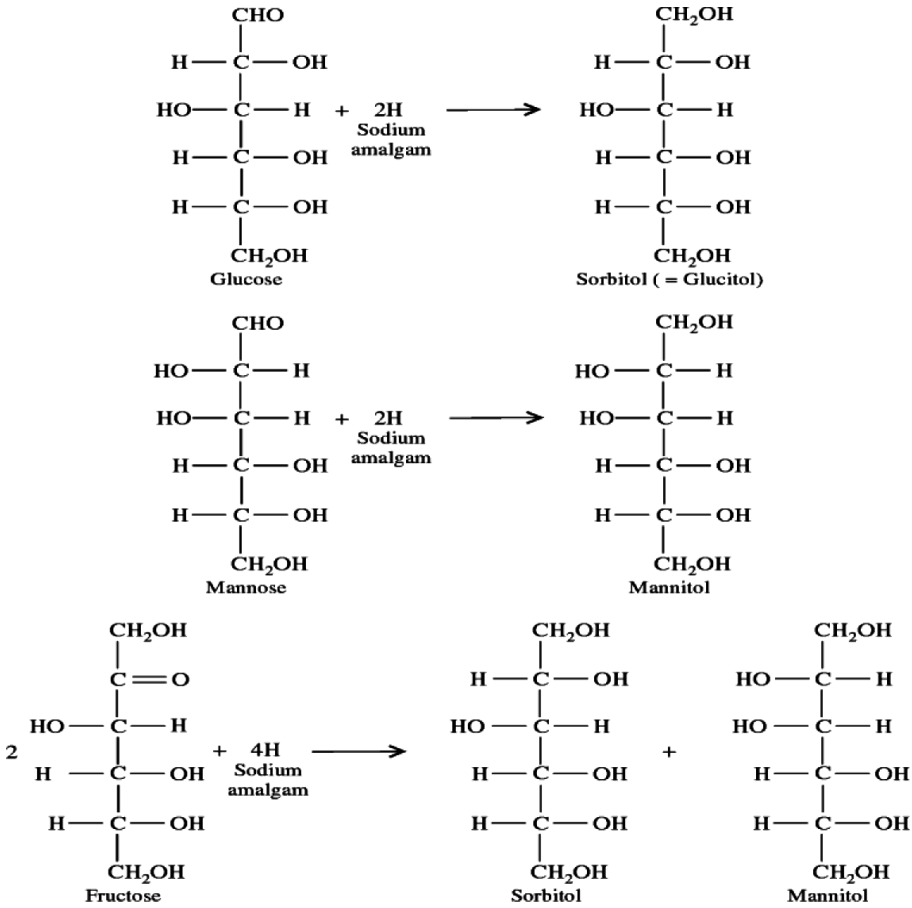
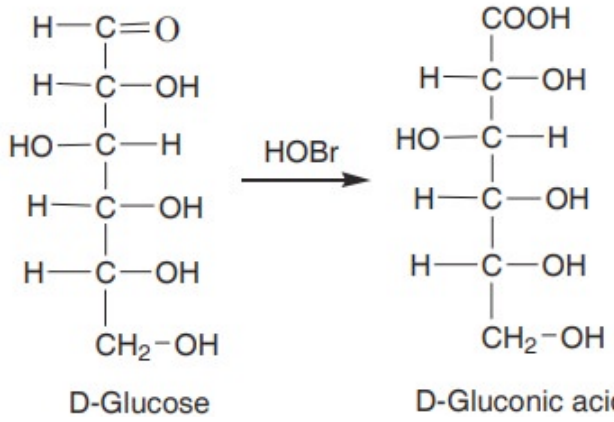


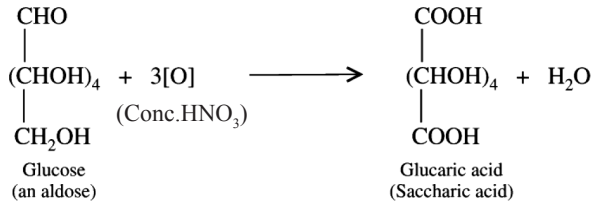
Fig 2.17 α and β D- fructose

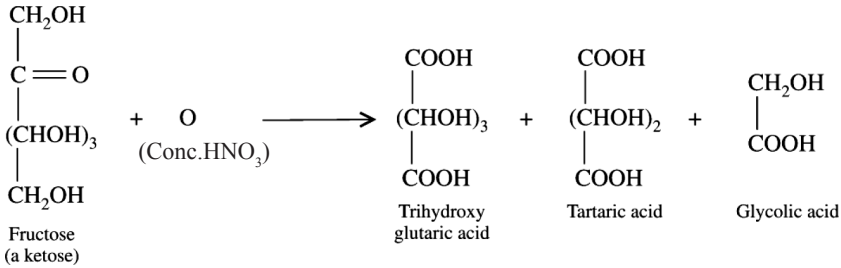
10. **Reducing agents (oxidation):** Monosaccharides act as best reducing agents. They readily reduce oxidizing agents such as ferricyanide, hydrogen peroxide or cupric ion. In such reaction, the sugar is oxidized at the carbonyl group and the oxidizing agent becomes reduced. Glucose and other sugars capable of reducing oxidizing agents are called reducing sugars. This property is useful in the analysis of sugars. Glucose reduces Tollen's reagents Fehling's solution, Benedict's reagents etc. At the same time glucose is oxidized to gluconic acid.

    a) **With mild oxidants (like HOBr):** Only the aldehyde group is oxidized to produce monocarboxylic acids. Ketoses do not respond to this reaction. Hence, this reaction is used to distinguish aldoses from ketoses.

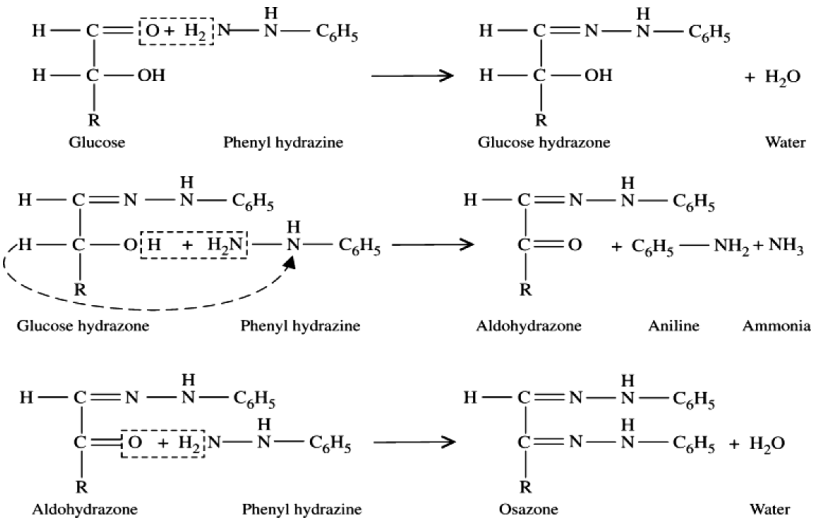


b) With strong oxidants (like Conc. HNO_3): Both the aldehyde group (or ketone group) and the primary alcohol group are oxidized to yield dicarboxylic acids.

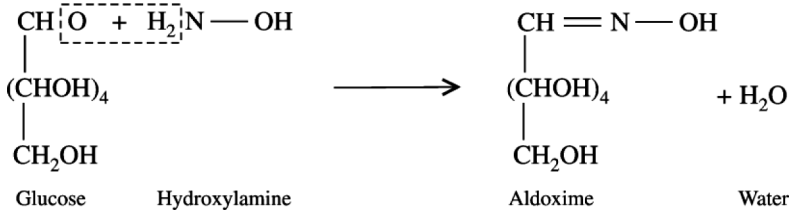




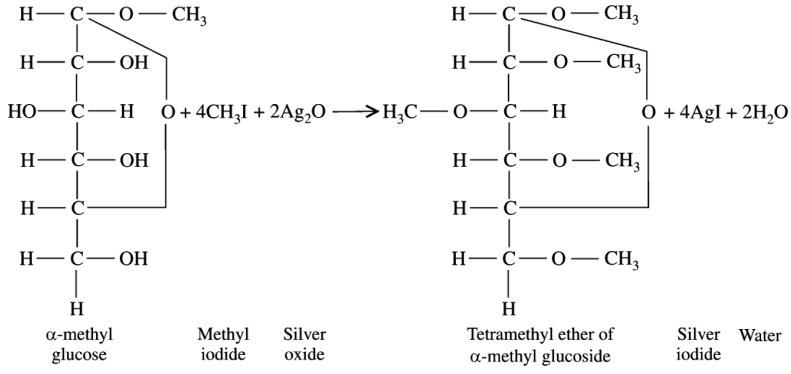
11. **Formation of Osazone:** Aldoses and ketoses react with phenylhydrazine. Glucose consumes 3 molecules of phenylhydrazine and produces osazone, aniline and ammonia. Reaction with phenylhydrazine involves only 2 carbon atoms, namely the carbonyl carbon atom (the aldehyde or ketone group) and the adjacent one. First of all, one molecule of phenylhydrazine reacts with one molecule of aldose or ketose to form a molecule of hydrazone. With a second molecule of phenylhydrazine, the hydrazone is oxidised to aldohydrazone and the phenylhydrazine itself is reduced to aniline and ammonia. Finally, a third molecule of phenylhydrazine reacts with aldohydrazone to produce Osazone.



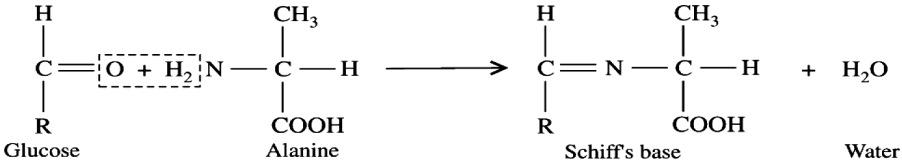
12. **Formation of Oximes:** Aldose and ketose react with hydroxylamine to form oximes.



13. **Reaction with methyl iodide (Etherification):** The alcoholic OH groups of monosaccharides are converted to ether groups upon treatment with methylating agents.



14. **Reaction with alanine:** The aldehyde group of carbohydrates condenses with the amino group of alanine to form Schiff's base.



15. **Reaction with Hydrogen cyanide:** When hydrogen cyanide is added to sugars, cyanohydrin is produced.

Glucose + Hydrogen cyanide → Glucose cyanohydrin

16. **Caramelization:** When monosaccharide is added with concentrated alkali, it is burnt and this process is called Caramelization.

17. **Fermentation:** Glucose gives ethyl alcohol and CO_2 during fermentation by Zymase.

Zymase

Glucose ----------→ Ethyl alcohol

2.8 STRUCTURE OF FRUCTOSE

2.8.1 Occurrence

- Fructose was discovered by French chemist Augustin-Pierre Dubrunfaut in 1847.
- The name Fructose was coined in 1857 by the English chemist William Miller.
- Fructose is found in trees, honey, vine fruits, flowers, berries and most root vegetables.
- It is often bound with Sucrose to form a disaccharide.
- Commercially this sugar has been derived from corn, sugarcane and sugar beets.

2.8.2 Structure

- Fructose (fruit sugar) is a simple, levorotatory monosaccharide (counterclockwise rotation of plane polarized light).
- It has the same molecular formula ($C_6H_{12}O_6$) as glucose but with a different structural arrangement of atoms.
- It is an isomer of glucose.
- Like glucose, fructose is a hexose (six carbons) sugar, but it contains a keto group instead of an aldehyde group, making it a Ketohexose.

- In straight chain structure, six carbon atoms are arranged in a chain. The two end carbons have primary alcoholic groups. The 2nd carbon is a keto group. The other three carbons 3, 4, 5 have secondary alcoholic groups.

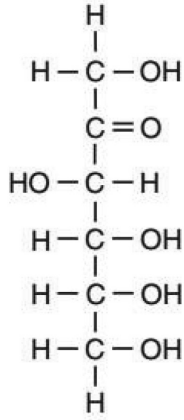


Fig 2.18 Open Chain Structure of Fructose

- Fructose can also exist in ring form. Its open-chain structure is able to cyclize (form a ring structure) because a ketone can react with an alcohol to form a hemiketal.
- Specifically, the C-2 keto group of a fructose molecule can react either with its C-6 or C-5 hydroxyl groups to form an intra molecular hemiketal.

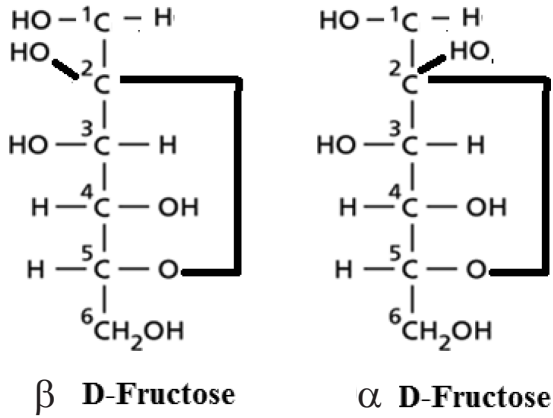


Fig 2.19 α and β D- fructose

- Fructose may form a six membered ring called a pyranose and five membered ring called a furanose.

- In solution, fructose exists as a mixture of 70% fructopyranose and about 22% fructofuranose.

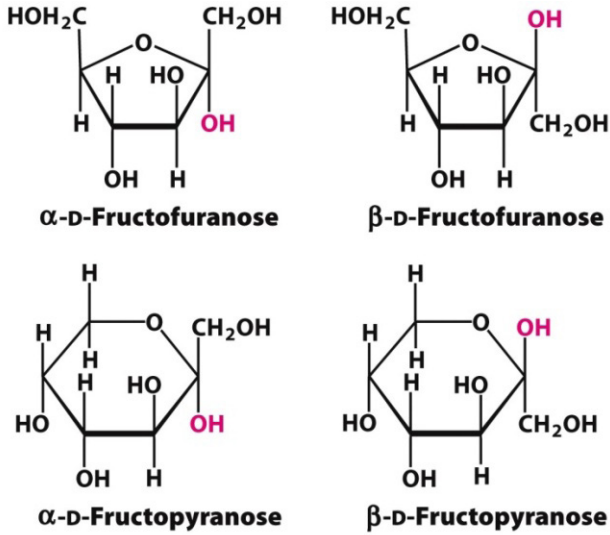


Fig 2.20 Pyranose and Furanose Ring Structure of Fructose

2.8.3 Properties

- It is a sugar with crystalline state and sweet taste. It is highly soluble in water.
- It occurs in plants and honey in the form of oligosaccharides and polysaccharides. It is found in free state in the seminal plasma.
- It is a reducing sugar. It reduces Tollen's reagent and Fehling's solution.
- It is an optically active compound. It is levorotatory. So it is called as levosugar. Its specific rotation is -92^0.
- It plays an important role in cellular metabolism. In the intestine and liver, fructose is converted into glucose and then used by the organs.

2.9 DISACCHARIDES

- Disaccharides are formed by the union of 2 monosaccharides. In other words, disaccharides yield 2 monosaccharides on hydrolysis.
- In general, these are colourless, crystalline substances, sweetish to taste, readily soluble in water and easily hydrolysed by enzymes and dilute mineral acids.

- The common disaccharides have the general formula $C_{12}H_{22}O_{11}$. During hydrolysis, they take up one molecule of H_2O to form 2 hexoses.

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

- During the formation of disaccharide from 2 molecules of monosaccharides an intermolecular dehydration occurs.

$$C_6H_{12}O_6 + C_6H_{12}O_6 \rightarrow C_{12}H_{22}O_{11} + H_2O$$

Hexose + Hexose Disaccharide + Water

- The 2 monosaccharides are united between the first carbon of one monosaccharide with the second or the fourth carbon of another monosaccharide by a glycosidic linkage. Since C – 1 is the carbonyl carbon atom i.e., functional group, reducing property of at least one hexose unit is lost. Hence disaccharides are considered as glycosides in which both components are sugars. Disaccharides may be of two types, reducing and non – reducing.

GLYCOSIDIC LINKAGE

- A sugar molecule can combine with an identical or a different type of a sugar molecule. The linkage between two monosaccharide sugar molecules is called glycosidic linkage or glycosidic bond.
- A sugar molecule has several reactive hydroxyl groups. The hydroxyl group of number one carbon atom (C-1) is known as the glycosidic hydroxyl group. It is very reactive and readily forms a glycosidic linkage with a hydroxyl group of another sugar molecule.
- When such a linkage occurs, one hydrogen atom and one hydroxyl group are eliminated to form H_2O. This process is dehydration synthesis. The reverse process with the incorporation of the elements of water is called hydrolysis.
- A linkage between C – 1 of one monosaccharide unit and C – 4 of another is called the 1, 4 linkage. Such linkage occurs in disaccharides and in the unbranched chains of polysaccharides.
- The 1, 4 linkage between two hydroxyl groups in the α positions is called α - 1, 4 linkage (e.g., maltose). Similarly a linkage between two β hydroxyl groups is called as β – (1 $\rightarrow$ 4) linkage (e.g., in lactose).
- Linkage can also takes place between C – 1 of one monosaccharide unit and C – 6 of another. Such a linkage occurs at the point of branching of a chain and is known as (1 $\rightarrow$ 6) linkage (e.g., in isomaltose, starch and glycogen).

Table 2.1 DIFFERENCE BETWEEN REDUCING AND NON-REDUCING SUGAR

Reducing sugar	Non – Reducing sugar
1. Carbohydrates with a free aldehyde (at C-1) or a free ketone (at C-2) group. 2. They are in hemiacetal or hemiketal form. 3. They exhibit mutarotation. 4. They form osazones with phenyl hydrazine. 5. They form oximes with hydroxylamine. Examples – Glucose, Fructose, Lactose, Maltose, Cellobiose	1. Aldehyde or ketone group is not free but instead utilized in bond formation. 2. They are in acetal or ketal form. 3. They do not exhibit mutarotation. 4. They do not form osazones 5. They do not form oximes Examples – Sucrose, Glycogen, Inulin.

2.10 SUCROSE (CANE SUGAR)

2.10.1 Occurrence

- Sucrose is the common sugar of commerce and kitchen and is widely distributed in all photosynthetic plants.
- It is otherwise called as "table sugar" or "household sugar".
- It occurs in very large amounts in sugar cane, beet root, pineapple, maple fruits, carrot, sweet potato and honey.
- Nectar of flowers is rich in sucrose.
- It is also present in varying amounts in different plant organs such as fruits, seeds, flowers and roots.

2.10.2 Structure

- It is a disaccharide composed of one molecule of α - D - glucopyranose and one molecule of β - D fructofuranose which are condensed together by α (1 --> 2) glycosidic linkage.
- The linkage exists between aldehyde group of glucose and keto group of fructose. As a result, no reducing group remains free in sucrose molecule.

- Since the functional groups of both glucose and fructose are blocked in the linkage, this disaccharide is called as non- reducing sugar.

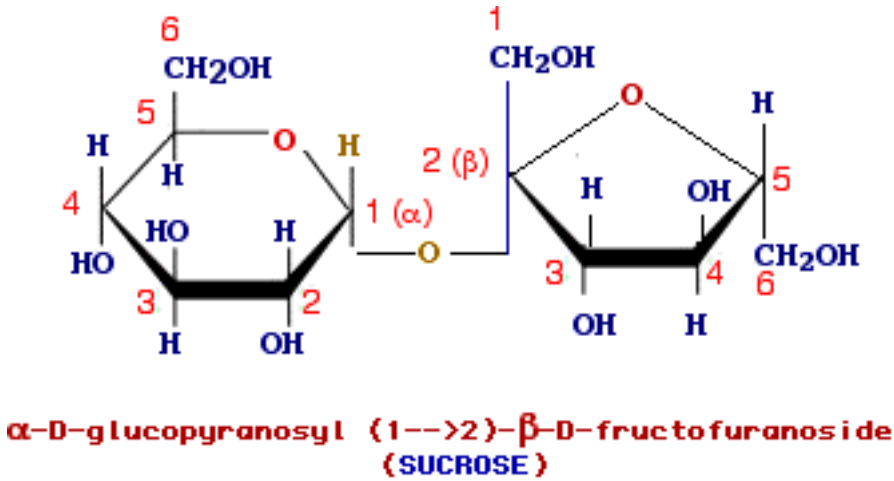


Fig 2.21 Structure of Sucrose

2.10.3 Properties

- It is white crystalline solid, soluble in water with a melting point of 180^0. When heated above its melting point, it forms a brown substance known as Caramel.
- It is the sweetest of 3 common disaccharides (maltose, lactose and sucrose). It is also sweeter than glucose.
- It has no free aldehyde or keto group because the linkage is between aldehyde group of glucose and keto group of fructose. Hence, it is a non-reducing sugar.
- It does not exhibit mutarotation and cannot exist in α and β forms.
- Since it is a non-reducing sugar, it does not reduce Fehling's and Benedict's solutions. It can't reduce Barfoed's solution. It cannot form osazones with phenyl hydrazine.
- Sucrose is dextrorotatory and its specific rotation is + 66.7^0.
- On hydrolysis, it gives one molecule of glucose and one molecule of fructose. Since, fructose is more strongly levorotatory than the dextrorotatory glucose, the mixture (product) after hydrolysis will become

levorotatory. This reaction is known as "inversion of sugar". Because the dextrorotatory cane sugar is converted into levorotatory product due to hydrolysis. The mixture of glucose and fructose is called "invert sugar". This invert sugar is sweeter than sucrose. Hydrolysis may be carried out by acids, enzymes (invertase or sucrose) and bacteria.

$$C_{12}H_{22}O_{11} + H_2O \text{ ----------> } C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose Glucose Fructose

- Sucrose is not fermented directly by yeast.

2.10.4 Functions

- It serves as a nutrient.
- Invert sugars are used in candy both because of its sweetness and do not crystalline.
- It is used to produce artificial sweetener of no nutritive value. e.g. Saccharin. It is developed especially for obese or diabetics. Saccharin is 400 times sweeter than sucrose.

2.11 MALTOSE (MALT SUGAR)

2.11.1 Occurrence

- Maltose does not occur abundantly in nature.
- Sprouting cereal grains are rich in amylase which split the starch to dextrins and maltose.
- Malt (prepared from sprouting barley) is an excellent source of maltose.
- It is the intermediate product in the breakdown of starch by the enzyme amylase in the alimentary canal.

2.11.2 Structure

- It is composed of 2 glucose residues which are condensed together through glycosidic linkage. The linkage exists between the OH group of C-1 of one glucose residue and OH group of C-4 of another glucose residue. This linkage is known as α-(1→4) glycosidic linkage.
- Here, there is a free aldehyde group in the second glucose residue. So, maltose is considered as reducing sugar.

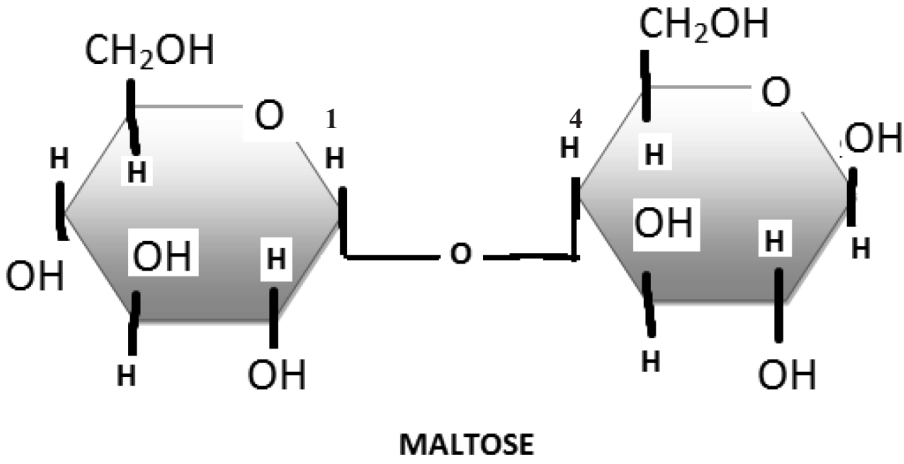


Fig 2.22 Structure of Maltose

2.11.3 Properties

- Maltose is a white crystalline solid, with a melting point 160 - 165^{0}C.
- It is soluble in water and dextrorotatory.
- It is hydrolyzed by the enzyme maltase to glucose and the products are absorbed.
- Since it has free aldehyde group, it shows mutarotation. The final rotation is +130^0 and can exist in all the three forms α, β and aldehyde form. In all the three forms, the linkage is α (1→4) glycosidic linkage. Among these three forms, β-form is most common.
- It can reduce Fehling's and Benedict's solution since it is a reducing sugar and cannot reduce Barfoed's solution, since it is a disaccharide.
- It forms osazone with phenyl hydrazine and maltosazone crystals have a characteristic petal like appearance and cluster of them looks like a sunflower and are readily identifiable when seen under microscope.

2.12 LACTOSE

2.12.1 Occurrence

- Lactose is a disaccharide purely of animal origin. It is commonly called as milk sugar. It is present in the milk of mammals. Lactose is found in the urine of pregnant and lactating women.

2.12.2 Structure

- Lactose is composed of α-D-galactose and α-D-glucose held together by β - 1, 4 glycosidic linkage between glucose and galactose molecules. It exhibits mutarotation due to the presence of a carbonyl group on the carbon atom 1 of glucose unit. The enzyme lactase hydrolysis lactose into glucose and galactose.

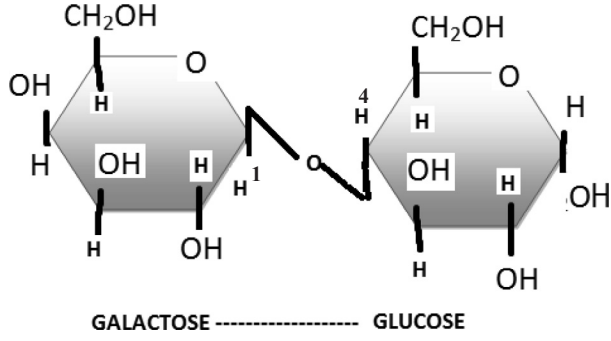


Fig 2.23 Structure of Lactose

2.12.3 Properties

- It is less soluble in water and less sweeter than sucrose.
- Lactose has low calories among sugars.

2.13 PROPERTIES OF OLIGOSACCHARIDES:

- Maltose is a reducing sugar. It reduces Fehling's solution and Tollen's reagent. This shows the presence of a free aldehyde group.
- It exhibits mutarotation.
- The anomeric carbon of C_1 glucose is free, hence lactose exhibits reducing property.
- Lactose from ozazones. It also reduces Fehling's solution.
- Lactose is hydrolysed by acids or by the enzyme lactase.
- Sucrose is a non-reducing sugar. The potential aldehyde group of glucose and the ketone group of fructose are blocked in the linkage and has no free reducing group.
- It does not reduce Tollen's and Fehling's solutions and does not form osazone.
- Sucrose on hydrolysis by dilute acids or the enzyme sucrase or invertase gives a mixture of glucose and fructose. It is called as invert sugar.

- Sucrose is dextrorotatory ($+62.5^0$) but its hydrolytic products are levorotatory because fructose has a greater specific levo-rotation than the dextrorotation of glucose. As the hydrolytic products invert the rotation, sucrose is known as invert sugars and the process is called as inversion.

2.14 FUNCTIONS OF OLIGOSACCHARIDES

- Cell recoginition - The oligosaccharides and glycoproteins present on the surface of cell membrane serve as identifiers. That assists the cells in their recognition and cell adhesion.
- Recognition of immunoglobulin - The liver cells recognize immunoglobulin with the help of oligosaccharides.
- Nitrogen fixation - The oligosaccharides present on the wall of nitrogen fixing bacteria help in the binding of bacteria to the root hairs of leguminous plants.

2.15 POLYSACCHARIDES

- Polysaccharides (variously called as glycanes or polyholosides or polyosides) are high molecular weight carbohydrates.
- On hydrolysis, they yield mainly monosaccharides or products related to monosaccharides.
- They may also be regarded as polymeric anhydrides of simple sugars. D – Glucose is the commonest component of polysaccharides.
- The various polysaccharides differ from one another not only in the composition of the constituents of monosaccharides but also in the molecular weight, in the nature of the chain (linear or branched), in the type of glycosidic bond (α or β) and in the type of linkage (1-2 or 1-3 or 1-4 or 1- 6) involved in the respective monosaccharide units.
- Majority of carbohydrates of nature occur as polysaccharides. Chemically, the polysaccharides may be distinguished into homopolysaccharides (homoglycanes) and heteropolysaccharides (heteroglycanes).
- Homopolysaccharides are composed of only one type of monosaccharides. On hydrolysis, they yield only one type of monosaccharides. E.g. Starch, Cellulose, Chitin, Glycogen.
- Heteropolysaccharides are composed of mixture of monosaccharides. On hydrolysis, they yield a mixture of monosaccharides. E.g. Hyaluronic acid, Chondroitin, Heparin, Agar –Agar.

- Based on their functional aspect, the polysaccharides may be grouped into two categories. They are structural (indigestible) polysaccharides (e.g. Cellulose, Pectin and Chitin) and storage (digestible or nutrient) polysaccharides (e.g. Starch, Glycogen and Inulin).

2.15.1 Types of polysaccharides

Polysaccharides are classified into two main types.

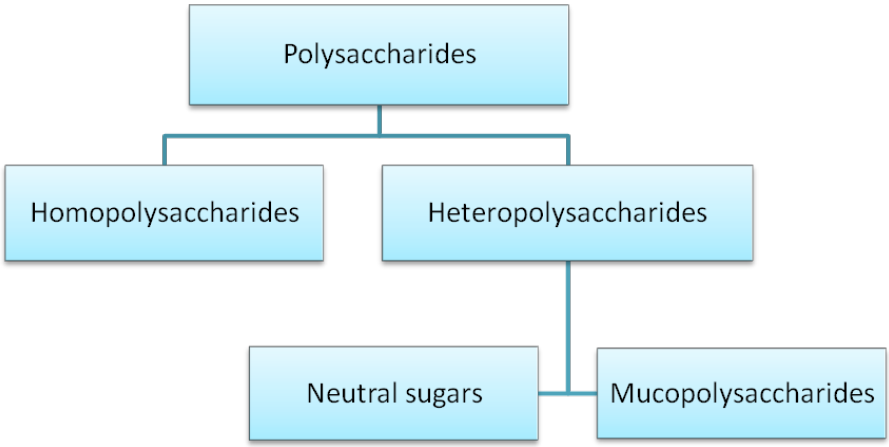


Fig 2.24 Classification of Polysaccharides

2.16 HOMOPOLYSACCHARIDES

Homopolysaccharides are non-sugars composed of only one types of monosaccharides. On hydrolysis they yield only one type of monosaccharides.

E.g. Starch, Glycogen, Inulin, Cellulose, Pectin, Chitin etc.

2.17 STARCH (HOMOPOLYSACCHARIDE AND STORAGE POLYSACCHARIDE)

2.17.1 Occurrence

- It is the most important reserve food material of the higher plants and is found in cereals, legumes, potatoes and other vegetables.
- More than half the carbohydrate ingested by humans is starch.
- Sago starch is obtained from sago palm (*Meteroxylon rumphii*), arrowroot from *Maranta arundinacea* and tapioca from *Manihot utillissima*.

- They usually occur as compact insoluble grains inside the plant cells.
- The bulk of our diet which consists of rice, wheat and vegetables is a good source of starch.

2.17.2 STRUCTURE

- Starch may occur either spherical, or oval in shape.
- They differ in size according to their sources.
- Generally, they appear to consist of concentric rings with a hilum in the centre.
- It consists of two components - amylose (15-20%) and amylopectins (80-85%).
- Starch from waxy corn consists of amylopectin component and no amylose.

Amylose structure

- It has a molecular weight range of 10,000 to 50,000. It is a long unbranched straight chain.
- Amylose forms the inner portion of starch grains and is soluble in water and less viscous.
- It may be formed in plant cells by elimination of water from glycosidic OH group of one α-D-glucose molecule and alcoholic OH group on carbon 4 of the adjacent α-D-glucose molecule. The linkage is α 1-4 glycosidic linkage.

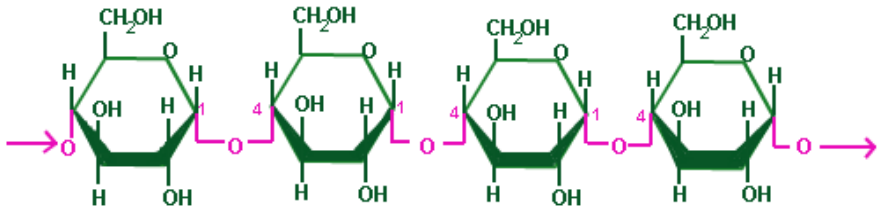


Fig 2.25 Structure of α - amylose

Amylopectin Structure

- In amylopectins, glucose molecules are arranged in highly branched form.
- They have much larger molecular weights of up to 1 million and the chains have at least 80 branches, each branch at interval of 24-30 glucose units.

- Branch point occurs at the 6th carbon atom of glucose molecule. Hence amylopectins have both (1-4) and (1-6) glycosidic linkages.
- Amylopectins form the outer covering of starch grain and is insoluble in H_2O.

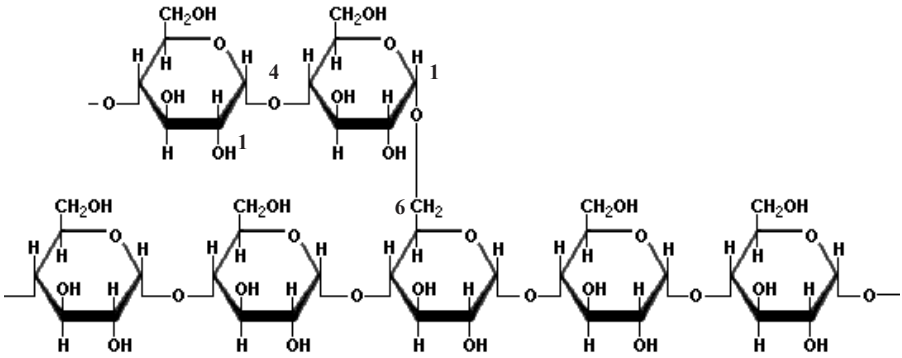


Fig 2.26 Structure of amylopectin

Table 2.2. DIFFERENCES BETWEEN AMYLOSE AND AMYLOPECTIN

Property	Amylose	Amylopectin
1. Molecular Weight	Low, about - 60,000	High, about 1 million
2. Number of glucose units	About 300 units	About 1000 units
3. Solubility in water	Highly soluble	Sparingly soluble or insoluble
4. Colour with dilute iodine solution	Blue colour	Violet colour
5. Branching	Absent	Present
6. Type of linkage	Only α (1- 4) glycosidic linkage is present.	Both α (1- 4) and α (1- 6) glycosidic linkages are present.

2.17.3 Properties

- Starch is a white, soft, amorphous powder and lacks sweetness.
- It is insoluble in water, alcohol and ether at ordinary temperature.
- The specific rotation of starch is + 196º.
- Starch breaks down into larger fragments called dextrins on heating in the presence of moisture. These dextrins are responsible for the stiffness of clothes which have been starched and ironed. It is called as "Washer Man's Starch". They are also used as adhesives on paper products.

- Since number of exposed OH groups are present in the structure, starch forms turbid colloidal solution when it is extracted from granules with hot water.
- Starch as a whole is insoluble in cold water. When it is heated with water the amylopectin absorbs water, swells up and bursts to form a paste, with amylose, diffusing into the water. This is carried out at a temperature between 60° - 80° C. This mixture of amylose and amylopectin in water is called as starch paste and is used as an adhesive.
- The enzyme amylase from saliva and pancreatic juice can hydrolyse starch to larger units called "dextrin" and finally to maltose. The dextrins give a bluish colour with iodine in the early stages of hydrolysis and later do not give any colour at all. They are accordingly called as

  Amylodextrins - give blue colour with iodine
  Erythrodextrins - give red colour with iodine
  Achrodextrins - give no colour with iodine.
- Hydrolysis of starch can also be brought about by boiling with dilute acids.
- Starch is a glucosan since it yields only glucose molecules on hydrolysis.

2.18 CELLULOSE (HOMOPOLYSACCHARIDE AND STRUCTURAL POLYSACCHARIDE)

2.18.1 Occurrence

- Cellulose is a structural polysaccharide acts as a chief constituent of the fibrous parts of plants and is the most abundant organic material in nature.
- Cellulose is often found associated with other structural substance such as lignin.
- Flax, ramic and cotton contain 97-99% of cellulose. Wood contains 41-53% of cellulose. Cercal straws contain 30-43% of cellulose.
- Cellulose used for experimental studies is prepared from raw cotton by extraction with organic solvents to remove lipids and other soluble impurites.
- It is also present in certain tunicates.

2.18.2 Structure

- Its molecular weight ranges between 2,00,000 and 20,00,000 corresponding to 1,250-12,500 glucose units per molecule.
- It is a linear unbranched structure.

- It is made up of β-D glucose units which are linked by β (1-4) linkages. The linkage involves glycosidic OH group of one β-D-glucose unit and alcoholic OH group at C-4 of the adjacent β-D- glucose molecule.
- It resembles the structure of amylose in starch except that the glucose units here are linked together by β (1-4) linkages where as in amylose it is α (1-4) linkage.
- Due to the difference in chemical structure, cellulose is not acted upon by the enzyme amylase which is present in the digestive juices.

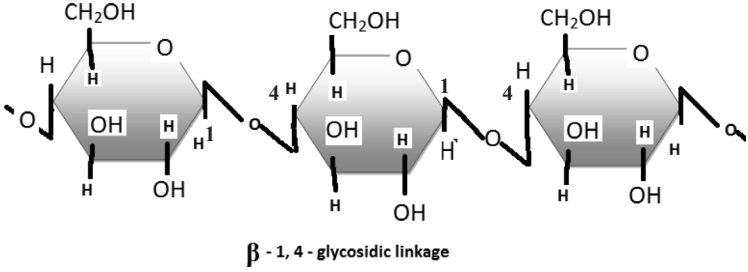


Fig 2.27 Structure of Cellulose

2.18.3 Properties

- It is fibrous, tough, white solid, insoluble in water but soluble in ammoniacal cupric hydroxide solution and in HCl solution of zinc chloride.
- It gives no colour with iodine and lacks sweetness.
- Because of the lack of chemical reactivity, cellulose is of no nutritive value.
- In man, cellulose is not digested because of the absence of appropriate hydrolyzing enzyme (cellulase).Hence it serves as an important source of "bulk" in the diet.
- However, microorganisms such as *Trichonympha* can digest cellulose because these microoranisms secrete an enzyme called cellulase which can break β (1-4) linkages.
- Wood-rot fungi and bacteria also produce cellulase. So that, they can easily digest cellulose.
- Cellulose is a chief food material for ruminants. As they are having cellulase secreting microorganisms in their digestive tract, they can easily digest cellulose.
- On partial hydrolysis, a disaccharide called cellobiose is formed.
- Cellulose is a relatively inert material and it is completely hydrolysed only under most drastic conditions.

- For e.g. it is hydrolysed to glucose when treated with conc. H_2SO_4 or HCl or with conc. NaOH.

2.19 GLYCOGEN

2.19.1 Occurrence

- Glycogen is a homopolysaccharide. It is a glucan because on hydrolysis it yields glucose.It is the major reserve carbohydrate in animals. So it is called as animal starch. It is stored mainly in the liver and muscles of all animals. It is a white powder. It does not dissolve in water. The solution gives reddish brown colour with iodine.

2.19.2 Structure

- It is a branched polymer of glucose with α - 1, 4 and α-1, 6 types of linkages. The external branches of glycogen molecules contain 6 to 7 glucose units. The internal branches contain only 3 glucose units.
- In the main stem, glucose units are linked by α-1, 4 linkage. In the branching points they are linked by α-1, 6 linkage or α-1, 4 linkage. Glycogen resembles amylopectin of starch chemically.
- It is a non reducing sugar.The liver glycogen supplies glucose to all tissues through the blood.
- The blood always contains 1% glucose. When it exceeds 1%, the excess glucose is transported to the liver. This process is called glycogenesis.
- When blood sugar level is below 1% the liver glycogen is changed into blood glucose. This process is called glycogenolysis.
- Muscle glycogen is utilized as the energy source during muscle contraction.

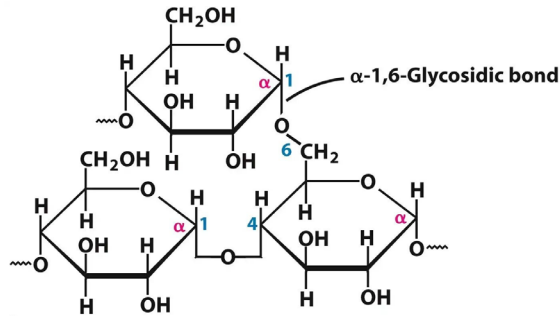


Fig 2.28 Structure of Glycogen

2.19.3 Properties

- Glycogen is a non-osmotic molecule, so it can be used as a solution to store glucose in the cell without disrupting osmotic pressure.

2.20 CHITIN

It is a homopolysaccharide.
It is formed of N-acetyl glucosamine.
It is related to cellulose. The alcoholic OH group on carbon atom 2 of β-D glucose units is replaced by N-acetylamino group.
The N-acetyl glucosamine units are linked by β-1, 4 glycosidic linkage.
It is found in the exoskeleton of insects and crustaceans and in the cell walls of fungi.
On hydrolysis with mineral acid, it gives glucosamine and acetic acid.
Chitin is decomposed to N-acetyl glucosamine by chitinase present in the gastric juice of snails or from bacteria.

2.21 HETEROPOLYSACCHARIDES

Heteropolysaccharides are non-sugars composed of a mixture of monosaccharides. On hydrolysis, they yield a mixture of monosaccharides.

E.g. Neutral sugars such as hemicelluloses, gums etc.

Mucopolysaccharides such as hyaluronic acid, chondroitin, chondroitin sulfate,

keratosulfate, heparin etc.

2.21.1 Classification of Heteropolysaccharides

Heteropolysaccharides are further classified into two types, namely

- Neutral sugars
- Mucopolysaccharides

Neutral sugars - It gives more than one type of sugar units on hydrolysis and sometimes non sugar components also.

Mucopolysaccharides - Mucopolysaccharides are long chain of sugar molecules that are found throughout the body, often in mucus and in fluid around the joints. They are more commonly called glycosaminoglycans. These are

gelatinous substances. They are heteropolysaccharides. E.g. Hyaluronic acid, Agar agar, Chondroitin.

2.22 HYALURONIC ACID

It is a heteropolysaccharide and mucopolysaccharide. Hyaluronic acid is also called hyaluronan.

2.22.1 Occurence

It is an anionic, nonsulfate glycosaminoglycans distributed widely throughout connective, epithelial and neural tissues.

2.22.2 Structure

It is a straight chain polymer of disaccharides which form the repeating unit. Each disaccharide unit is formed of D-glucuronic acid an N-acetyl D-glucosamine linked by β-1, 3 linkage.

On Hydrolysis, hyaluronic acid yields an equimolecular mixture of D-glucuronic acid, D-glucosamine and acetic acid.

Hyaluronic acid is split by the enzyme hyaluronidase. The sperm is rich in hyaluronidase and hence can advance better in the cervical canal and finally fertilize the ovum.

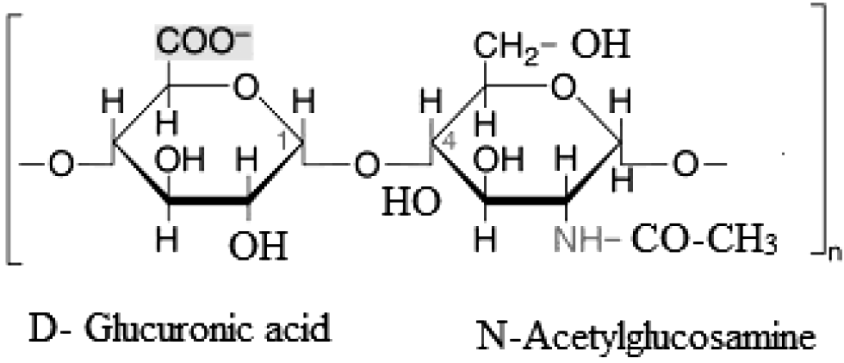


Fig 2.29 Structure of Hyaluronic acid

2.22.3 Properties

- It is highly viscous substances
- It acts as a lubricant and as biological cement in connective tissues.

2.23 CHONDROITIN

Chondroitin is a mucopolysaccharide.

2.23.1 Occurrence

It is found in cartilages and is also component of cell coats.
It is a parent substance for chondroitin sulfate.

2.23.2 Structure

It is a straight chain polymer of disaccharides which form the repeating units. Each disaccharide is linked to the next by β-1, 3 linkages.

Each disaccharide unit is formed of a D-glucuronic acid and N-acetyl D-galactosamine joined by β - 1, 3 linkages.

Thus chondroitin is similar to hyaluronic acid. In chondroitin, galactosamine is present instead of glucosamine.

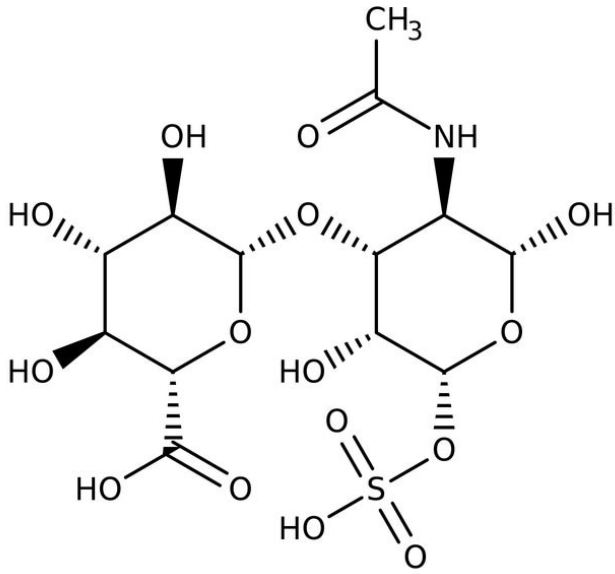


Fig 2.30 Structure of Chondroitin

2.23.3 Properties

- It provides resistance to compression.
- Along with glucosamine, chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis.

2.24 AGAR-AGAR

2.24.1 Occurrence

Agar- Agar is a commercially important product. It is used extensively in biology laboratories for the production of culture media for bacteria, fungi etc. It is also used in the preparation of medicines and in cosmetics and leather industry.

2.24.2 Structure

Agar-Agar is a heteropolysaccharide because it yields a mixture of monosaccharides on hydrolysis. It has a gel-like consistency.

It consists of D and L galactose in a ratio of 9:1.

It is produced by certain red algae such as Gelidium, Gracilaria, Gigartina, Eucheuma, Campylaephora, Hypner etc.

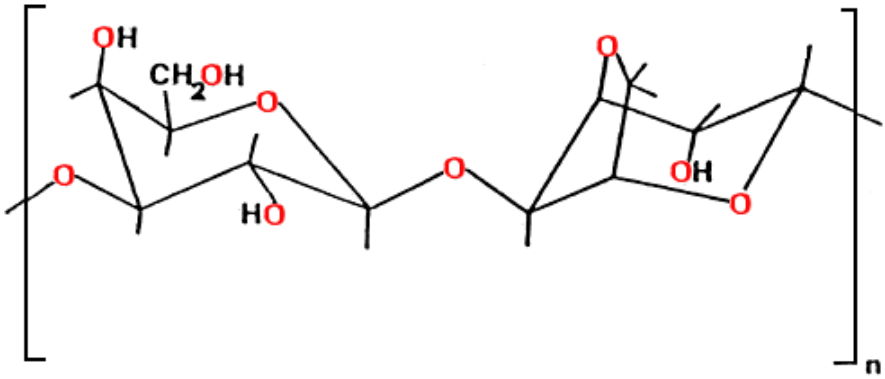


Fig 2.31 Structure of Agar Agar

2.24.3 Properties

It is acidic in nature. Hence it is an acidic heteropolysaccharide. That acidity is due to the presence of sulphuric acid.

2.25 HEPARIN

Heparin is a heteropolysaccharide because it yields mixtures of monosaccharides and their derivates.

It is a mucopolysaccharide because it has gel- like consistency.

It contains uronic acid and sulfuric acid and is acidic in nature.

Hence it is also called acidic heteropolysaccharide.

It functions as an anticoagulant because it prevents coagulation or clotting of blood.

It is a straight chain polymer composed of D-glucuronic acid and D-glucosamine-N-sulfate. It contains an additional O-sulfate group at C_6.

The two molecules are linked by 1 → 4 linkages only and 1 → 3 linkages is absent.

The D-glucuronic acid is esterified at carbon atom number 2.

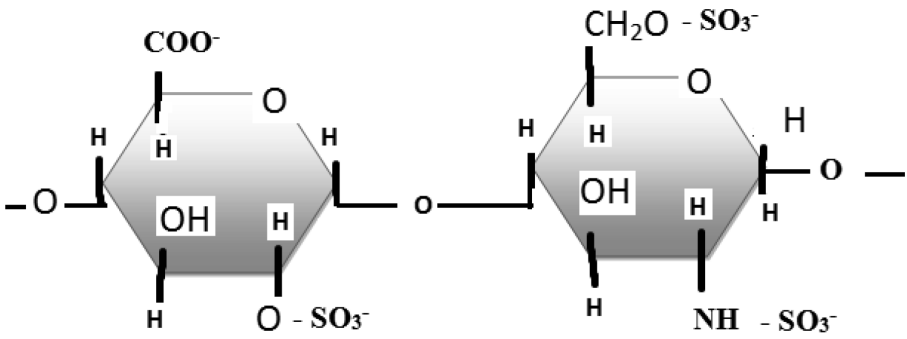


Fig 2.32 Structure of Heparin

2.26 FUNCTIONS OF CARBOHYDRATES

- Carbohydrates provide energy for body functions and for doing work.
- They are structural components of many organisms.
- They exert a sparing action on protein.
- They provide the carbon skeleton for the synthesis of some non essential amino acids and fats.
- Some carbohydrates are present as tissue constituents.
- Starch forms the main source of carbohydrates in the diet.
- Glycogen is the major reserve carbohydrate in animals and is often called animal starch. It is stored in liver and muscle of animals.
- Cellulose is widely distributed in plant sources. It occurs in the cell walls of plants where it contributes to the structure. It is the

main constituents of the supporting tissues of plants and forms a considerable part of vegetables.

- Pectin and hemicelluloses are present in fruits of many plants and serve as jelling agents.
- Hyaluronic acid occurs in synovial fluid in skin and in tissues. It acts as cementing substances in tissues and also acts as a lubricant. It is also present in vitreous humor.
- Heparin is used in medicine as an anticoagulant and prevents blood clotting.
- Keratan sulphate is an important component of cartilage and cornea.
- Lactose is otherwise called as milk sugar. It is present in milk and is made up of monosaccharides (i.e.) glucose and galactose.

$$\text{Glucose} + \text{Galactose} \longrightarrow \text{Lactose}$$

- Maltose is otherwise known as malt sugar and is present in germinating cereals, malt etc. It is the intermediate product in the hydrolysis of starch by amylase in the alimentary canal. It is made up of 2 molecules of glucose.

$$\text{Glucose} + \text{Glucose} \longrightarrow \text{Maltose}$$

- Sucrose is otherwise called as table sugar or cane sugar. It is the common sugar and is widely distributed in all photosynthesis plants. It does not exist in the body but occurs in sugarcane, pineapple, sweet potato and honey. It is made up of glucose and fructose.

UNIT III

AMINO ACIDS AND PROTEINS

Aminoacids: Structure, Specific rotation, Distribution and location in proteins, Classification: Based on Composition, Number of Amino and Carboxylic groups, Polarity of R group, Metabolism, Nutritional requirements, Non standard protein amino acids, Non protein amino acids, Physical and Chemical properties.

Proteins: Biologically important proteins, Sources, Nutritive value, Biological functions, Elemental composition, Classification – Based on solubility and shape, Structure complexity. Ramachandran plot. Structural organization of proteins: primary, secondary, tertiary and quaternary structure. - Bonds involved in protein structure, Properties, Tests for proteins.

3 AMINO ACIDS AND PROTEINS

3.1 STRUCTURE OF AMINO ACIDS

- Each amino acid is a nitrogenous compound having both an acidic carboxyl (COOH) group and a basic amino (NH_2) group.
- The amino acids are regarded as building blocks of proteins.
- The general formula of an amino acid is presented below

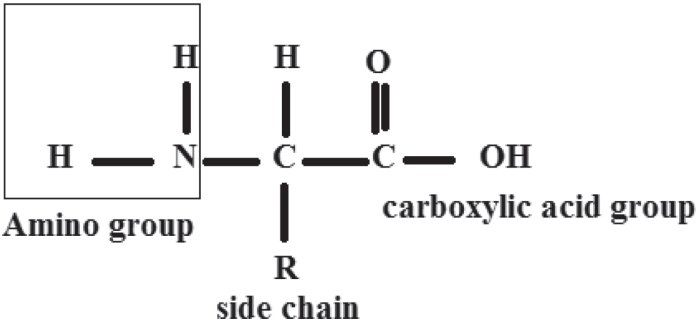


Fig 3.1 Structure of Amino acid

- "R" stands for the side chains that are different for each amino acid. "R" can be as simple as a hydrogen atom (H) or a methyl group (CH_3) or a more complex structure.
- The first carbon is the part of the carboxyl group. The second carbon, to which the amino group is attached, is called the α carbon.
- The α carbon of most of the amino acids is joined by covalent bonds to four different groups of atoms.
- Thus, the α carbon atom in all amino acids is asymmetric except in glycine, where the α carbon is symmetric.
- Amino acids (except glycine) exist in two optically active forms due to the presence of asymmetrical carbon atom: those having - NH_2 group to the right are designated as D-forms and those having - NH_2 group to the left as L-forms.

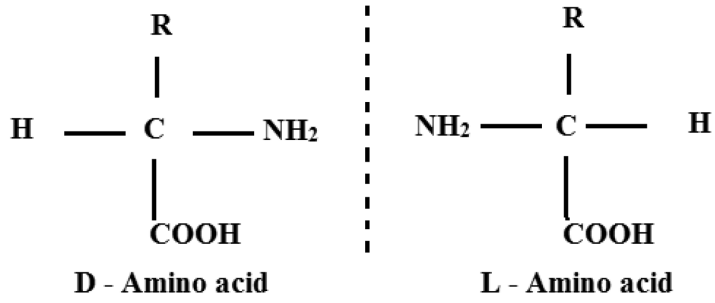


Fig 3.2 D and L forms of Amino acid

- However, the two amino acids, threonine and isoleucine have two asymmetric carbon atoms each and thus have $2^n = 2^2 = 4$ optical isomers.
- At pH 7.0, both the carboxyl and amino groups are ionized. The amino acids found in the proteins belong to the L- series.

3.1.1 Specific Rotation

- It is interesting to note that the amino acids found in the proteins belong to the L-series.
- Many of the naturally occurring L-amino acids rotate the plane of polarized light to the left (i.e., they are levorotatory) while others rotate the plane of polarized light to the right (i.e., they are dextrorotatory).
- Thus, it is evident that the symbols D and L do not identify the property of light rotation, i.e. D-isomers can be either dextrorotatory (d) or levorotatory (l); similarly, L-isomers can be either dextrorotatory (d) or levorotatory (l).
- However, to minimize confusion, the symbols d and l are usually not used nowadays. Moreover, the DL nomenclature has limitations because it describes the asymmetry of only one carbon atom in a compound and many biomolecules contain two or more asymmetric carbon atoms.
- The R and S classification or RS notation of isomers, introduced in 1956 by Robert Cahen, Christopher Ingold and Vladimir Prelog are currently being used in chemistry. This is more useful for defining the asymmetry of biomolecules because it accounts for all asymmetric carbons in an isomer.
- If any atom (other than H) or group on the asymmetric carbon is on the right side, that asymmetric carbon is designated as R (from *rectus*[L] = right); conversely, if any atom (other than H) or group is on the left side, the asymmetric carbon is then designated as S (from *sinister*[L] =left).

3.1.2 Distribution in Proteins

- The distribution of the 20 amino acids is not uniform in all proteins. Nearly 40% by weight of fibroin and 25% by weight of collagen are accounted for glycine.
- Fibroin is all rich in alanine (30% by weight). Serine and threonine predominate in casein and phosvitin.
- Collagen (in connective tissue), gliadin (in wheat) and zein (in corn) are rich in proline. Human serum albumin with 585 amino acid residues has only one tryptophan moiety.
- The pulse is notable as it lacks S-containing amino acid, methionine (Met) but contain good amount of the basic amino acid, lysine (Lys); whereas cereals lack lysine but have sufficient quantity of methionine. When combined, these alter the deficiency of each other through mutual supplementation and are better utilized in human body.

3.1.3 Location of Proteins

- Amino acids with uncharged polar side chains are relatively hydrophilic and are usually on the outside of the proteins, while the side chains on non polar amino acids tend to cluster together on the inside.
- Amino acids with acidic or basic side chains are very polar and they are nearly always found on the outside of the protein molecules.

3.2 CLASSIFICATION OF AMINO ACIDS

3.2.1 On the Basis of the Composition of the Side Chain or R Group

Based on the composition of the side chain, the twenty amino acids may be grouped into following 8 categories.

- **Simple amino acids**: These have no functional groups in the side chain, e.g. Glycine, Alanine, Valine, Leucine and Isoleucine.
- **Hydroxy amino acids**: These contain a hydroxyl group in their side chain, e.g. Serine and Threonine.
- **Sulfur-containing amino acids:** These possess a sulfur atom in the side chain, e.g. Cysteine and Methionine.
- **Acidic amino acids**: These have a carboxyl group in the side chain, e.g. Aspartic acid and Glutamic acid.

- **Amino acid amides**: These are derivatives of acidic amino acids in which one of the carboxyl group has been transformed into an amide group (-CO.NH2), e.g. Asparagine and Glutamine.
- **Basic amino acids**: These possess an amino group in the side chain, e.g. Lysine and Arginine.
- **Heterocyclic amino acids**: These amino acids have a ring in their side chain which possess at least one atom other than the carbon, e.g. Tryptophan, Histidine and Proline.
- **Aromatic amino acids**: These have a benzene ring in the side chain, e.g. Phenylalanine and Tyrosine.

3.2.2 On the Basis of the Number of Amino and Carboxylic Groups

McGilvery and Goldstein (1979) have classified twenty amino acids as follows:

- **Monoamino-monocarboxylic amino acids:**
  - ♦ **Unsubstituted** – Glycine, Alanine, Valine, Leucine, Isoleucine
  - ♦ **Heterocyclic** – Proline, Histidine
  - ♦ **Aromatic** – Phenylalanine, Tyrosine, Tryptophan
  - ♦ **Thioether** – Methionine
  - ♦ **Hydroxyl** – Serine, Threonine
  - ♦ **Mercapto** – Cysteine
  - ♦ **Carboxamide** – Asparagine, Glutamine
- **Monoamino-dicarboxylic amino acids**: Aspartic acid, Glutamic acid
- **Diamino-monocarboxylic amino acids**: Lysine, Arginine,

3.2.3 On the Basis of Polarity of the Side Chain or "R" Group

Amino acids is based on the polarity of the R groups present in their molecules, it is classified as follows.

- **Amino acids with non polar R groups:**
  The R groups in this category of amino acids are hydrocarbon in nature and thus hydrophobic. This group includes five amino acids with aliphatic R groups (alanine, valine, leucine, isoleucine, proline), two with aromatic rings (phenylalanine, tryptophan) and one containing sulfur (methionine).

- **Amino acids with polar but uncharged R groups:**
  The R groups of these amino acids are more soluble in water i.e. more hydrophilic than those of the non polar amino acids because they contain functional groups that form hydrogen bonds with water. This category includes 7 amino acids, viz., glycine, serine, threonine, tyrosine, cysteine, asparagine and glutamine. The polarity of these amino acids may be due to either a hydroxyl group (serine, threonine, tyrosine) or a sulfhydryl group (cysteine) or an amide group (asparagines, glutamine).
- **Amino acids with negatively charged (=acidic) R groups:**
  These are monoamino-dicarboxylic acids. In other words, their side chain contains an extra carboxyl group with a dissociable proton. The resulting additional negative charge accounts for the electrochemical behaviour of proteins. The two amino acids which belong to this category are aspartic and glutamic acid.
- **Amino acids with positively charged (=basic) R groups:**
  These are diamino-monocarboxylic acids. In other words, their side chain contains an extra amino group which imparts basic properties to them. Lysine, arginine and histidine belong to this category.

3.2.4 Based on Metabolism

- Purely Ketogenic: Leucine is purely ketogenic because it is converted to ketone bodies.
- Ketogenic and Glucogenic: Lysine, Isoleucine, Phenylalanine, Tyrosine and Tryptophan are partially ketogenic and partially glucogenic. During metabolism, part of the carbon skeleton of these amino acids will enter the ketogenic pathway and the other part to glucogenic pathway.
- Purely glucogenic: All the remaining 14 amino acids are purely glucogenic as they enter only into the glucogenic pathway.

3.2.5 Based on Nutritional Requirements

The amino acids may further be classified according to their essential nature for growth.

- **Essential or indispensible amino acids:**
  - ♦ Isoleucine, Leucine, Threonine, Lysine, Methionine, Phenylalanine, Tryptophan and Valine are essential amino acids.

- **Partially essential or semi-essential: amino acids**
  - ♦ Histidine and Arginine are semi essential amino acids.
- **Non essential or Dispensable: amino acids**
  - ♦ The remaining 10 amino acids are non-essential, because their carbon skeleton can be synthesized by the body. The non essential amino acids are alanine, aspargine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, tyrosine.

3.3 NON STANDARD PROTEIN AMINO ACIDS

- In addition to the above-mentioned twenty standard amino acids, several other amino acids exist.
- As an example, hydroxyproline has a limited distribution in nature but constitutes as much as 12% of the composition of collagen, an important structural protein of animals.
- Similarly, hydroxylysine is also a component of collagen, where it accounts for about 1% of the total amino acids.
- N-methyl lysine is found in myosin, a contractile protein of muscle. Another important nonstandard or less common amino acid is γ-carboxyglutamate, which is found in the blood clotting protein, prothrombin as well as in certain other proteins that bind Ca^{2+} in their biological function.

3.4 NON PROTEIN AMINO ACIDS

- There are some 300 additional amino acids which are never found as constituents of proteins but which either play metabolic roles or occur as natural products.
- Non protein amino acids are L-ornithine, L-citrulline, β-alanine, creatine and γ-amino butyrate (play metabolic role).
- L-ornithine and L-citrulline occur in free State in the animal tissues and are metabolic intermediates in the urea cycle.
- Higher plants are especially rich in non protein amino acids. These non protein amino acids are usually related to the protein amino acids as homologues or substituted derivatives. They have a limited distribution, sometimes to a single species even.
- L-azetidine-2-carboxylic acid, a homologue of proline, accounts for 50% of the nitrogen present in the rhizome of Solomon's seal, *Polygonatum multiflorum*.

- Orcylalanine is found in the seed of cornocockle, *Agrostemma githago*. It may be considered as a substituted phenylalanine.
- Furthermore, in the toxic polypeptides of *Amanita phalloides*, in addition to hydroxyleucine, allo-threonine is also found.

3.5 PHYSICAL PROPERTIES OF AMINO ACIDS

- Amino acids are colourless crystalline substance.
- They are generally soluble in H_2O, acids, alkalies, but sparingly soluble in organic solvents.
- They have melting points ranging between 200°C to 300°C or even more. Many of the amino acids undergo more or less decomposition at or near the melting point.
- Amino acids are usually sweet, tasteless or bitter.
  - ♦ For example glycine, alanine, valine, proline, hydroxyl proline, serine, tryptophan and histidine are sweet.
  - ♦ Leucine is tasteless.
  - ♦ Isoleucine and arginine are bitter and sodium glutamate acts as valuable flavouring agent for the preparation of certain sauces and dishes.
- All amino acids except glycine are optically active since they contain asymmetric carbon atoms.
- Amino acids contain acidic and basic groups. They can both donate and accept protons, hence they are said to be amphoteric in nature. The name zwitter is derived from the German word which means "hybrid". Zwitterion (or) dipolar ion is a hybrid molecule containing positive and negatively ionic groups. Basically the proton shifts from carboxyl group to amino group of the self molecule at normal pH cellular levels.

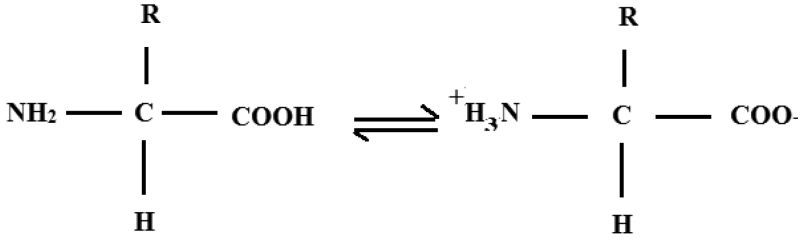


Fig 3.3 Zwitterion formation

- According to zwitter ion theory, amino acid possessing double charges (both +ve and –ve) are called zwitter ions which are electrically netural. It has been found out that in an acid solution (low pH), amino acids carry positive charge and hence they move towards cathode in an electric field.
- In alkaline solution (high pH), the amino acids carry negative charge and therefore move towards anode in electric field. But at around neutrality, the amino acids exist as inner salts or zwitter ions which do not migrate to either electrode in an electric field.

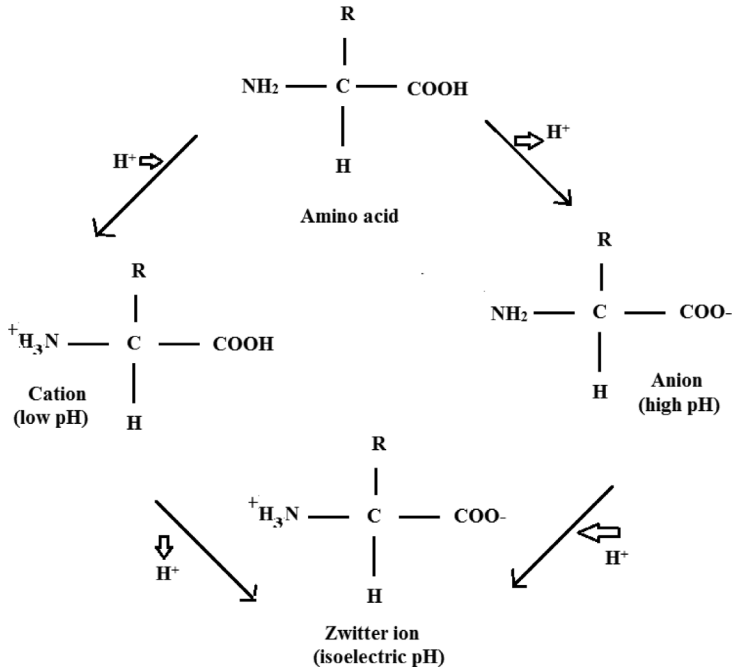


Fig 3.4 Existence of Amino acid as Cation, Anion and zwitter ion

- **Isoelectric pH:** At particular pH, amino acids do not migrate towards cathode (or) anode under the influence of electric field, is simply called "**Isoelectric point**". Isoelectric pH is defined as the pH at which a molecule exists as a Zwitter ion (or) dipolar ion and carries no net charge. Thus, the molecule is electrically neutral, but the charge will cancel each other. This is symbolized by pI. Individual amino acids have their own constant isoelectric points.

3.6 CHEMICAL PROPERTIES OF AMINO ACIDS

Chemical reactions of amino acids due to carboxyl and amino groups:

3.6.1 Due to Carboxyl group

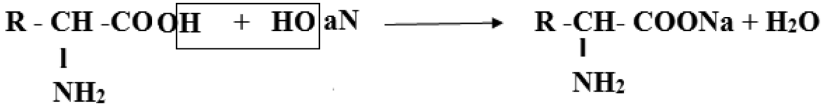
a) Decarboxylation

The amino acids will undergo alpha decarboxylation to form the corresponding "amines". Thus important amines are produced from amino acids.

- **Histidine** → Histamine + CO_2
- **Tyrosine** → Tyramine + CO_2
- **Tryptophan** → Tryptamine + CO_2
- **Lysine** → Cadaverine + CO_2
- **Glutamic acid** → Gamma Amino Butyric Acid (GABA) + CO_2

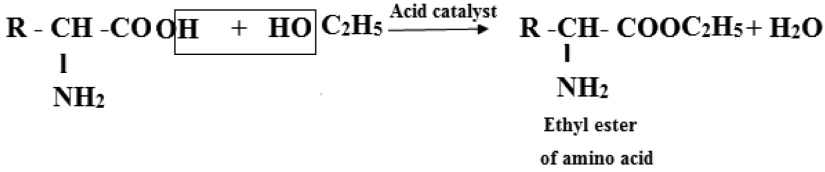
b) Reaction with Alkalies (Salt formation):

The carboxyl group of amino acids releases a H^+ ion with the formation of carboxylate (COO^-) ions. These may be neutralized by cations like Na^+ and Ca^{+2} to form salts. Thus amino acids react with alkalies to form "Salts".



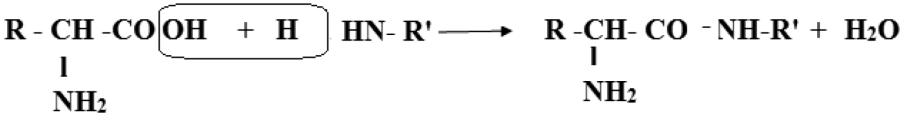
c) Reaction with Alcohols (Esterification):

The amino acids react with alcohol to form "Ester".



d) Reaction with Amines:

Amino acid reacts with Amines to form "Amides".



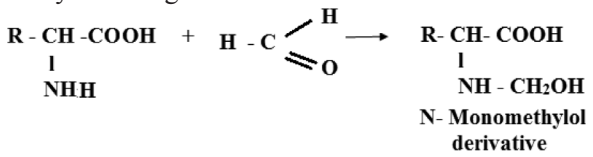
3.6.2 Due to Amino group

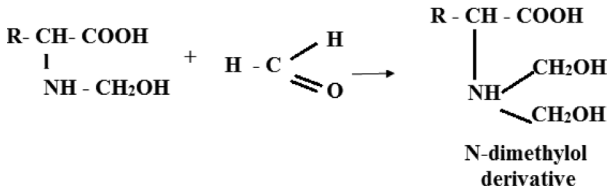
a) Reaction with Mineral acids (Salt formation)

- When the amino acids are treated with mineral acids (like HCl), they form "Acid Salts".

b) Reaction with Formaldehyde

- When the amino acid reacts with two molecules of formaldehyde it forms "N-dimethylol derivative" (Hydroxy-methyl derivative). This reaction is done in two steps. These derivatives are insoluble in water and resistant to attack by microorganisms.



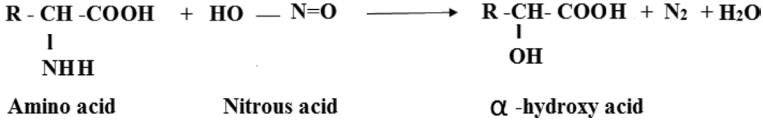


c) Reaction with Benzaldehyde:

- The amino acid reacts with benzaldehyde it gives "Schiff's base".

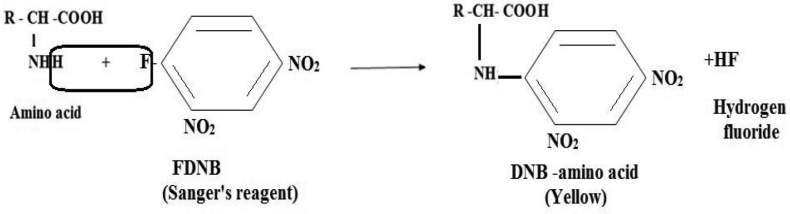
d) Reaction with Nitrous acid (Van Slyke reaction):

- When the amino acids react with nitrous acid (HNO_2) to liberate N_2 gas, it produce the corresponding "α-hydroxyl acid". The imino acids Proline and Hydroxyproline do not respond to this reaction.



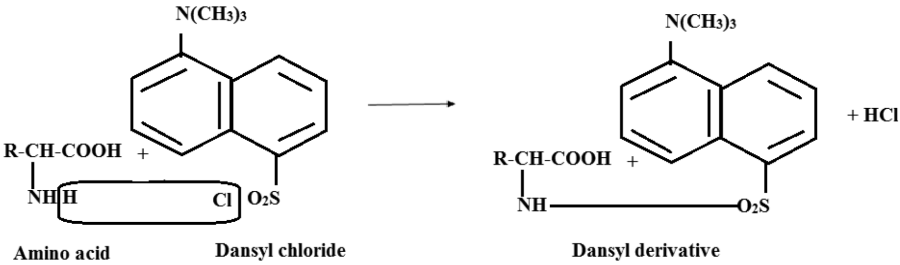
e) Reaction with Sanger's reagent:

- "1-Fluoro-2, 4-dinitrobenzene" is called Sanger's reagent (FDNB). In mildly alkaline solution, Sanger's reagent reacts with α-amino acid to produce a yellow coloured derivative, DNB-amino acid.



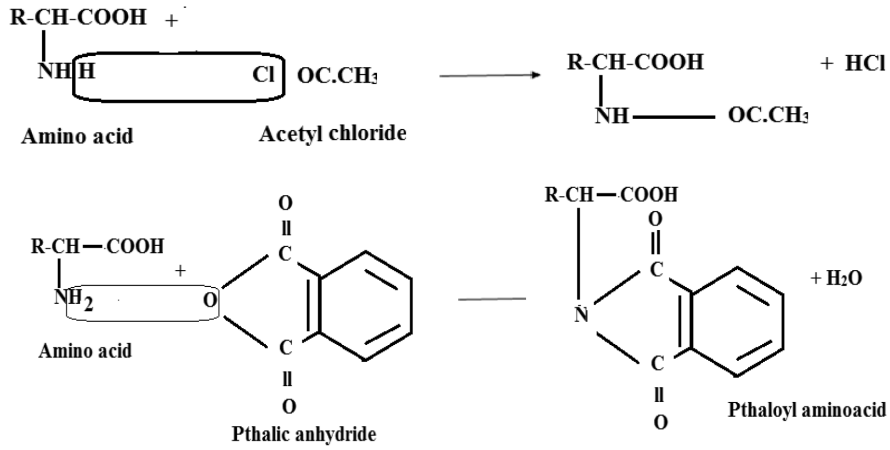
f) Reaction with DANSYl Chloride

- DANSYl chloride means "Dimethyl Amino Naphtha Sulphonyl Chloride". When the amino acid reacts with DANSYl chloride reagent, it gives a "Fluorescent DANSYl derivative".



g) Reaction with acylating agents (Acylation):

- When the amino acids react with "Acid chloride" and acid anhydride (Pthalic anhydride) in alkaline medium it gives "pthaloyl amino acid".

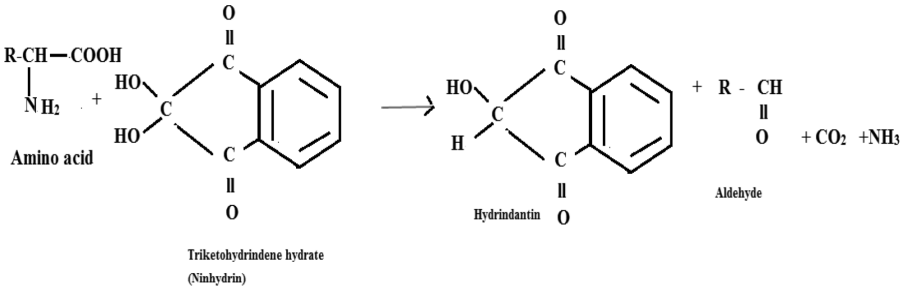


3.6.3 Due to amino & carboxyl group

Ninhydrin reaction

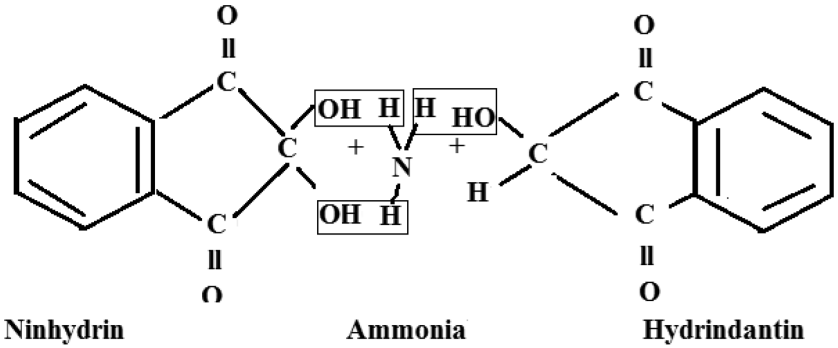
Step1

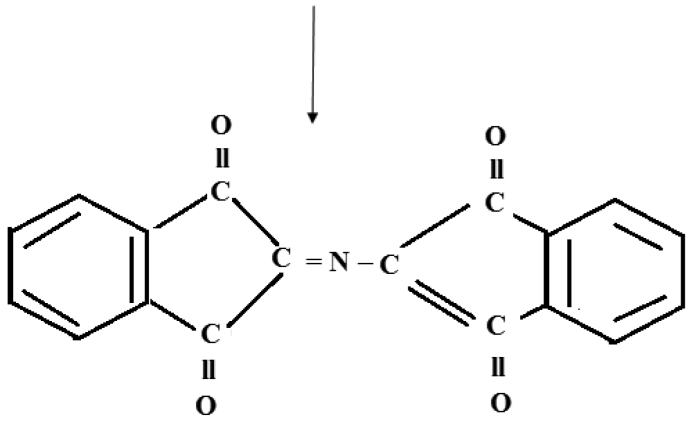
- Ninhydrin (=indane 1,2,3-trione hydrate) is a powerful oxidizing agent and causes oxidative decarboxylation of α-amino acids producing CO_2, NH_3 and an aldehyde with one less carbon atom than the parent amino acid.



Step2:

- The reduced ninhydrin then reacts with the liberated NH_3 and a molecule of ninhydrin, forming Blue-colored Ruheman's complex.





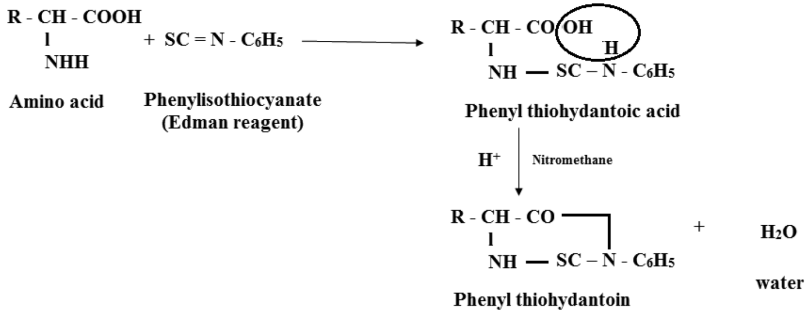
Diketohydrindylidene diketohydrindamine (Ruheman's purple)

This reaction is a very sensitive reaction and it is used for amino acid and imino acid identification. When amino acids (or) imino acid react with Ninhydrin molecule it gives colour. When it gives **Purple colour Ruheman's complex** –

the unknown sample is amino acids (which have primary amine $–NH_2$) or it gives yellow colour – the unknown sample is imino acid (**-NH-**).

Reaction with Edmann's degradation:

- Edmann's reagent is **"phenylisothiocyanate"**. When amino acids react with Edmann's reagent it gives "*phenyl thiohydantoic acid*" and finally it turns into cyclized form "*Phenyl thiohydantoin*" (Edmann's derivative).



3.7 PROTEINS

Protein is a macromolecule composed of one or more polypeptide chains possessing a characteristic amino acid sequence. It is a polymer of amino acids. Protein is the essential constituents of living cells. Proteins make up 12 % of the protoplasm. They are the body-builders. The term protein is derived from Greek: Proteuo = Primary or holding first place.

- The term protein was first proposed by **Berzelius.**
- They contain carbon, nitrogen, hydrogen, oxygen and sometimes sulphur. They are constructed largely of **amino acids.**

3.8 BIOLOGICALLY IMPORTANT PROTEINS

Thousand of proteins exist in the biological system. A few commonly available proteins are given below.

1. Plasma albumin
2. Ovalbumin in egg
3. Lactalbumin in milk
4. Plasma globulin
5. Serum globulin
6. Ovaglobulin in egg
7. Myosin in muscles

8. Edestin in hempseed
9. Glutenin in wheat
10. Oryzenin in rice
11. Zein in corn
12. Hordein in barley
13. Secalin in rye
14. Keratin in hair
15. Collagen in tendons
16. Elastin in ligaments
17. Fibroin in silk
18. Histones in nucleus
19. Sturin in sperm
20. Salmin in sperm
21. Mucin in saliva
22. Casein in milk
23. Vitelline in egg
24. Lipoprotein of blood
25. Nucleoproteins
26. Haemoglobulin
27. Myoglobulin
28. Cytochromes
29. Flavoproteins
30. Insulin
31. Oxytocin
32. Lactic dehydrogenase
33. Isozyme
34. Chymotrypsin

3.9 SOURCES OF PROTEIN

- Proteins are obtained from both animal and plant sources. The animal sources of proteins include milk, egg, meat, fish, liver etc. Plant sources of proteins are pulses, nuts and cereals.

3.10 NUTRITIVE VALUE

- The nutritive value of protein is based on two factors, namely amino acid composition and digestibility.
- All proteins do not contain all the essential amino acids. Only a few types of proteins contain the complete set of essential amino acids. Such proteins are called first class proteins and their nutritive value is high.
- Almost all the animal proteins contain all the essential amino acids. Eg. milk, milk products, meat, fish, kidney, liver, shell fish, soya beans etc.

3.11 BIOLOGICAL FUNCTIONS OF PROTEIN

- **Enzyme catalysts** – The chemical reactions in the biological system are catalyzed by enzymes. They exhibit enormous catalytic power. Thousands of enzymes have been identified in the biological system. The striking fact is that all the enzymes are nothing but proteins.
- **Transport -** Proteins transport ions and small molecules. Haemoglobin, a conjugated protein of blood, transport oxygen. Myoglobin, a muscle protein, transports oxygen in the muscles. Transferrin carries iron in the plasma of blood. The lipoproteins of plasma carry lipids from the liver to other organs. The membrane proteins transport glucose, amino acids and other nutrients across the membrane into the cell.
- **Storage** - Certain proteins function as a storage molecule. Ferritin, a protein stores iron in the liver. Seeds store nutrient proteins. Eg. Wheat, corn, rice etc.
- **Nutrients** - Certain proteins function as nutrients. The egg contains ovalbumin. The milk contains casein.
- **Contraction and Movement** - The contraction of muscle is brought about by two fibrous protein called actin and myosin. The microtubules of flagella and cilia are built on tubulin, a protein.
- **Mechanical Support -** Many proteins serve as supporting filaments, cables or sheets to give biological structures, strength, support and protection. Collagen, a fibrous protein is the major component of tendons, cartilage and leather. Ligaments contain elastin, a structural protein. Keratin, an insoluble protein, is the main component of hair, finger nails and feathers. Fibroin is the major component of silk fibres and spider web.
- **Immune Protection** - Many proteins defend organisms against invasion by other species. Invading bacteria virus, etc. elicit the production of

antibodies by lymphocytes. Antibodies neutralize the foreign germs. Antibodies are protein and immunoglobulins.

- **Blood Clotting -** Bleeding is stopped by the formation of clot. Clotting is brought about by blood clotting proteins such as fibrinogen and thrombin.
- **Transmission of Nerve Impulse** - The nerve impulse is transmitted through synapse with the help of receptor proteins. Receptor proteins at the synapse are triggered by acetylcholine.
- **Gene Expression -** In a cell, all the genes are not functioning at a time. Only a few genes are active and the other genes remain in an inactive condition. The inactivation of genes is brought about by repressor proteins especially in bacteria.
- **Hormonal Action** - Many hormones are proteins. Eg. Insulin, growth hormone, parathyroid hormone etc.
- **Thermoregulation** - The blood plasma of some antartic fish contains antifreeze proteins, which protect the blood from freezing.

3.12 ELEMENTAL COMPOSITION OF PROTEINS

All proteins contain C, H, O, N and generally S. Many proteins contain P also. Elements such as I, Fe, Cu and Zn are also occasionally present. Proteins are made up of Amino Acids.

Proteins are polymers of amino acids. The amino acids are the building blocks or monomers of proteins. An amino acid consists of five components namely

1. An amino group (NH_2)
2. A carboxyl group (COOH)
3. A hydrogen atom
4. An R group or a side chain or alkyl
5. A carbon atom.

There are more than 100 amino acids. All proteins of the biological system from bacteria to man are constructed out of 20 amino acid only. This is just like the 26 letters of English alphabet can make up many thousands of words. Similarly, the 20 amino acids make up thousands of proteins. For example, a bacterial cell contains 1000 to 2000 proteins and the human cell contains as many as 100000 protein molecules.

3.13 CLASSIFICATION OF PROTEINS

Proteins are macromolecules made up of amino acids. They are classified in two ways.

1. On the basis of their solubility or shape.
2. On the basis of increasing complexity of structure.

3.13.1 Classification of proteins on the basis of solubility or shape

Proteins are classified into two groups on the basis of their solubility or shape. They are globular proteins and fibrous proteins.

1. GLOBULAR PROTEINS

- Globular proteins are spherical in shape.
- They are soluble in water
- They are highly branched
- The polypeptide chains are cross linked by the usual peptide bonds.
- The globular protein molecules are tightly folded into spherical or globular shapes

Example : Enzymes, Protein hormones, Antibodies, Haemoglobin and Myoglobin

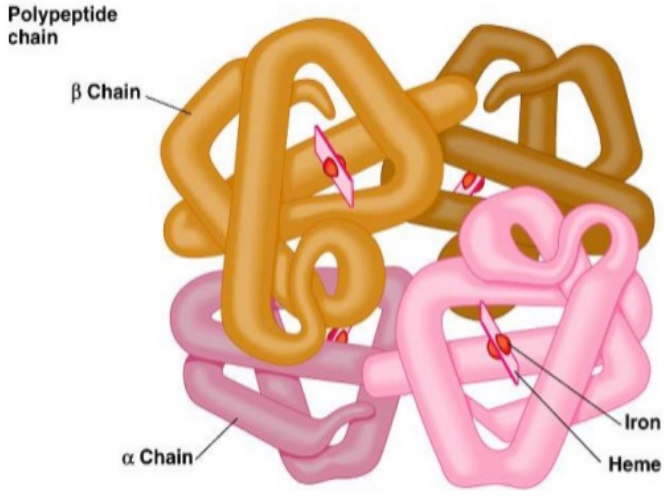


Fig 3.5 Globular proteins

2. FIBROUS PROTEINS

- Fibrous proteins are insoluble in water.
- They are in the form of fibres.
- They are highly resistant to digestion by proteolytic enzymes.
- They are unbranched.
- They are linear molecules.
- The long linear protein chains are held together by intermolecular hydrogen bonds.
- They are not folded into globular molecules.
- They serve as structural proteins.

Example : Collagen of tendons, Elastin of connective tissue, Fibroin of silk, Keratin of hair, Actin and Myosin.

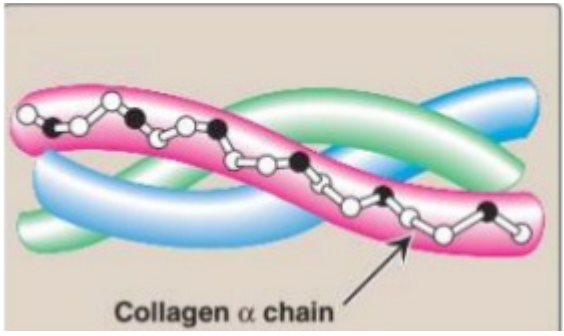


Fig 3.6 Fibrous proteins

3.13.2 Classification of proteins Based on the increasing complexity of structure

On the basis of increasing complexity of structure, proteins are classified into three groups. They are:

1. Simple proteins
2. Conjugated proteins
3. Derived proteins

SIMPLE PROTEINS

The proteins which yield amino acids or their derivatives on hydrolysis are called simple proteins. Simple proteins are further classified into 7 subtypes on the basis of the decreasing solubility.

1. Albumins

Albumins are simple proteins soluble in water. They are coagulated by heat. They are precipitated by saturated ammonium sulphate salt solution. They are deficient in glycine.

Eg: Plasma albumin, serum albumin of egg, ovalbumin of egg, white lacatalbumin of milk.

2. Globulins

Globulins are insoluble in water, but soluble in dilute solution of neutral salts. They are coagulated by heat. They are precipated by lower concentrations of salts such as ammonium sulphate or sodium sulphate. Globulins are precipated by saturated NaCl solution. They are glycine.

Eg: Plasma globulin, ovaglobulin in egg white, myosin in muscles and edestin in hemp seed.

3. Glutelins

Glutelins are insoluble in water and dilute solutions of neutral salts. But they are soluble in acids and bases. They are coagulated by heat. They are rich in arginine, proline and glutamic acid.

Eg: Glutenin in wheat, oryzenin in rice.

4. Prolamines

Prolamines are soluble in 70 to 80% ethyl alcohol. But they are insoluble in water, absolute alcohol and other neutral solvents. They are not coagulated by heat. They contain large amount of protein. They are deficient of lysine.

Eg: Zein from maize, glaidin from wheat, ordain from barley.

5. Albuminoids or Scleroproteins

These proteins are insoluble in water, dilute solutions of neutral salts, acid, bases and 60 to 80% ethyl alcohol. But they are soluble in long boiling concentration acid solutions.

Eg. Keratin in hair, feathers, nails and horn, collagen in tendons, skin and bone, elastin in ligaments and fibroin in silk.

6. Histones

Histones are simple proteins soluble in water and dilute acids, but insoluble in ammonia. They are not coagulated by heat. Histones are rich in basic amino acids

like histidine and arginine, but deficient in tryptophan and contain little cystine or methionine. Histones are combined with nucleic acids and haemoglobulin.

7. Protamines

Protamines are soluble in water and ammonium hydroxide. They are not coagulated by heat. They are more basic than histones. They contain large quantities of arginine. Tyrosine and tryptophan are absent.

Eg. Salmine from salmon sperm, clupeine from hering, sturine from sturgeon.

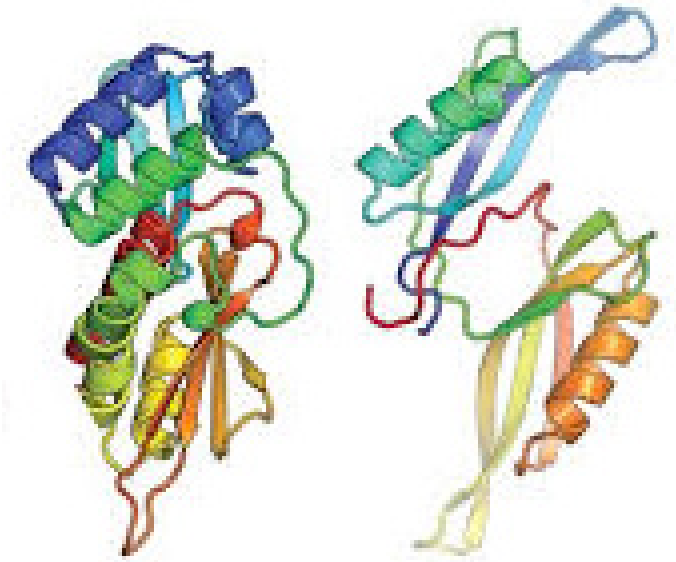


Fig 3.7 Protamines

CONJUGATED PROTEIN

Conjugated proteins are proteins united with non-protein substances. The non-protein substances linked to proteins are referred to as prosthetic group.

The protein part is called apoprotein. The prosthetic group and apoprotein are together called holoprotein. So conjugated protein, on hydrolysis yield non-protein substances in addition to amino acids.

The conjugated proteins are further classified into 5 sub types.

1. Glycoprotein or Mucoproteins

Glycoproteins contain carbohydrates as the prosthetic group. On hydrolysis, they yield amino sugars. E.g. Mucin in saliva, egg albumin, serum albumins and serum globulins.

2. Phosphoproteins

Phosphoproteins contain phosphoric acid as the prosthetic group. The phosphoric acid is attached to the hydroxyl group of protein by an ester linkage. E.g. Casein in milk and vitelline in egg yolk.

3. Lipoprotein

Lipoproteins contain phospholipids or cholesterol as the prosthetic group. E.g. Lipoproteins of blood serum.

4. Nucleoprotein

Nucleoproteins contain nucleic acid as the prosthetic group. E.g. Nuclein, nucleohistone etc.

5. Chromoproteins

Chromoproteins are coloured proteins. These are simple proteins linked to a metallic prosthetic group which gives the colour to the protein. Eg. Haemoglobin, haemocyanin, cytochromes, flavoproteins, chlorophyll etc.

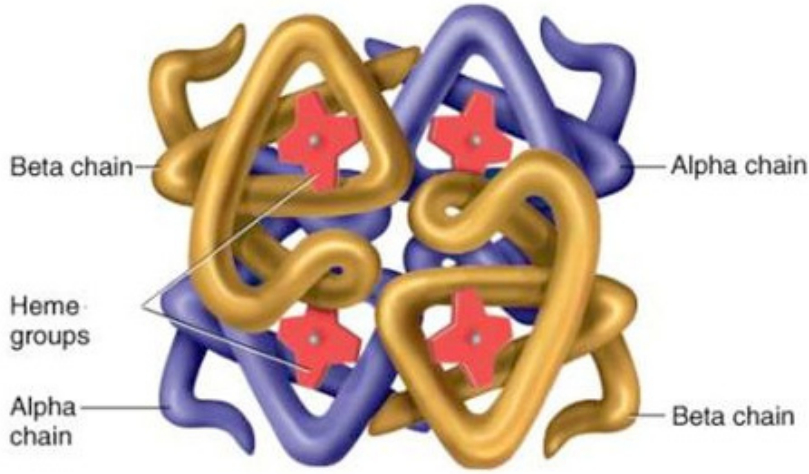


Fig 3.8 Chromoproteins

DERIVED PROTEINS

Derived proteins are the intermediate products formed from natural proteins when they are hydrolyzed by heat, acids, alkalies or enzymes. The derived proteins are of two types. They are primary derived proteins and secondary derived proteins.

a. Primary Derived proteins:

Primary derived proteins are the derivatives of proteins in which the size of the protein molecule is not materially altered. There are 3 types of primary derived proteins.

1. PROTEANS

Proteans are denatured proteins. They are the first products produced by the action of acids, enzymes or water on proteins. They are insoluble in water.

Eg. Edestan derived from edestin, Fibrin derived from fibrinogen, Myosan derived from myosin.

2. METAPROTEINS

Metaproteins are derived by the further action of acid or alkali on proteins. These are insoluble in water, but soluble in dilute acid or alkali.

3. COAGULATED PROTEINS

Coagulated proteins are insoluble protein products produced by the action of heat or alcohol on protein. Coagulated egg white is an example of this type.

a. Secondary Derived Proteins:

Secondary derived proteins are the products of proteins in which definite hydrolysis has taken place. The molecules are smaller than those of the original protein. They may be mainly of three types, namely proteases, peptones, polypeptides.

1. **Proteoses:** Proteoses are soluble in water. They are not coagulated by heat. They are precipitated by saturating their solutions with ammonium sulphate.
2. **Peptones:** Peptones are soluble in water. They are not coagulated by heat. They are also not precipitated by saturating their solutions with ammonium sulphate.
3. **Polypeptides:** Polypeptides are the derivates of proteins containing many amino acid units.

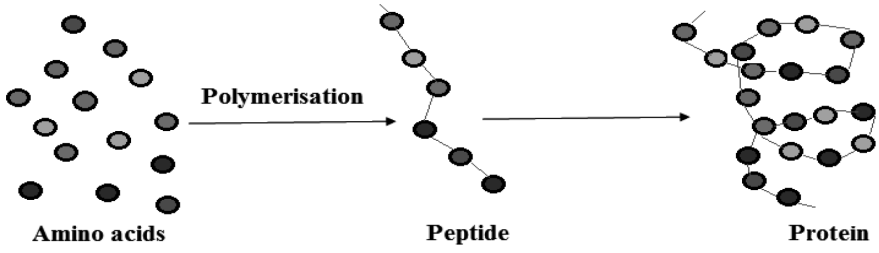


Fig 3.9 Aminoacids to Proteins

3.14 RAMACHANDRAN PLOT

- The secondary structure of proteins can be determined with the help of Ramachandran plot.
- The dihedral angles ψ against φ of amino acid residues in protein structure can be known with the help of Ramachandran plot.
- The two torsion angles of the polypeptide chain, also called Ramachandran angles.
- It describe the rotations of the polypeptide backbone around the bonds between N-Cα (called Phi, φ) and Cα-C (called Psi, ψ).

3.15 STRUCTURE OF PROTEIN

- The structure of protein depends upon its molecular size and shape. Four levels of structural organisation are primary, secondary, tertiary and quaternary structures.
- Some proteins have only first level and some other proteins have first and second levels and so on.
- These structures were first worked out by two American Scientists L. Pauling and Corey by using X- ray crystallography and later by Linderstrom – Lang.

3.15.1 PRIMARY STRUCTURE (1°)

- This refers to the number, nature and sequence of amino acids along the peptide chain. The amino acids are linked with each other by peptide bonds only.
- The formation of disulfide bridges is also included in this organisation. The primary structure of proteins may consist of one or more peptide chains.

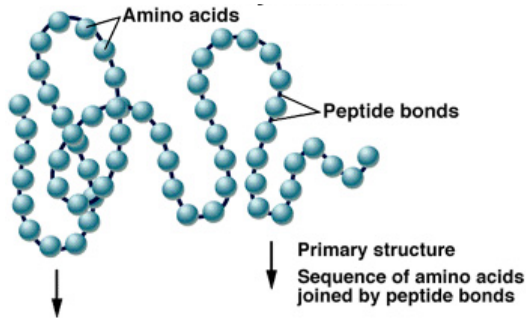
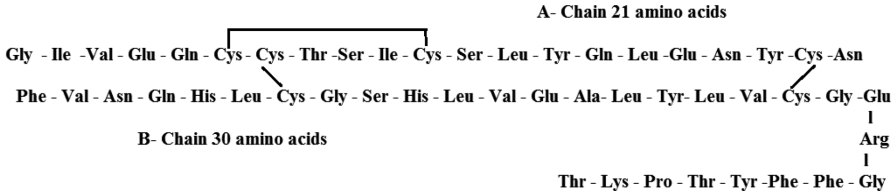


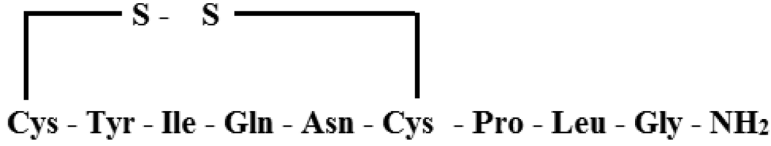
Fig 3.10 Primary Protein Structure

Examples

- Insulin is an excellent example where two peptide chains are linked together by two disulfide bonds. Both peptide and disulfide bonds are stable and individually or collectively they maintain the linear form of the protein molecule.



- **Oxytocin** – a hormone stimulating the contraction of smooth muscles especially during child birth is another example. Here, the intra-disulfide bond occurs between two cysteine units which are separated from each other in the peptide chain by four different amino acids.



Oxytocin

3.15.2 Secondary (2°) Structure: (Helix Formation)

- If the peptide and disulfide bonds alone present in proteins, these molecules would have behaved as irregularly coiled peptide chains of considerable length.
- But the native proteins show regularly coiled structure which involves the formation of hydrogen bonds between the folding of the peptide chain. This brings the second level of organisation called secondary structures.
- Due to the folding and hydrogen bonding between neighbouring amino acids results in the formation of a rigid and tubular structure called as helix.
- Two types of helix conformations have been recognised. They are α type – and β type.

α HELIX:

Some structural proteins are found to be in a coiled form. Details of the α– helix have been furnished by Nobel prize winner Lineaus Pauling and his co-workers in 1951.

- The α - helix is a spiral, formed by coiling of the linear polypeptide chain around the fibre axis.
- Each turn of the helix is 5.4 A° long and contains 3.6 amino acid residues, length of each residue being 1.5A°.
- This helical structure is stabilized by non - covalent hydrogen bonds linking the amide nitrogen with the carbonyl oxygen.
- The side chains of the residues project outwards. But the amino acids proline and hydroxy proline do not fit into the normal α-helix. So, they disrupt the α helical structure and cause sharp bends or changes in the direction of the chain. Because of this characteristic feature, proline and hydroxy proline along with glycine, serine and asparagine are called as "helix breaker".
- The α– helix may be either right handed or left handed. The right handed α–helix is favoured for L amino acids. Best example for α helix is α keratin which makes up the protein of hair, wool, fur, claws, hooves and feathers. The α helix is found in both fibrous and globular proteins.

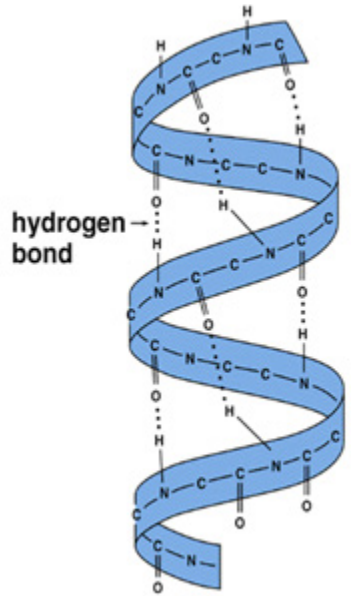
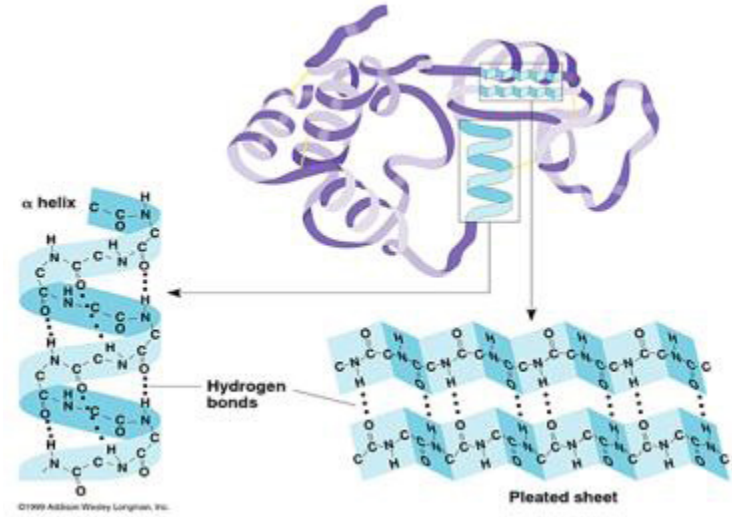


Fig 3.11 Secondary Structure of Proteins

β - PLEATED SHEET

- Pauling and Corey proposed another arrangement of protein, the β-pleated sheet structure. In α helix, the polypeptide chain is condensed whereas, in β-pleated sheet structure it is almost fully extended.

- In the β - pleated sheet structure, 2 or more peptide chains are linked together laterally by hydrogen bonds.
- The chains may be parallel or anti-parallel. In a β- pleated sheet, when the adjacent polypeptide chains run in opposite directions, the structure is termed as anti-parallel whereas, if the chains run in the same direction, it is termed as parallel.
- The anti-parallel structure permits more number of hydrogen bonding than parallel chain structure.
- Most heat treatment and stretching of α keratin convert it into β - keratin by breaking the stabilising hydrogen bonds.
- β helix is found in silk, muscle and contractile fibres. Anti-parallel structure is found in silk fibroin.



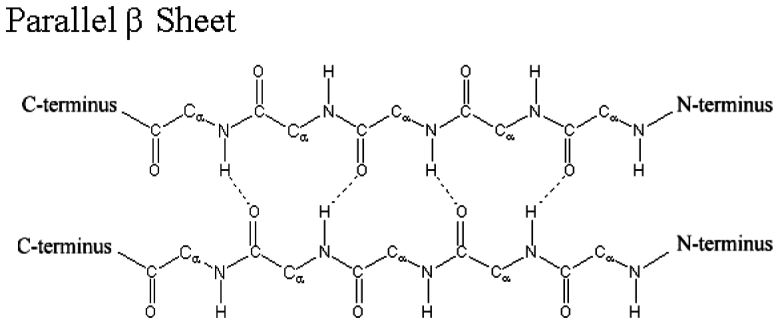
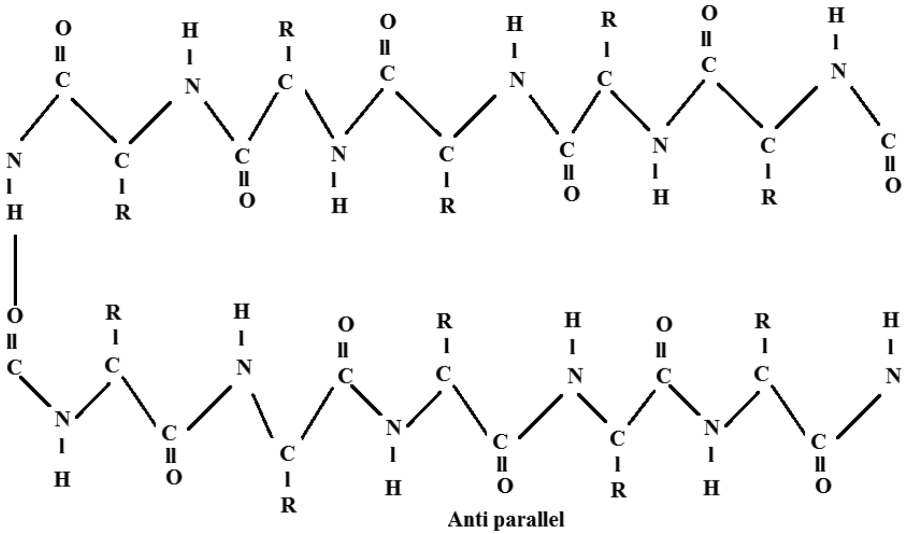


Fig 3.12 β - Pleated Sheet



3.15.3 TERTIARY STRUCTURE OF PROTEIN

- When a protein chain is folded in a three dimensional arrangement, a compact and tightly folded structure is formed and designated as tertiary structure.
- Most of the native proteins occur in this state and the shape is stabilized by intermolecular bonds like
  - ♦ Interpeptide hydrogen bonds
  - ♦ Side chain hydrogen bonds
  - ♦ Ionic bonds and
  - ♦ Hydrophobic interactions, so that the distant regions of the chain are brought closer.
- The folded proteins may be spherical, globular or ellipsoidal. The tertiary structure refers the folding of the helices of globular proteins. X-ray crystallography studies reveals the 3D structure.
- The tertiary structure describes the conformation of the entire protein. Example is myoglobin.
- Myoglobin is the first protein whose tertiary structure was established by Kendrew using X-ray diffraction technique.
  - ♦ Molecular weight of myoglobin is 16,700.
  - ♦ It is an iron containing protein acting as storehouse of oxygen in the muscle.
  - ♦ In contains a single polypeptide chain of 153 amino acids.

- ♦ There are no SH groups, hence no disulfide bonds.
- ♦ The myoglobin chain is folded back on itself in a complex arrangement without any symmetry within the molecule.
- ♦ The backbone of the molecule is made up of 8 more or less straight helical segments interrupted or alternated by non – helical bends which twist to produce the characteristic folding. The longest helical segment has 23 amino acids and the shortest has only seven residues.
- ♦ All the helical segments are right handed α – helix.
- ♦ The tertiary structure gives the myoglobin a very compact structure with little space which can accommodate only four water molecules.
- ♦ All the polar R – groups except two are located on its outer surface and all of them are hydrated whereas, non polar R – groups are located in the interior of the molecule.
- ♦ Each of the 4 proline residues occurs at a turn. Other turns and bends are occupied by serine, threonine and asparagines.

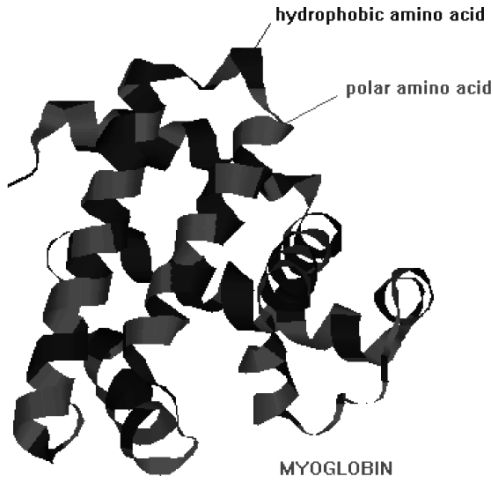


Fig 3.13 Tertiary Structure of Protein

3.15.4 Quaternary Structure: (protein – protein interaction)

- This is the fourth level of organisation in the protein structure shown by proteins containing more than one polypeptide chain.
- These chains may be identical (homogenous quaternary structures) in their primary structure.

- The specific association of number of sub units into a complex, large – sized molecules is called as quaternary structure.
- Again the same forces which are involved in maintaining the tertiary structure are also involved in the formation of quaternary structure to link the various polypeptide chains which are otherwise called as subunits or monomers or promoters.
- Each of the subunits is characterised by its own secondary and tertiary structures.

E.g. Haemoglobin

- Haemoglobin is a protein consists of 4 polypeptide chains of 2 types; two alpha chain and two beta chain.
- The alpha chain has valine at N – terminal and arginine at the C terminal and contains 141 amino acid residues.
- The beta chain has valine at N- terminal and histidine at the C-terminal and contains 146 amino acid residues. So, the protein has the total of 574 amino acid residues in its structure.
- Each of the 4 chains has its own characteristic 2^0 and 3^0 structure.
- Like myoglobin, this protein also contains number of straight chain segments of alpha helix interrupted by non – helical chains.
- The alpha and beta subunits are held together as a pair by ionic and hydrogen bonds. The 2 pairs are then joined with each other by additional ionic (salt) bonds, hydrogen and hydrophobic bonds.
- Thus the four subunits fit together almost tetrahedrally to produce the characteristic quaternary structure of haemoglobin.

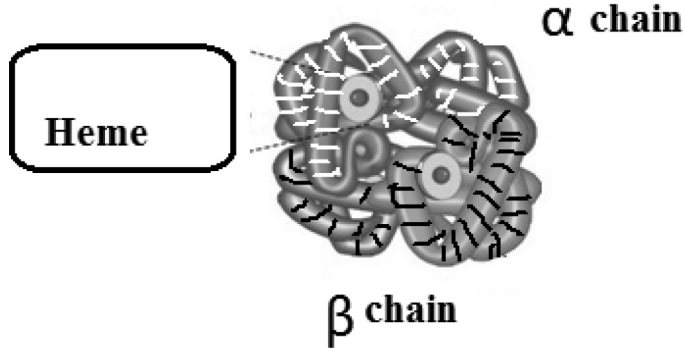


Fig 3.14 Haemoglobin

3.16 BONDS INVOLVED IN PROTEIN STRUCTURE

Proteins are the polymers of amino acids monomers. Any two amino acids monomers are linked together by a chemical bond. There are five types of bonds which occur in proteins. They are the following:

1. Peptide bond
2. Disulfide bond
3. Hydrogen bond
4. Non polar or hydrophobic bond
5. Ionic bond

3.16.1 Peptide bond

Peptide bond is an amide bond when the CO group of COOH group of the amino acids linked with the NH group of NH_2 group of the adjacent amino acid [-CO-NH-]. The peptide bond produces linear primary structure of proteins.

3.16.2 Disulfide Bond [-s-s-]

Disulfide bond is a covalent bond formed between two polypeptide chains by a cystine residue. The disulfide bond is formed by the oxidation of thiol [-SH] group of two cysteine molecules. This result in the formation of molecules of cysteine an amino acid with a disulfide bridge. Oxytocin, a hormone of pituitary gland has a disulfide bond. Here the disulfide bond links two cysteine unit of the same polypeptide chain. Insulin is another example for disulfide bond. In insulin, two polypeptide chains are linked together by 2 disulfide bonds. Another disulfide bond connects the amino acid 6 and 11 in A chain. The disulfide bond produces a linear form and gives the primary structure of protein

3.16.3 Hydrogen bond [>O….HN<]

Hydrogen bond is a weak electrostatic attraction between one electronegative atom and a hydrogen atom covalently linked to a second electronegative atom. The formation of a hydrogen bond is due to the tendency of hydrogen atom to share electrons with two neighbouring atoms, especially O and N. For example the carbonyl O of one peptide bond shares its electrons with the hydrogen atom of another peptide bond. An interaction set in between a C=O group and the proton of an NH or OH group if these groups come within a distance of about 2.8A°. This secondary valence bond is symbolized by a dotted line CO….H-N. Silk fibroin is an example of the presence of hydrogen bond involving the imide

(>NH) and carbonyl (C=O) groups of the peptide bond. Here the hydrogen bonds link the vicinal peptide chains. In keratin of wool, the hydrogen bonds link the side chain so that a single peptide chain is held in a coiled or helical nature and produce secondary structure of protein.

3.16.4 Non Polar or Hydrophobic Bond

The association of non polar groups with each other in aqueous systems because of the tendency of the surrounding water molecules to seek their most stable is called non polar or hydrophobic bond. Many amino acids like alanine, leucine, isoleucine, methionine, tryptophan, phenylalanine and tyrosine have little attraction for water molecules in comparison to the strong hydrogen bonding between water molecules. Such R groups can unite among themselves with the elimination of water to form linkages between various segments of a chain or between different chains. This is very much like the coalescence of oil droplets suspended in water.

3.16.5 Ionic or Electrostatic Bond

Ionic bonds are formed by ionization. The bond is formed by the transfer of electrons (ions) from one atom to another. Ions possessing similar charge repel each other whereas the ions having dissimilar charge attract each other. For example, divalent cations like magnesium may form electrostatic bonds with 2 acidic side chains. Another instance of ionic bonding may be the interaction between the acidic and basic groups of the constituents' amino acids. Ionic bonds occur between positively charged groups and negatively charged groups. The ionic bonds maintain a folded nature of proteins and procedure tertiary structure.

3.17 PROPERTIES OF PROTEIN

Proteins have the following properties

- Most of the proteins are hydrophilic colloids. A few proteins such as insulin, tobacco mosaic viruses etc are crystalline in nature.
- Proteins have no characteristic colour except chromoproteins. Chromoproteins are coloured and the colour is imparted by the metallic prosthetic group.
- A pure protein is tasteless and odourless.
- Proteins are highly viscous in nature. Generally fibrous proteins are more viscous than globular protein.
- The molecular weight of proteins varies from 30,000 to a few million. Protein molecules are much smaller. In fact, the molecular weight of a typical protein is comparable to that of the smallest nucleic acid molecule,

the tRNA. Typical polypeptide chains have molecular weights ranging from 15,000 to 70,000. The average molecular weight of an amino acids is 110, which means that typical polypeptide chains contain some 135 to 635 amino acids. The molecular weight is determined by ultracentrifuge.

- All the proteins are levorotatory. The property is due to the presence of α-amino acids, which are the building blocks of proteins.
- Peptides and proteins undergo hydrolysis by means of dilute HCl or H_2SO_4, alkali or enzyme into their constituent amino acids.
- When proteins are brought into contact with water, protein molecules absorb water and swell up. The polar group likes –COOH, $-NH_2$ and OH become hydrated. Electrolytes, alcohols or sugars in high concentration will complete for the water of hydration, dehydrate the protein and precipitate from solution.
- Most of the proteins are hydrophilic colloids. They are precipitated or coagulated in solutions alkaline to the isoelectric pH by positive ions such as Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}. At this pH the protein has a negative charge.
- Negative ions combine with proteins in solutions more acidic than the isoelectric pH to form salts. The common precipitants in this group are trichloracetic acid, tungstic acids, phosphotungstic acid, sulphosalicylic acid and tannic acids.
- The solubility of many proteins is increased in the presence of small concentrations of various neutral salts. This is referred to as salting in of proteins. Salting in protein is caused by forces of attraction between salt and protein at low salt concentrations leading to increased solubility.
- As the concentration of the neutral salt is increased, the solubility increases to maximum and then starts decreasing and finally the protein is precipitated. This is referred to as salting out. The salting out of proteins is caused by the competition between the protein and the salt at high concentrations. The salting out of proteins is an effective method of purification of proteins.
- Proteins are oxidized by putrefaction processes to form the products such as amino nitrogen compounds, CO_2 and water. The bad smell of the dead and decaying bodies is due to the putrefaction of the protein.
- In proteins there are several free – NH_2 groups from basic amino acids and –COOH groups from acidic amino acids. The groups can ionize in solution producing both anions and cations. Thus the protein molecules exist as zwitterions in solution. It can combine with both acids and bases. Thus proteins are Amphoteric compounds.

- As each protein contain one free NH_2 group and another free carboxyl group, the protein has an ionizing property. Due to these ionisable groups in the protein chains, the proteins also have some definite isoelectric pH at which they do not migrate in an electric field. At the isoelectric pH, the number of positive charges is equal to the number of negative charges, giving a net charge of zero.
- The isoelectric pH of the proteins has been found to depend upon the relative number of acidic or basic groups, which are rendered by amino acids. Serum albumin, which has many acidic amino acids, has an isoelectric pH of 4.7. Giladin with many basic amino acids has 9.0 isoelectric pH.
- Swelling capacity, viscosity and solubility are minimal at the isoelectric pH.
- Proteins are cations at pH values lower than the isoelectric pH and anions at pH values higher than the isoelectric pH.
- Proteins undergo remarkable changes in their solubility, optical rotation and biological properties, when they are treated with heat, X-rays, ultra violet rays, light, alcohol, acetone, aqueous potassium iodide, urea, detergents, etc. These changes occurring in proteins are called denaturation. The changed proteins are called denatured proteins and the unchanged proteins are called native proteins.

3.18 TESTS FOR PROTEINS

Proteins give characteristic colours on treatment with specific reagents. These reactions help to identify proteins. Hence they are called tests for proteins. There are 6 tests to identify proteins. They are the following.

- Biuret test
- Millon's test
- Ninhydrin test
- Xanthoproteic test
- Heller's test
- Nitroprusside test

3.18.1 Biuret Test

The protein solution is warmed gently with 10% solution of sodium hydroxide and then a drop of very dilute copper sulphate solution is added. The formation of reddish violet colour indicates the presence of peptide link –CO-NH. This test is answered by all proteins, peptones and peptides except dipeptide.

The name biuret is derived from the fact that the test is also positive for the compound biuret, H_2N. CONH. $CONH_2$ obtained from urea by heating.

Dipeptides do not answer the biuret test, while all other peptides answer the test. Hence biuret test is important to know whether hydrolysis of proteins is complete or not. If the biuret test is negative, hydrolysis is complete atleast to the dipeptide stage.

3.18.2 Millon's Test

Millon's reagent is a solution of mercuric and mercurous nitrates in nitric acid containing a little nitrous acid. Proteins on adding Millon's reagent followed by heating the solution give a red precipitate or red colour. The test is responded by the proteins having tyrosine. The hydroxyphenyl group of tyrosine is the structure responsible for this test. The non proteinous material having phenolic group, also responds the test.

3.18.3 Ninhydrin Test

When ninhydrin is added to protein solution and the mixture is heated to boil, a blue to violet colour appears. It is a test used to test the presence of amino acids.

3.18.4 Xanthoproteic Test

When a few drops of nitric acid is added to the protein solution, yellow precipitate appears. Xanthoproteic reactions are due to the presence of amino acids namely tyrosine, phenylalanine and tryptophan. The yellow precipitate is due to the formation of metaproteins insoluble in nitric acid.

3.18.5 Heller's Test

In a test tube containing concentrated nitric acid, small quantity of protein solution is slowly added. The two solutions are mixed slowly by rotating on the palm. A white ring appears at the junction of the two solutions.

3.18.6 Nitroprusside Test

Proteins containing free –SH groups (of cysteine) give a reddish colour with sodium nitroprusside in ammoniacal solution.

UNIT IV

LIPIDS AND FATTY ACIDS

Structure, classification, properties and biological significance of lipids. Simple lipids –Monoglycerides, Diglycerides, Triglycerides and Wax - Compound lipids - phospholipids and glycolipids and derived lipids – steroids- cholesterol, ergosterol, lanosterol - terpenes and carotenoids). Fatty acids - saturated, unsaturated fatty acids, hydroxy or oxygenated, cyclic, essential, non-essential fatty acids.

4 LIPIDS

The lipids are a heterogeneous group of compounds related to fatty acids. They are formed of 3 fatty acids joined to an alcohol. Fatty acids and alcohol are the building block components of lipids. Chemically, lipids are defined as the esters of alcohol and fatty acids. The term lipid was first introduced by the German Biochemist Bloor in 1943.

E.g. Fats, Oils, Waxes etc.

4.1 STRUCTURE OF LIPIDS

- Lipids are esters (ester is a compound formed by the combination of an acid with a glycerol with the removal of water) of glycerol and fatty acids.
- Lipid is made up of a glycerol and three fatty acids. Such a lipid is called a triglyceride or a neutral fat.

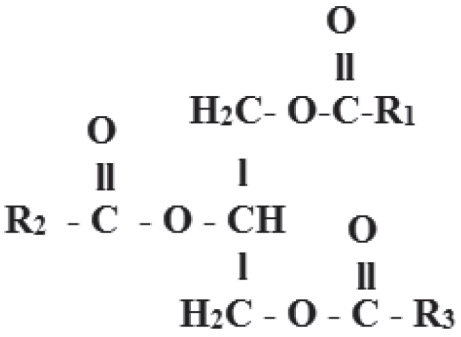


Fig 4.1 Structure of a Simple Lipid

4.2 CLASSIFICATION OF LIPIDS

Lipids are generally classified into three types. They are

1. Simple lipids or homolipids
2. Compound lipids or heterolipids
3. Derived lipids

4.2.1 Simple Lipids or Homolipids

- These are the esters of fatty acids with alcohols like glycerol (in neutral fats) and cetyl alcohol (in waxes).
- Example - fats, oils and waxes.

4.2.2 Compound Lipids

- Compound lipids are the lipids linked to non-lipids. They are also called heterolipids.
- The compound lipid consists of three components namely glycerol, fatty acid and a nonlipid. The non-lipid may be a phosphoric acid or a carbohydrate.
- Compound lipids are of two types. They are phospholipids (phosphatids) and glycolipids (cerebrosides).

4.2.3 Derived Lipids

- Derived lipids are the products of hydrolysis of simple lipids and compound lipids
- Example - steroids, terpenes, fatty acids, alcohols, aldehydes and ketones.

4.3 PROPERTIES OF LIPIDS

Lipids have the following important properties.

- Lipids are insoluble in water but soluble in non-polar organic solvents, such as acetone, alcohol, chloroform, benzene and ether.
- They contain a large proportion of carbon and hydrogen bonds and release large amount of energy on breakdown.
- Fats on hydrolysis with the enzyme lipase, yield fatty acids and glycerol.
- Fats containing saturated fatty acids are solids. Example - animal fats.
- Fats containing unsaturated fatty acids are liquids. Example - plant fats.
- Lipids are greasy to touch and they leave an oil impression on paper.
- Pure fats are colourless and odourless.
- Fats are sparingly soluble in water, i.e. fats are hydrophobic. They are highly soluble in organic solvents like alcohol, ether etc.
- Melting point of fatty acids increases with increase in molecular weight. Saturated fatty acids having higher melting points than fatty acids with unsaturated bonds.

- Specific gravity is less than water. So they are floating on the water surface. Solid fats are lighter than liquid fats (oil).
- Due to the presence of double bonds in unsaturated fatty acids, geometrical isomerism (cis-trans) is possible.
- Fats are bad conductors of heat.
- In water, fats are broken into minute droplets and dispersed. This process is called emulsification. Emulsion is a mixture of lipids and water.
- When a liquid fat is placed on water, it spreads uniformly over the surface of water to form a thin layer. This phenomenon is called spreading.
- The conversion of a fat into glycerol and 3 molecule of sodium salt of higher fatty acids (soap) by boiling with sodium hydroxide is called saponification.
- Rancidity is ill-smelling of fat. It is caused by rancidification. Rancidification is due to auto-oxidation of fats. The fat which has become rancid has a disagreeable odour and taste and is unfit for consumption. Rancidity occurs in two ways. They are hydrolytic rancidity and oxidative rancidity.

  a. Hydrolytic Rancidity: When butter or other fats are stored, they become rancid and unpalatable. This is due to the activity of microorganisms. Micro-organisms secrete enzymes like lipase. Lipase hydrolyses the fat into fatty acid and glycerol. The fatty acid gives unpalatable smell. This is known as rancidity. Rancidity may be prevented by refrigeration or by excluding water.

  b. Oxidative Rancidity: Oils and unsaturated fatty acids are easily oxidized at the double bonds by atmospheric air at room temperature to yield short chain fatty acids (with C_4 to C_{10}) and aldehydes. Rancid taste and odour of the fats are due to the atmospheric oxidation. This is called oxidative rancidity.
- The unsaturated fatty acids are saturated by the reaction with H_2 in the presence of nickel or platinum or palladium as catalyst. If there is one double bond, one molecule of H_2 will be taken. If two bonds are present, 2 hydrogen molecules will be taken. Thus for saturation, the number of hydrogen molecule taken will be equal to the number of double bonds.
- Unsaturated fatty acids and esters are giving addition reactions with halogens in the presence of acetic acid or methanol at room temperature.

- Fatty acids with unsaturated bonds are oxidized by ozone or potassium permanganate. Reaction with ozone is called ozonolysis and the product is an ozonide. The ozonides on hydrolysis give two molecules of aldehydes.
- When fats are heated with $NaHSO_4$ (sodium bisulphate) or potassium hydrogen sulphate, acrolein (unsaturated aldehyde) having a pungent odour is formed. This is a test for a fat containing glycerol.

4.4 BIOLOGICAL SIGNIFICANCE OF LIPIDS

Lipids constitute an essential component of cell. So it has enormous biological importance.

- Lipid contains large amount of energy. One gram of lipid on oxidation releases 9.3 kilocalories of heat. But the same amount of carbohydrate releases 4.5 kilocalories only.
- Lipids are insoluble in water. So they are readily stored in the body as a food reserve. Eg.Triacylglycerol is a food reserve. It is used as a substrate for oxidation.
- Lipids constitute an important component of cell membrane.
- Phospholipids transport cations across the lipid layer of biomembranes.
- Lipids serve as an electro insulating material in the myelin sheath of neurons.
- Subcutaneous fats of mammals act as an insulator against excessive heat loss to the environment.
- Amphipathic lipids are emulsifiers. The process of emulsification is of great metabolic significance. In fact, the fats have to be emulsified before they can be absorbed by the intestinal wall. This process is accomplished by the bile juice secreted by the liver.
- Lipids of connective tissue of internal organs protect them from eventual damage on exposure to mechanical action. E.g.Triacylglycerides subcutaneous fat deposits act as an insulator against mechanical trauma.
- Under physiological conditions, certain lipids function as solvents to dissolve other lipids. E.g. Bile acids are solvents for insoluble vitamins in the intestine.
- The major group of hormones is formed of steroids. They regulate a large variety of physiological functions. E.g. Sex hormones and adreno corticoids.
- Prostaglandins are modulators of hormones, derived from fatty acids.

- Vitamin – D, calciferol is a steroid derivative. It possesses a steroid structure. It is synthesized from cholesterol.
- Lipids act as carrier of natural fat soluble vitamins such as A, D and E.
- Lipids are essential for the activation of enzymes. Eg. Glucose-6-phosphatase, β – hydroxybutyric dehydrogenase.

4.5 SIMPLE LIPIDS (HOMOLIPIDS)

- A simple lipid is formed when three molecules of fatty acids combine with one molecule of glycerol. In this process, three molecules of water are released. They are further classified into fats and waxes.

Fats - They are esters of fatty acids with a trihydroxy alcohol, glycerol. A fat is solid at room temperature.

Waxes - They are esters of fatty acids with high molecular weight of monohydroxy alcohols.

- When three palmitic acids are combined to glycerol, it is called tripalmitin.
- When three stearic acids are combined to glycerol, it is called tristearin.
- When three oleic acids are combined to glycerol, it is called triolein.
- When three fatty acids of a lipid are similar, the fat is called a neutral fat. Eg. Tripalmitin, tristearin, triolein etc.
- When the three fatty acids in a fat are different, the fat is called a mixed fat.
- When the mixed fat contains two palmitic acids and one stearic acid, the fat is called dipalmito stearin.

4.5.1 Monoglycerides

- When only one fatty acid is combined to a glycerol, it is called monoglyceride.
- When the fatty acid is palmitic acid, then it is called monopalmitin.
- When the palmitic acid is attached to the first carbon atom of glycerol, then the monopalmitin is named as 1-monopalmitin. If it is attached to the second carbon, it is called 2-monopalmitin.

4.5.2 Diglycerides

- When two fatty acids are combined to a glycerol, the fat is called a diglyceride. When the fatty acid is palmitic acid, the diglyceride is called a dipalmitin.
- When the palmitic acids are attached to first and second carbon atoms of glycerol, the fat is called 1,2 dipalmitin. Similarly, there are 1,3 dipalmitin and 2,3 dipalmitin etc.

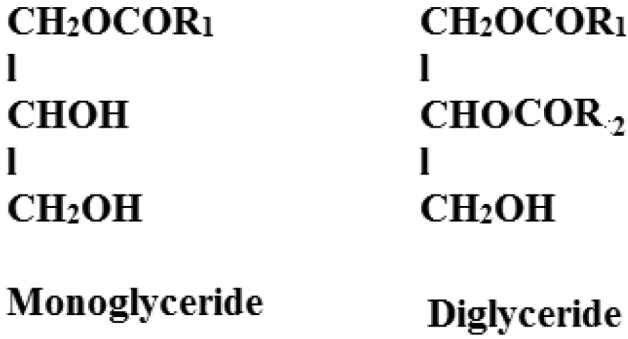


Fig 4.2 Monoglyceride and Diglyceride

4.5.3 Triglyceride

- Triglyceride is a simple and most abundant lipid. It is often referred to as fat or neutral fat.
- Glycerol reacts with 3 molecules of fatty acids to give a molecule of triglyceride with 3 water molecules.
- There are many different types of triglyceride, with the main division between saturated and unsaturated types. Saturated fats are "saturated" with hydrogen — all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These have a higher melting point and are more likely to be solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These have a lower melting point and are more likely to be liquid at room temperature.
- A glycerol reacts with three palmitic acids to form tripalmitin.
- A glycerol reacts with three oleic acids to form triolein.
- A glycerol reacts with three stearic acids to form tristearin.

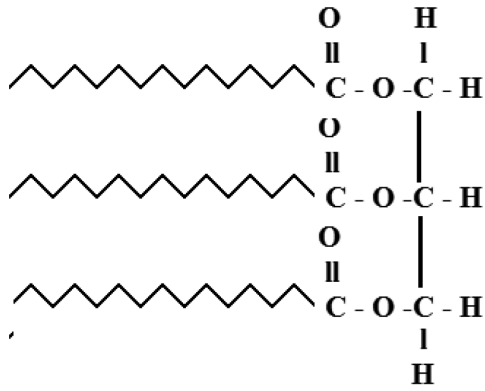


Fig 4.3 Triglyceride

4.5.4 Waxes

- Waxes are simple lipids. They are solid lipids. Wax means the material of honey comb.
- They are esters of fatty acids with monohydric alcohols of higher molecular weight.
- The fatty acids of waxes have 14C atoms to 36C atoms.
- Alcohols have 16C atoms to 36C atoms.
- Waxes are secreted by bees, cutaneous glands and plants.
- Hair, wool and fur are coated with wax.
- Waxes are acting as a protective coating to keep the skin pliable, lubricated and water proof.
- Due to the deposition of waxes on the leaves of certain plants, they are shiny.
- Waxes are the chief storage form of fuel in plankton.
- Waxes act as major food and store lipids in them.
- The common fats are

  i. Sperm whale wax (Spermaceti)
  ii. Bees wax
  iii. Carnauba wax
  iv. Ambretolide

- The bees-wax contains palmitic acid and myricyl alcohol and it is called myricyl palmitate.
- The sperm whale wax contains palmitic acid and cetyl alcohol and it is called cetyl palmitate.

Palmitic acid + Myricyl alcohol ⟶ Myricyl palmitate

Palmitic acid + Cetyl alcohol ⟶ Cetyl palmitate

- Carnauba wax is the hardest known wax. It contains fatty acids esterified with tetracosanol and tetratriacontanol.

Ambretolide is composed of hydroxy fatty acid and alcohol.

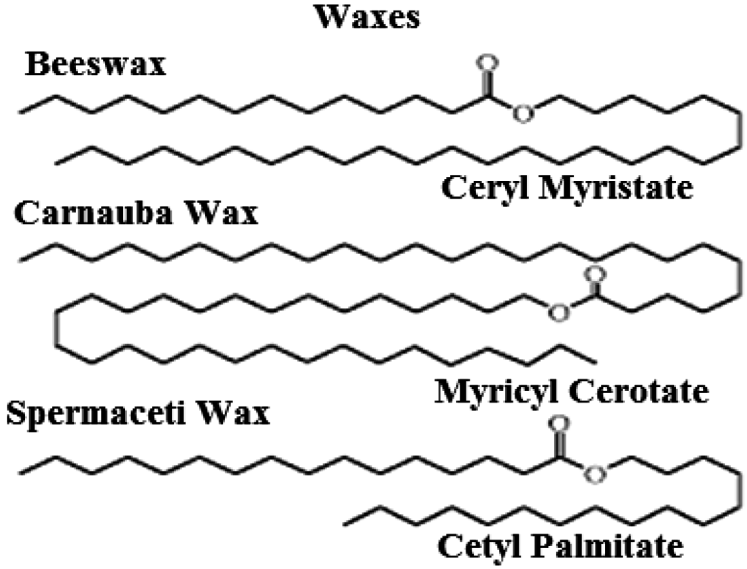


Fig 4.4 Common Fats

Properties of Waxes

- They have fully reduced hydrocarbon chain.
- They are insoluble in water.
- They are resistant to atmospheric oxidation.
- They have high melting points.
- They are chemically inert.
- They are not digested by enzymes but may be split by hot alcoholic KOH.
- Bees wax is used in sealing the honey comb cells to store the food for the winter season.

- Waxes provide water barrier quality for aquatic animals, insects and birds.
- Waxes serve as protective coatings on fruits and leaves.

4.6 COMPOUND LIPIDS

- Compound lipids are the lipids linked to non-lipids. They are also called heterolipids.
- The compound lipid consists of 3 components, namely glycerol, fatty acid and a non-lipid. The non-lipid may be a phosphoric acid or a carbohydrate.
- Compound lipids are of two types. They are phospholipids (phosphatids) and glycolipids (cerebrosides).

4.6.1 Phospholipids

- Phospholipids are compound lipids. They are formed by glycerol, phosphoric acid and fatty acids. They are also called phosphatids.
- They are the structural component of membranes and so are abundant in brain, kidney etc.
- Due to the presence of phosphoric acid group, phospholipids behave as polar lipids.
- They are hydrophilic in nature.
- Phospholipids are amphipathic.
- Phospholipids are further classified into
  1. Lecithins
  2. Cephalins
  3. Plasmologens
  4. Phosphoinositides
  5. Phosphingosides

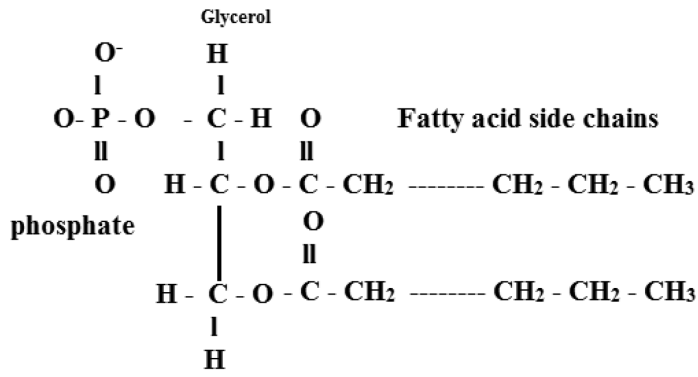


Fig 4.5 Phospholipid

4.6.1.1 Lecithins

- Lecithins are compound lipids. They are phospholipids.
- Lecithins are the esters of glycerol with fatty acids.
- Two hydroxyl groups of glycerol are linked to two fatty acid molecules.
- The third hydroxyl group of glycerol is linked to a phosphoric acid molecule.
- The phosphoric acid group links with choline. Choline is a nitrogenous base.
- It is found in all animals and plants. In animals, it is found in the brain, nerve tissues, sperm and egg yolk. In plants it is abundant, in seeds and sprouts. Soya bean is the main source.
- Lecithins are yellowish grey solids, soluble in ether, alcohol etc.
- Lecithins are required for normal transport and utilization of other lipids particularly in the liver.
- They swell in water and form colloidal solutions.
- They are dextrorotatory.
- Lecithins on hydrolysis yield choline and two molecules of fatty acids. On partial hydrolysis by lecithinase, (found in snake venoms) only one fatty acid molecule is removed. The product without one fatty acid molecule is called lysolecithin.
- When lysolecithin is injected into the blood stream either by snake bite or by syringe, rapid rupture of red blood corpuscles takes place. This is called hemolysis.
- It has an important role in fat metabolism in liver.
- There are twenty forms of lecithins in human red blood corpuscles.

Functions of Lecithin

- It is a component of plasma membrane.
- It transports fats and oils through the plasma membrane.
- It brings about cell communication.
- It is an emulsifier. Hence it helps to absorb fats and oils from the digested food.
- It is a source of B vitamins such as choline and inositol.
- Lecithin improves memory.
- Daily intake of lecithin improves brain chemical activity.
- It improves learning.

- Lecithin is essential for liver metabolism.
- It supports regeneration of liver cells.
- It helps to heal liver from hepatitis.
- It needs for healthy gall bladder and heart.
- It helps to reduce cholesterol.
- It is used to treat treat anxiety and eczema.
- It acts as moisturizer on the skin.
- It relieves arthritis.
- It is an ingredient in eye medicines.
- It is used as food additive.

4.6.1.2 Cephalins

- Cephalins are compound lipids. They are phospholipids. They are otherwise called phosphatidyl ethanolamine.
- They resemble lecithins except that the choline is replaced by ethanolamine or serine. Cephalin consists of 4 components, namely glycerol, fatty acids, phosphoric acid and ethanolamine.
- Cephalin is the ester of glycerol and fatty acids.
- The two hydroxyl groups of glycerol are linked to two fatty acids.
- The third hydroxyl group of glycerol is linked to a phosphoric acid.
- The phosphoric acid is linked to ethanolamine.
- There are two types of cephalins. They are α-phosphatidyl ethanolamine and α-phosphatidyl serine.
- Cephalins are more acidic than lecithins.
- Cephalins are less soluble in alcohols than lecithins.
- Venoms containing lecithinase split off the fatty acids from cephalins leaving hemolytic lysocephalins.
- Cephalin occurs in all cells, soyabean oil etc. It is abundant in brain cells. The important fatty acid constituents of cephalin are stearic, oleic, linoleic and arachidonic acids.

Functions of Cephalins

- Cephalin is a component of plasma membrane.
- It is found in the inner lipid layer of plasma membrane.
- It maintains the fluidity of the plasma membrane.

- The brain cells contain 45% of cephalins.
- It is involved in membrane fusion in cytokinesis.
- It helps for the secretion of lipoprotein in the liver.
- It propagates infectious prions without the help of nucleic acids.
- It improves blood clotting.
- It causes deterioration of processed foods such as chocolate, soyabean milk, infant formula etc.
- It causes vascular diseases, diabetes and cancer.

4.6.1.3 Plasmologens

- Plasmologens are phospholipids.
- These are found in brain, muscle and seeds of higher plants.
- These are not significantly found in plant tissues.
- Structurally, plasmologens resemble lecithins and cephalins except in having one unsaturated ether group in the place of fatty acid groups.
- It is soluble in all lipid solvents. Since the base is a nitrogen base it may be choline, ethanolamine or serine. Due to this, plasmologens are of three types.

Serine cephalin - This compound contains amino acid serine instead of ethanolamine.

Phosphatidyl inositol - This cephalin contains inositol instead of serine. On hydrolysis, the phosphoinositides yield 1 mole of glycerol, two moles of fatty acid, 1 mole of inositol and 1,2 or 3 moles of phosphoric acid.

Accordingly mono, di or tri phosphoinositides are formed.

4.6.1.4 Phosphoinositides

- In these phospholipids a cyclic hexahydroxy alcohol inositol replaces the base. So these lipids are otherwise known as lipoinositol.
- Occurrence - Phospholipids of brain tissues, soyabeans and also in nervous tissue.
- They play an important role in transport processes in cells.

4.6.1.5 Phosphingosides (Sphingomyelins)

- These are phospholipids. Sphingomyelins differ radically from cephalin in composition.

They contain fatty acid, phosphoric acid, choline and a complete amino alcohol sphingosine. The fatty acid is attached to amino group of sphingosine by amide linkage. The phosphoric acid is attached to one of the hydroxyl group of sphingosine.

- On hydrolysis, the phosphingosides yield equimolecular mixture of fatty acid, phosphoric acid, choline and sphingosine or dihydrosphingosine (sphinganine). No glycerol is formed.
- It is commonly found in nerve tissue especially in the myelin sheath of the nerve. So the name is sphingomyelins. It is found in brain and spinal cord and in plant seeds.
- Sphingomyelins are electrically charged molecules. They contain phosphocholine as their polar head groups.
- Sphingosine carries the phosphoric acid on its primary alcohol group and the fatty acid by amide linkage on its primary amino group.
- It is absent in plants and micro-organisms.
- In a syndrome called Niemann-Pick disease, a rare genetic disorder (inherited as an autosomal recessive condition) the sphingomyelins are stored in the brain in large quantities. The disease is due to the deficiency of the enzyme sphingomyelinase.

4.6.2 Glycolipids

- Glycolipids are compound lipids containing sugar and high molecular weight fatty acids.
- It is found in the brain, adrenals, kidney, spleen, liver, leucocytes, thymus, lungs, retina, egg-yolk and fish sperm.
- Cerebrosides and gangliosides are important glycolipids.
- Cerebroside is relatively abundant in liver, spleen and medullated nerve fibres.
- Cerebroside is made up of 3 components namely,
  i. Sphingosine
  ii. Fatty acid
  iii. Carbohydrate

4.6.2.1 Sphingosine

The sphingosine is an amino alcohol with a long unsaturated hydrocarbon chain.

The molecular formula is $C_{16}H_{37}NO_2$.

It is a nitrogen containing base.

4.6.2.2 Fatty acid

The fatty acid is linked to the amino group of sphingosine by an amide linkage.

The sphingosine and fatty acid together form a waxy substance called ceramide.

4.6.2.3 Carbohydrate

The carbohydrate is linked to the primary alcoholic group of sphingosine through a glycosidic linkage.

The carbohydrate may be glucose or galactose.

If the carbohydrate is a glucose unit, then the cerebroside is called glucocerebroside.

If the carbohydrate is a galactose unit, then the cerebroside is called galactocerebroside.

4.7 SULFUR CONTAINING GLYCOLIPIDS

Glycolipids containing sulfur are called sulfolipids and sulfatides.

4.7.1 Sulfolipids

- Sulfolipids are widely distributed in plants.
- It is localised in chloroplasts.
- It is also found in the chromatophores of photosynthetic bacteria.
- The sulfur in this lipid is in the sulfonic group with a hexose. So it is called as sulfolipids.

4.7.2 Sulfatide

- It is a sulfur containing glycolipid. It is a sulfate ester analogue to phrenosin.
- It is present in the white matter of brain.
- The sulfate is present in the ester linkage at C_3 of galactose portion of the molecule.
- Members of this cerebroside sulfuric esters are called as sulfatide.

4.8 DERIVED LIPIDS

- Derived lipids are the substances produced from simple and compound lipids through the process of hydrolysis. There are many different types of derived lipids, including alcohols, monoglycerides, diglycerides, fatty acids, steroids, terpenes and carotenoids, with the last three groups being the most common.
- Steroids are derived lipids that are found in almost every species of animal and do not contain fatty acids. Terpenes are found mainly in plants and this group includes substances such as natural rubber and many essential oils. Carotenoids are a type of tetraterpene produced only by plants, although they are widespread in both plants and animals as they remain in the body after carotenoid producing plants are eaten.

4.8.1 Steroids

- The steroids are often found in association with fat. Since they contain no fatty acids, they are non-saponifiable, i.e., cannot be hydrolyzed by heating with alkali to yield soaps of their fatty acid components.
- All steroids may be considered as derivatives of a fused and fully saturated ring system called cyclopentanoperhydrophenanthrene or sterane.
- This system consists of 3 cyclohexane rings (A, B and C) fused in nonlinear or phenanthrene manner and a terminal cyclopentane ring (D).

Steroids may be divided in the following manner

- Sterols: Cholesterol, Ergosterol, Coprosterol; Bile acids: Glycocholic acid and Taurocholic acid; Sex hormones: Testosterone, Estradiol; Vitamin D: Vitamin D_2 and D_3; Adrenocortical hormones: Corticosterone; Cardiac glycosides: Stropanthin; Saponins: Digitonin. Some common steroids are: Cholesterol

4.8.2 Cholesterol

- Cholesterol has a molecular formula, $C_{27}H_{45}OH$.
- In addition to an OH group at C_3, there is a double bond at C_5.
- The hydroxyl group constitutes its polar head and the rest of the molecule is hydrophobic.

- It is a white crystalline solid and is optically active, [α] D 39°.
- It has a melting point of 149°C.
- Cholesterol is generally believed to be notorious as a major cause of heart disease.
- There are 2 types of cholesterol, the low density lipoprotein cholesterol (LDL-C) and the high-density lipoprotein cholesterol (HDL-C).
- It is the principal sterol of higher animals and is especially abundant in nerve tissues and in gallstones.
- It occurs either free or as fatty esters in all animal cells.
- It was first isolated in 1784, from human gallstones which consist almost entirely of cholesterol and hence so named (cholesterol literally means 'solid alcohol from bile').
- Its main sources are fish liver oils and the brain and spinal cord of cattle.
- Cholesterol is not found in plant fats.

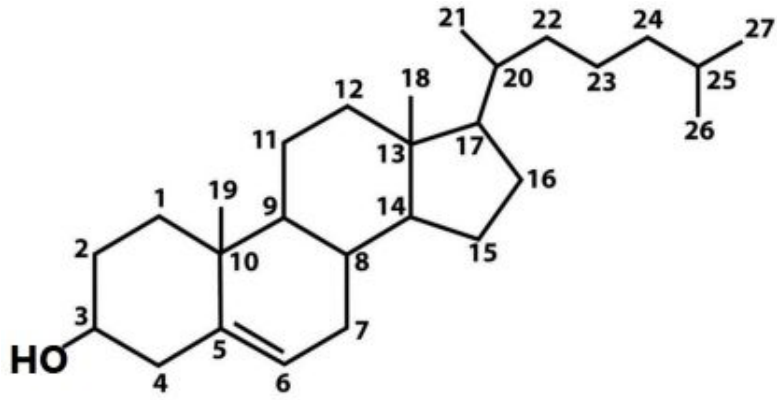


Fig 4.6 Structure of Cholesterol

4.8.3 Ergosterol

- It is present in ergot (hence its nomenclature), yeast and the mould Neurospora.
- Its parent hydrocarbon is ergostane, $C_{28}H_{50}$.
- Ergosterol has a molecular formula, $C_{28}H_{43}OH$ with one **OH group at C_3** and **3 double bonds at C_5, C_7 and C_{22}**.
- It is also **optically active**, [α] D -135°.

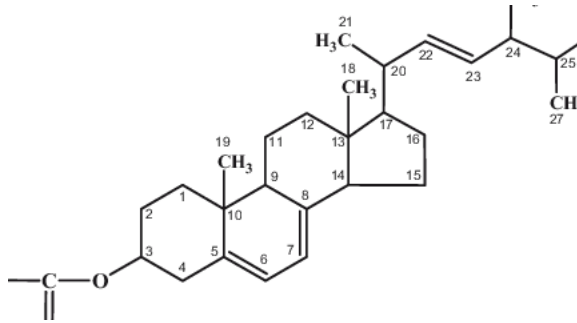


Fig 4.7 Structure of Ergosterol

4.8.4 Lanosterol

- It is a major constituent of wool fat and is also present in minor quantities in liver and yeast.
- Lanosterol is a C30 compound with twin methyl groups at C_4 and a third angular methyl group on C_{14}.
- There are 2 double bonds at C_8 and C_{24}.
- It is an intermediate in the biosynthesis of cholesterol.

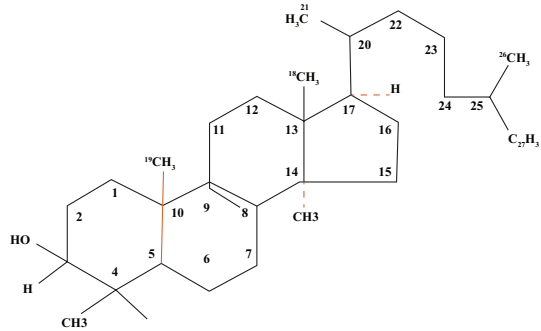
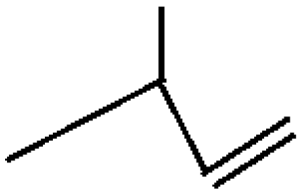


Fig 4.8 Structure of Lanosterol

4.9 TERPENES

1. Terpenes are a group of derived lipids.
2. They are non-saponifiable lipids found in plants.
3. They cannot yield soap on hydrolysis.
4. Chemically, terpenes are hydrocarbons**.**
5. They have less than 40 carbon atoms.
6. Terpenes belong to isoprenoid groups.



$$CH_3$$
$$|$$
$$_2HC = C - CH = CH_2$$

ISOPRENOID

Fig 4.9 Isoprenoid

7. Terpenes are constructed out of isoprene units. Each isoprene unit has two ends, namely a head and a tail. The head is the branched end and the tail is the unbranched end.

8. Each terpene has two or more isoprene units. The isoprene units are joined in a head to tail fashion. That is, the tail end of one isoprene unit is joined to the head end of another isoprene units.

9. Terpenes are classified into 6 types based on the number of isoprene units present in them. They are the following:

Type of Terpene	Number of Carbon atoms	Isoprene Units
Hemiterpene (half)	C5	1
Monoterpene	C10	2
Sesquiterpene (one and a half)	C15	3
Diterpene	C 20	4
Sesterterpene	C25	5
Triterpene	C30	6
Tetraterpene	C40	8
Rubber	>500	>100

- **Monoterpenes:** Monoterpenes contain 2 units of isoprene E.g. *Myrcene* from oil of bay, *geraniol* from rose oil, *limonene* from lemon oil and *menthol* from pepper mint oil. The fragrances of many plants arise from these terpenes. The monoterpenes are used as perfumes.
- **Sesquiterpenes:** Sesquiterpenes contain 3 units of isoprene. They are also used as perfumes. E.g. *Farnesol* found in essential oils.
- **Diterpenes:** Diterpenes contain 4 units of isoprene. They are found as substituent of the resins and balsams.
- **Triterpenes:** Triterpenes contain 6 isoprene units. They do not occur in nature. But they are produced as intermediate products during the biosynthesis of cholesterol.

- **Tetraterpenes:** Tetraterpenes contain 8 isoprene units. E.g. Carotenoids.
- **Polyterpenes:** Polyterpenes contain more than 8 units of isoprene units. E.g. Rubber. It is present in the latex of many tropical plants. A molecule of rubber is composed of about 500 to 5000 isoprene units.

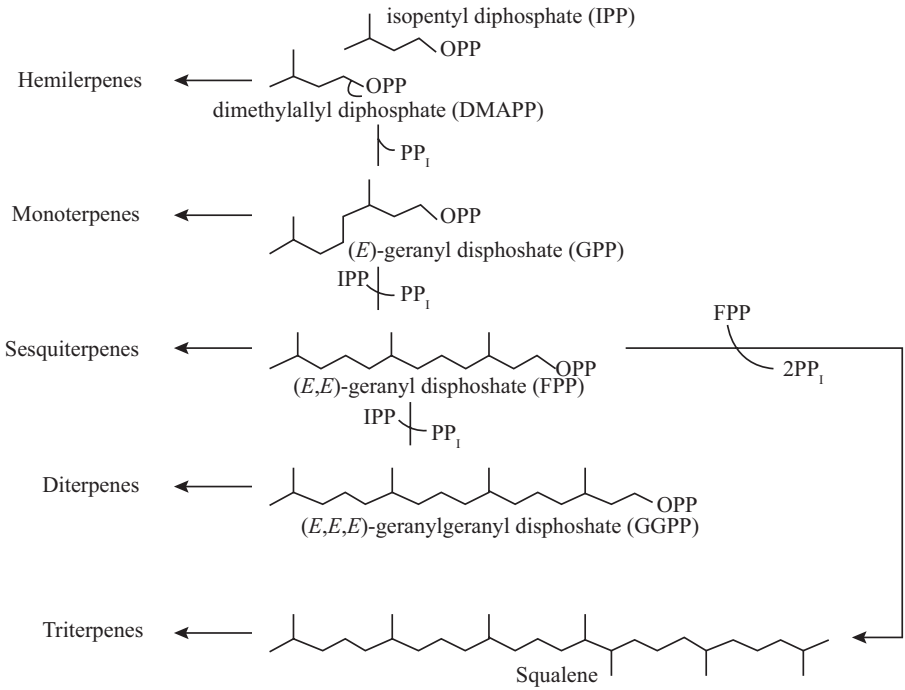


Fig 4.10 Terpenes

4.10 CAROTENOIDS: (CHROMOLIPIDS)

- Carotenoids are a group of derived lipids. They are also called chromolipids or lipochromes.
- They are non saponifiable lipids.
- They cannot yield soap on hydrolysis.
- They are exclusively of plant origin, but are distributed both in plants and animals. They are found in tomato, carrots and leaves.
- Chemically, carotenoids are hydrocarbon.
- They have 40 carbon atoms.
- They are isoprene derivatives.
- They are tetraterpenes containing 8 isoprene units.

- They have high degree of unsaturation containing many conjugated double bonds.
- They are coloured red or yellow.
- They exhibit isomerism and exist in many forms. Examples: Lycopene found in tomato, Alpha and beta carotene found in carrot, Xanthophylls.

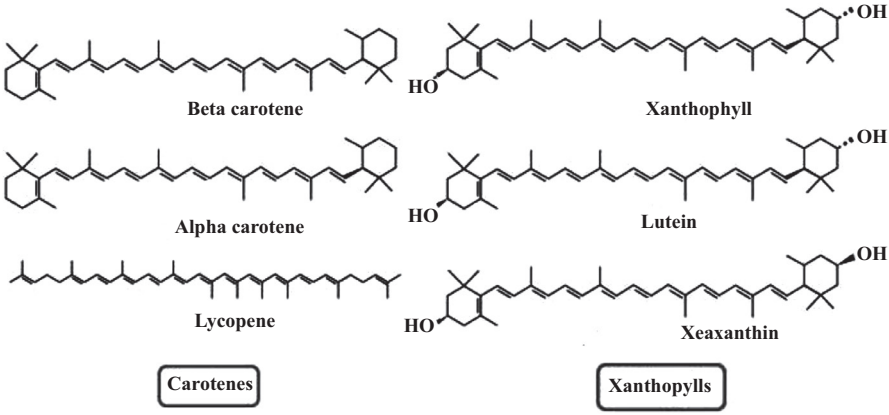


Fig 4.11 Carotenoids

4.10.1 Lycopene

Lycopene is a carotenoid found in tomato and other fruits. It is a tetra terpene. It is a highly unsaturated, unbranched, long chain hydrocarbon (polytene). It is composed of two identical units (C_{20} H_{28}) joined by a double bond between C_{15} and C^{1}_{15}. Each of these two units, in turn is derived from 4 isoprene units (C_5 H_8). A molecule of lycopene contains 13 double bonds of which 11 are conjugated.

4.10.2 Carotene

Carotene is a carotenoid found in carrots. Carotene was first isolated from carrot by Wackenroder. As it was isolated from carrot, it was named as carotene. Alfalfa is another source of carotene. Carotene is a tetraterpene. It is a highly unsaturated, unbranched long chain hydrocarbon (polytene). It exists in three forms, namely alpha- carotene, beta- carotene and gamma- carotene. Alpha carotene is a violet crystal, beta carotene is a red crystal and gamma carotene is a dark red crystal. Beta – carotene is the precursor of vitamin A. The carotene has the molecular formula like that of lycopene ($C_{40}H_{56}$). It is a derivative of isoprenoid. Carotene is formed of two identical units and each unit is formed of $C_{20}H_{28.}$ The two units are joined by a double bond between C_{15} and $C^{1}_{15.}$ Each of these units is derived from 4 isoprene units, namely C_5 $H_{8.}$ Alpha and Beta- carotene have two rings one

at each end. Each is formed by the closing of the end of lycopene chain by the disappearance of double bonds. The ring is structurally related to ionone alpha and beta- carotene differs in the position of double bonds in the rings.

4.10.3 Xanthophylls

Xanthophylls is a yellow carotenoid pigment. Xanthophylls are found in leaves corn and crustaceans. The Xanthophylls of leaf are called lutein, that of corn is called Zeaxanthine and that of crustaceans is called as taxanthine. Lutein is derived from alpha- carotene while Zeaxanthine is derived from beta-carotene. Xanthophylls are characterized by the presence of hydroxyl groups (OH) in ionone rings of carotenes, in the para position to the long chain.

4.11 FATTY ACIDS

- Fatty acids are aliphatic straight chain hydrocarbon compounds with a terminal carboxyl group.
- They are the building blocks of lipids.
- There are about 200 fatty acids.

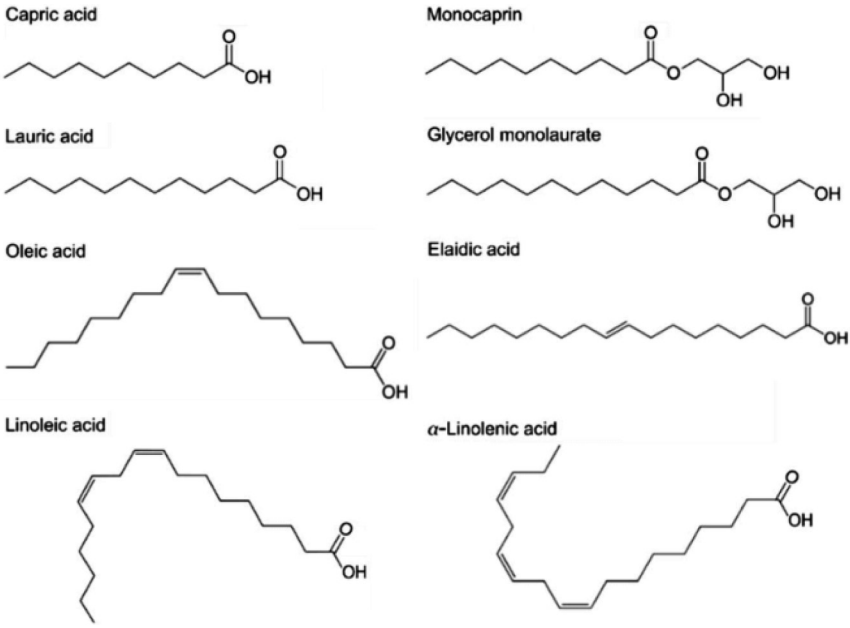


Fig 4.12 Structure of Fatty acids

- Fatty acids are long chain organic compounds containing even number of carbon atoms from 4 to 24.

- They have a single carboxyl group and a long non-polar hydrocarbon tail. The non-polar tail gives most lipids their water insoluble and oily or greasy nature.
- In fatty acid, carbon atoms are numbered starting at the carboxyl terminus.
- Carbon atoms 2 and 3 are often referred to as α and β respectively. The methyl carbon atom at the distal end of the chain is called ω carbon.
- Fatty acids do not occur in free or unbound form in cells or tissues. They are bound in lipids through covalent bonds. However, they are released on hydrolysis.
- The hydrocarbon chain is almost unbranched very rarely it is branched.
- Fatty acids with 16 and 18 carbon atoms are most abundant in nature.
- The fatty acid, containing a single bond, is called saturated fatty acid. The suffix ***–anoic*** indicates a fully saturated fatty acid.
- The fatty acid containing one or more double bonds is called unsaturated fatty acid. The suffix ***enoic***, ***dienoic*** and ***trienoic*** suggest the presence of one, two and three double bonds in the molecule.
- A C_{18} fatty acid without double bond is called octadecanoic acid; A C18 fatty acid with one double bond is called octadecenoic acid, with two double bonds is called octadecadienoic acid and with three double bonds is called octadecatrienoic acid.

4.11.1 Classification of Fatty Acids

Fatty acids are classified into two types based on the absence or presence of double bonds. They are

- Saturated fatty acids – without double bonds
- Unsaturated fatty acids – with one or more double bonds

The fatty acids containing hydroxyl groups are called hydroxy fatty acids or oxygenated fatty acids. The fatty acids containing ring structure are called cyclic fatty acids. Again, based on their requirement in the diet, fatty acids are classified into two types, namely

- Essential fatty acids
- Non-essential fatty acids

Thus fatty acids are classified into 6 types. They are the following:

1. Saturated fatty acids
2. Unsaturated fatty acids

3. Hydroxy fatty acids or oxygenated fatty acids
4. Cyclic fatty acids
5. Essential fatty acids
6. Non-essential fatty acids

4.12 SATURATED FATTY ACIDS

- The saturated fatty acids have single bonds. They have maximum possible number of hydrogen atoms. At one end, there will be an acid group (COOH). At the other end, there will be a methyl group (CH_3). In between these two groups, there will be CH_2 groups. The saturated fatty acids end with the suffix *–anoic*. Eg. *Octanoic acid*, *Decanoic acid*, *Butanoic acid* etc.
- The saturated fatty acids are straight chain acids. In addition to these straight chain acids, there are some branched chain acids, with odd or even number of carbon atoms. But these are the minor components of natural fats and oils. Isopalmitic acid, ante-isopalmitic acid, tuberculo-stearic acids are some examples of branched chain fatty acids identified in fats.

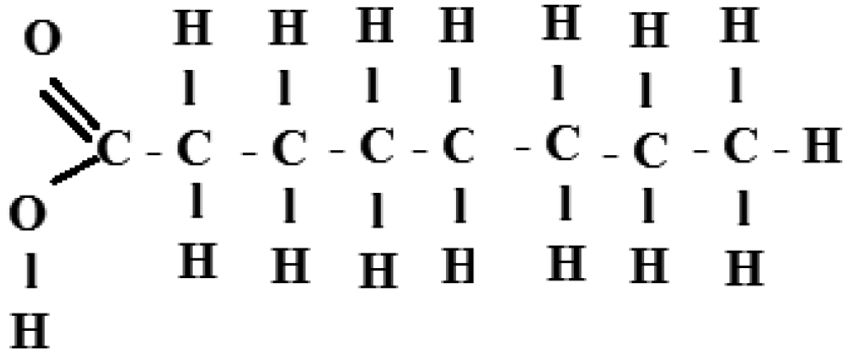


Fig 4.13 Saturated Fatty acids

4.13 UNSATURATED FATTY ACIDS

- The unsaturated fatty acids have one or more double bonds, i.e. 1 to 6 double bonds. These double bonds may occur after 9,12,15,18 etc carbon atoms. The unsaturated fatty acids are named with the suffix – enoic. Based on the number of double bonds, unsaturated fatty acids may be called as monoenoic (one =), dienoic (two =), trienoic (three =), tetraenoic (four =) and pentaenoic acids etc.

- In most of the unsaturated fatty acids, there is a single double bond lying between carbon atom 9 and 10. This is designated as δ^9. The symbol δ with the superscript number 9 indicates the position of double bond. When there are more than one double bonds, the additional, bonds occur between the δ^9 double bond and the methyl terminal end of the chain.
- The symbol 18:0 denotes a C_{18} fatty acid with no double bonds. The number 18:2 signifies that there are two double bonds. Similarly, the symbol 18:2, 9, 12 is used to denote an 18 carbon fatty acid with two double bonds in the 9 and 12 positions. When two or more double bonds are present in a fatty acid, the double bonds are never conjugated. But the double bonds are separated by a methylene group.
- Unsaturated fatty acid containing more than one double bond is called polyunsaturated fatty acid. The unsaturated fatty acids are common in living organism.
- Nemotinic acid is one of the few naturally occurring compounds containing allene group along with single, double and triple carbon-carbon linkages. It is excreted in the growth medium by Citrivorium mould. Santalbic acid, a major component of seed oil of sandal wood contains one acetylene group.
- Due to the presence of C=C in unsaturated fatty acids, geometrical isomerism is possible. Depending on the spatial arrangements of groups or atoms around the C=C two isomers are possible. They are cis and trans isomers. In cis isomers, identical groups are on the same side of the C=C. In trans isomers, identical groups are occupying opposite sides of C=C. Examples: Oleic acid and elaidic acid.
- In unsaturated fatty acid, if the double bonds are in alternate carbon atoms then it is said to be a conjugated unsaturated fatty acid. Example: α – eleostearic acid.
- If the double bonds are not in alternate positions, unsaturated fatty acid is called non conjugated fatty acid. Example : Linoleic acid
- The hydro carbon chain of saturated fatty acid is in a zig-zag manner. The zig-zag configuration is a stable configuration. Eg. Stearic acid.
- When a cis double bond is inserted into a stearic acid, an oleic acid is formed. Oleic acid has got the bent structure.

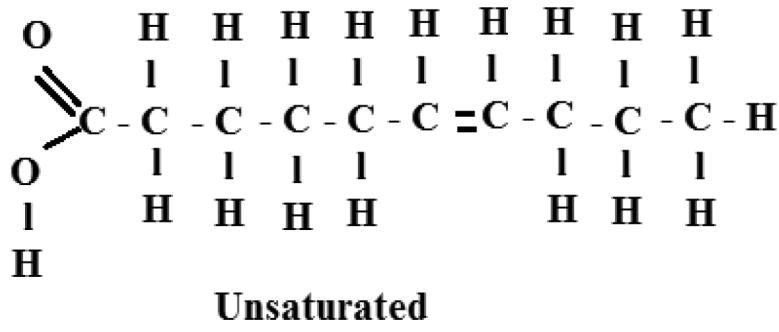


Fig 4.14 Unsaturated Fatty Acids

- Fatty acids containing hydroxyl groups are called hydroxy fatty acids.
- **Ricinoleic Acid -** It is a C_{18} acid. A double bond is present at C_9 carbon atom and hydroxyl group on C_{12}.
- **Cerebronic Acid -** It is obtained from animal lipid. It is also a hydroxy acid. The hydroxyl group is present at the C_2 carbon atom.

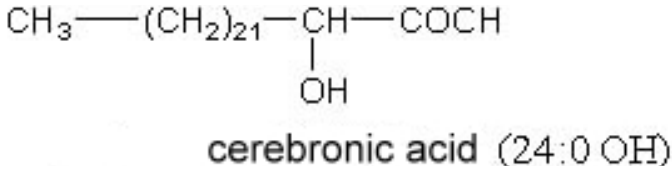


Fig 4.15 Cerebronic acid

4.14 CYCLIC FATTY ACIDS

- Fatty acids with cyclic structures are rare occurrence. Chaulmoogric acid, hydnocarpic acid, lactobacillic acid and sterculic acids are examples of cyclic fatty acids.
- **Chaulmoogric Acid -** It is present in chaulmoogra oil used in the treatment of leprosy. It has a cyclopentenyl ring in its structure.
- **Hydnocarpic Acid** - It is present in chaulmoogra oil.

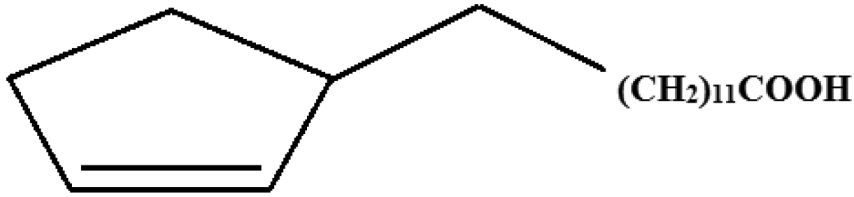


Fig 4.16 Cyclic Fatty Acids

4.15 ESSENTIAL FATTY ACIDS

Man cannot synthesize in his body certain fatty acids. These fatty acids must be included in the diet. Such, fatty acids which are not synthesized by man, but they must be included in the diet are called essential fatty acids. There are 3 essential fatty acids. They are linoleic acid, linolenic acid and arachidonic acid.

Essential fatty acids cannot be synthesized in the body of human beings.

- They must be included in the diet for maintaining normal health.
- The essential fatty acids are linoleic acid, linolenic acid and arachidonic acid.
- Most of the animal systems can interconvert these three essential fatty acids. Therefore, the diet should contain atleast any one of these essential fatty acids.
- The essential fatty acids are unsaturated fatty acids with one or more double bonds.
- Linoleic acid has 2 double bonds, linolenic acid has 3 double bonds and arachidonic acid has 4 double bonds.
- The essential fatty acids are present in vegetable oils. The best known source is the safflower oil.
- They are essential for active promotion of growth and for the maintenance of dermal integrity.
- Essential fatty acids are the important constituents of structural lipids of plasma membrane and mitochondrial membrane.
- They are found in high concentrations in reproductive organs.
- They are also present in the phospholipids.
- Arachidonic acids give rise to prostaglandins.
- They are involved in the genesis of fatty livers.
- They are essential for the metabolism of cholesterol.
- In infants, eczema is cured by feeding fats containing essential fatty acids.
- Deficiency of essential fatty acids, does not occur in man because the amount required is very less and absolutely fat free diet is practically unknown.

$CH_3 - (CH_2)_4 CH = CH - CH_2 - CH = CH - (CH_2)_7 - COOH$

LINOLEIC ACID

$CH_3 - (CH_2)_4 CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - (CH_2)_7 - COOH$

LINOLENIC ACID

$CH_3 - (CH_2)_4 CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - (CH_2)_3 - COOH$

Arachidonic acid

Fig 4.17 Essential Fatty Acids

4.16 NON-ESSENTIAL FATTY ACIDS

- Certain fatty acids can be synthesized in the tissues from other fatty acids. These fatty acids need not be included in the diet. Hence they are also called as non-essential fatty acids.
- Example - palmitoleic acid and oleic acid.
- They are unsaturated fatty acids each containing one double bond.
- Non-essential fatty acids are synthesized from their corresponding (stearic acid) saturated fatty acids by the introduction of a single double bond. They are synthesized in the liver.
- An enzyme system in liver microsomes catalyzes the conversion of stearic acid to oleic acid.

UNIT V

ENZYMES

Chemistry of enzymes, Prosthetic group (coenzymes), Nomenclature, Classification, Properties, Mechanism of enzyme action (lock and key hypothesis, induced fit theory) and its regulation, Coenzymes: Salient features and functions, Mechanism of Coenzyme action, Classification, Common coenzymes NAD, FAD, CoA, ATP, TPP, Co Q. Factors affecting enzyme activity.

5 ENZYMES

Enzymes are defined as the biocatalysts. Enzymes are catalysts for biochemical reactions in living cells. They are proteins. They are soluble and colloidal. They accelerate the rate of reactions without being lost in the process. Enzymes are located in the cells, cytoplasm, mitochondria, tissue and body fluids.

- Enzymes help to speed up reactions of digestion and metabolism.
- Cells cannot exist without enzymes.

The term enzyme was derived from Greek meaning "in yeast", because the yeast cells were the first to reveal enzyme activity in living organisms. It was first introduced by W. KUHNE in 1878. The study of enzyme is known as enzymology.

5.1 NOMENCLATURE OF ENZYMES

- The naming of enzymes is called as nomenclature of enzymes.
- Enzymes are named based on the substrate, the reaction, synthesis, chemical nature, etc.

1. Nomenclature based on substrate

Substrate is the substance on which an enzyme acts. Many enzymes are named by adding the suffix –ase to the name of substrate.

- The enzymes acting on carbohydrates are named as carbohydrases.
- The enzymes acting on proteins are named as proteases.
- The enzymes acting on lipids are named as lipases.
- The enzymes acting on nuclei acid are named as nuclease.
- The enzymes acting on maltose are named as maltase.
- The enzymes acting on lactose are named as lactase.
- The enzymes acting on sucrose are named as sucrase.
- The enzymes acting on urea are named as ureases.

2. Nomenclature based on reaction

The enzyme are highly specific to the reaction they catalyze. Hence this had necessitated their naming by adding the suffix –ase in the name of the reaction.

- The enzyme catalyzing hydrolysis is named as hydrolase.
- The enzyme catalyzing oxidation is named as oxidase.
- The enzyme catalyzing reduction is named as reductase.
- The enzyme catalyzing dehydrogenation is named as dehydrogenase.
- The enzyme catalyzing phosphorylation is named phosphorylase.
- The enzyme catalyzing transamination is named as transaminase.
- The enzyme catalyzing isomerization is named as isomerase.

3. Based on substrate and reaction

Some enzymes are named based on the substrate utilized and the type of reaction catalyzed.

- The enzyme removing CO_2 from pyruvic acid is named as pyruvic decarboxylase.
- The enzyme removing hydrogen from isocitric acid is named as isocitric dehydrogenase.

4. Based on synthesis

Enzymes are also named by adding suffix –ase to the substance to be synthesized.

The enzyme synthesizing citric acid is named as citric acid synthetase.

5. Based on discoverer

Certain enzymes are named by the discoverers of enzymes. Eg. Pepsin, Trypsin, Ptyalin, etc.

6. Based on enzyme commission

Commission on enzymes named the enzymes into 6 groups based on the chemical reaction catalyzed. They are

- Oxidoreductases
- Transferases
- Hydrolases
- Lyases
- Isomerases
- Ligases

Oxidoreductase: The enzymes catalyzing oxidation-reduction reactions are named as oxidoreductases E.g. Oxidase, Oxygenases, Dehydrogenases.

Transferases: The enzymes which catalyze the transfer of a group between two substances are called transferases.

E.g. Transaminase transfer amino group from one amino acid to another.

Hydrolase: The enzymes which catalyze the substrate by adding water across the bond they split are called hydrolases. E.g. Proteases, Esterases, Carbohydrases.

Lyases: The enzymes which remove the groups from substrates by mechanisms other than hydrolysis are named as lyases. E.g. Aldolase, enolase, fumarase etc.

Isomerase: The enzymes which interconvert isomers by rearrangement of atoms are named as isomerases. E.g. Phosphohexo isomerase

Ligases: The enzymes which link two substrate are called ligases. They are called synthetases. E.g. Acetyl CoA synthetase, Glutamine synthetase etc.

7. Based on E.C. Number

The commission on enzymes named the enzymes by a code number called enzyme commission number. Accordingly each enzyme is named by 4 digit number. E.g. 5. 2. 1. 3

The first number 5 represent the 5th class of enzyme Isomerase. The second number represents the sub-class, the third number represent the sub- sub class and the final number represents the enzyme.

5.2 CHEMISTRY OF ENZYMES

1. Proteins

Enzymes are the most specialized large protein molecules. They are made up of one or more polypeptide chains.

2. Simple enzymes

Some enzymes are simple proteins. On hydrolysis, they yield amino acid only.
Eg. Amylase, Urease etc.

3. Holoenzymes

Chemically enzymes are proteins. Some enzymes are simple proteins and others are conjugated proteins. The conjugated protein enzymes are made up of two components, namely a protein part called apoenzyme and a non-protein part. The two components together form a holoenzyme.

5.3 PROSTHETIC GROUP

The non-protein part of the enzyme may be firmly bond to the enzyme and cannot be separated by dialysis. This tightly bound non-protein part of the enzyme is called prosthetic group.

Co-enzyme

In the co-enzyme, the cofactor component is not firmly attached to the enzyme protein. Here the co-factor exist in free state in the solution. It makes a contact with the enzyme protein only at the instant of enzyme action.

5.4 PROPERTIES OF ENZYMES

- Most of the enzymes are simple or conjugated proteins. They exhibit all the properties of proteins.
- Enzymes are colloidal in nature.
- Denaturation is the change in structure and loss of activity. Enzymes are subject to denaturation by changing pH or increases in the temperature.
- Enzymes accelerate the speed of reactions.
- The enzymes promote a given reaction, but itself remains unchanged at the end of the reaction.
- Only a small amount of enzyme is required by a biological system for a complete reaction. A simple enzyme can act upon 5 lakhs substrate molecules per minute. This value is known as Turn-over number. The number of substrate molecules catalyzed by an enzyme is called turn-over number.
- Enzymes are sensitive to heat. They are destroyed by high temperature. Above 60°C the enzymes coagulate and become inactivated.
- Every enzyme has an optimum temperature at which the rate of activity is maximum. The enzyme is most active at the optimum temperature. The optimum temperature ranges between 30°C to 40°C for most of the enzymes. The optimum temperature for catalyst is 30°C.
- The rate of activity decreases when the temperature goes up or goes down from the optimum temperature.
- The activity of the enzyme steadily increases when the temperature is raised to the optimum level. The reaction velocity doubles for every rise in 10°C. This is called Q_{10} or temperature coefficient or temperature quotient.
- Most of the enzymes are characterized by the reversibility of their actions. That is, the enzymes act in either direction.

- Each enzyme will react with only one type of substrate or a group of related substrates. This property of enzyme is called specificity of enzymes.

5.5 CLASSIFICATION OF ENZYMES

In 1961, the enzyme commission of the International Union of Biochemistry proposed a comprehensive system for the classification of enzymes. According to this system, enzymes are classified into six major classes. They are follows:

- ♦ Oxidoreductases
- ♦ Transferases
- ♦ Hydrolases
- ♦ Lyases
- ♦ Isomerases
- ♦ Ligases

- Oxidoreductases are the enzymes catalyzing oxidation- reduction reactions are named as oxidoreductases. Oxidation means addition of oxygen or removal of hydrogen. Reduction means addition of hydrogen or removal of oxygen. The important sub classes are
- Dehydrogenases are enzymes that catalyze the removal of hydrogen from one substrate and pass it on to second substrate.

$$AH_2 + B \xrightarrow{\text{Dehydrogenase}} BH_2 + A$$

E.g. Alcohol dehydrogenase enzyme

- Oxidases are enzyme which catalyze the removal of hydrogen from a substrate and pass it to oxygen.

  E.g. Cytochrome oxidase enzyme

- Oxygenases are enzymes which catalyze the incorporation of oxygen directly into the substrate.
- Transferases are enzymes which catalyze the transfer of a group between two substances are called transferases.

$$AX + B \xrightarrow{\text{Transferase}} A + BX$$

eg. Transaminase transfer amino group from one amino acid to another.

- Transaminase is an enzyme which transfers amino group from alanine to oxaloacetic acid. The enzyme creatine phosphoryl transferase transfers phosphate group from creatine phosphate or adenine diphosphate.

- Hydrolase are the enzymes which catalyze the substrate by adding water across the bond they are called hydrolases. E.g. Proteases, esterases, carbohydrases.
- Proteases are enzymes that attack the peptide bonds on proteins and peptides. It is sub divided into peptidase and proteinases. It is otherwise known as exopeptidases.
- Carboxypeptidases require a free carboxyl group in the substrate and split the peptide bond adjacent to this group, liberating a free amino acid.
- Peptidases are the enzymes which act on peptide bonds adjacent to a free amino or carboxyl group.

1. **Esterases:** These enzymes catalyses hydrolysis of ester linkages. Eg. Liver esterase, Lipase, Nuclease etc.
2. **Carbohydrases:** These enzymes hydrolyze the glycosidic linkages of simple glycosides, oligosaccharides and polysaccharides.
   - Lyases are the enzymes which remove the groups of atoms from substrates leaving the double bond or add groups to double bond without hydrolysis, oxidation or reduction are named as lyases. E.g. Aldolase, Enolase, Fumarase etc.
   - Isomerases are the enzymes which interconvert isomers by rearrangement of atoms are named as isomerases.
   - These enzymes catalyze intra molecular rearrangements. The Phosphohexose isomerase catalyze the following interconversion

$$\text{Glucose-6-phosphate} \xrightarrow{\text{Phosphohexose isomerase}} \text{Fructose-6- phosphate}$$

   - Ligases are the enzymes catalyze synthesis reactions by joining two molecules coupled with that breakdown of a pyrophosphate bond of ATP $\longrightarrow$ ADP. The common Ligases are DNA Ligases, RNA synthetase, Glutamine synthetase.

5.6 MECHANISMS OF ENZYME ACTION

- The breaking of substrate into end product by an enzyme is called enzyme action. The compound on which the enzyme acts is called the substrate.
- Michaelis and Menton proposed a hypothesis for enzyme action. The enzyme action involves the following steps.

1. The enzyme molecule (E) combines with a substrate molecule (S) to form an enzyme-substrate complex. It is also called as Michaelis complex.
2. The enzyme contain specific sites for the attachment of substrates. These sites are called active sites or catalytic centres. They are made up of amino acid residues.
3. The active sites loosen the chemical bonds in the substrate and this leads to the breaking of substrates into end products.
4. Finally, the enzymes dissociates from the end product.
5. The enzyme is now free to combine with another molecule of substrate.

There are two hypothesis to explain the mechanism of the formation of enzyme-substrate complex.

1. Lock and Key hypothesis
2. Induced fit hypothesis.

5.6.1 Lock and Key hypothesis

- This hypothesis was proposed by Emil Fisher (1914)
- This theory explains the mechanisms of the formation of enzyme substrate complex. According to this hypothesis, the enzyme molecule has one or more specific points. These points are called active sites or active centers.
- The active sites exist in the enzyme in a rigid and proper conformation even in the absence of substrate.
- During enzyme action the substrate fits into the active site of the enzyme as a key fits into the lock.

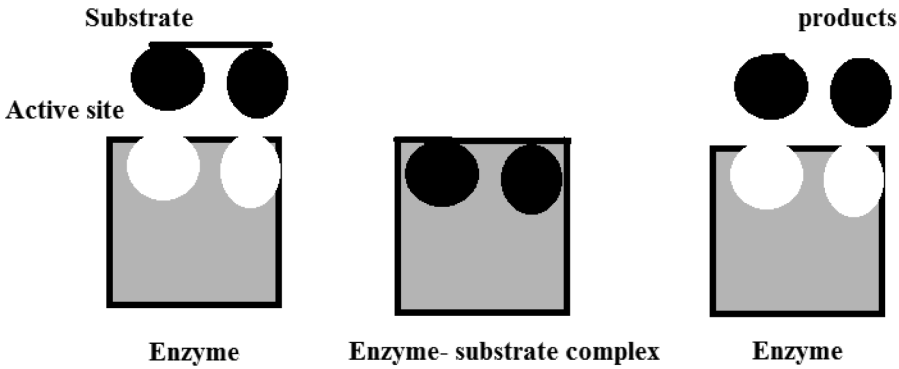


Fig 5.1 Lock and Key hypothesis

5.6.2 Induced Fit hypothesis

- This hypothesis was proposed by Koshland (1963). It explains the mechanism of the formation of enzyme-substrate complex.
- This theory says that the active site does not possess a rigid and performed structure. The region of the active site is flexible.
- When the enzyme reacts with the substrate, the substrate induces a conformational change in the active site of the enzyme.
- This change results in the development of attraction between enzyme and the substrate so that an enzyme - substrate complex is formed.
- It leads to the loosening of the chemical bonds linking the components of the substrate.
- As the reaction is completed the substrate is split into end product and enzyme is released.

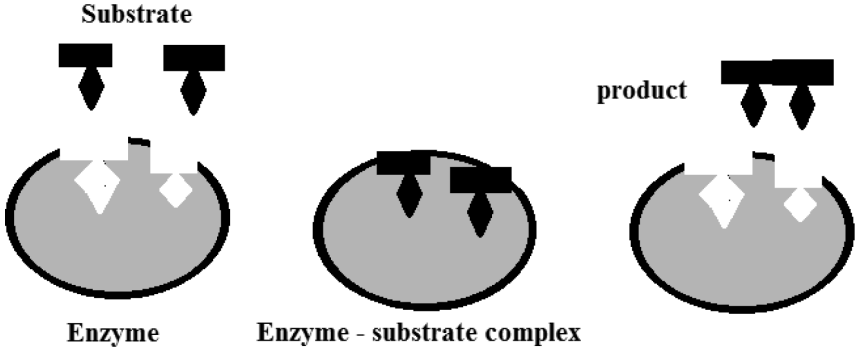


Fig 5.2 Induced Fit hypothesis

5.7 COENZYMES

- Coenzymes may be defined as non-protein organic substance loosely attached to the enzyme and can be separated by dialysis and is essential for enzyme action. E.g. NAD, NADP, ATP, UDP, CoA, TPP, FAD, FMN, Ubiquinone (CoQ), etc.
- Chemically enzymes are proteins. Some enzymes are simple proteins and others are conjugated proteins. The conjugated protein enzymes are made up of two components, namely a protein part called apoenzyme and a non-protein part. The two components together form a holoenzyme.

Holoenzyme ⟷ Apoenzyme + Non-Protein

- The non-protein part of the enzyme may be firmly bond to the enzyme and cannot be separated by dialysis. This tightly bound non-protein part of the enzyme is called prosthetic group. The loosely bound prosthetic group is the coenzyme.

Holoenzyme ⟷ Apoenzyme + Prosthetic group

Holoenzyme ⟷ Apoenzyme + Coenzyme

- The coenzyme is also called co-substrate or co-factor. It is an organic substance. It is a small molecule with low molecular weight. Hence it can be separated by dialysis. Coenzymes are heat stable. Most of them are derivatives of vitamin B complex. They are necessary for enzyme action and they accelerate the reaction rate.

5.7.1 Salient Features of Coenzymes

1. Coenzymes are non-protein organic substances loosely attached to enzymes and are easily separated by dialysis and are inevitable for enzyme action.
2. Coenzymes are also called co-substrates.
3. They are small molecules with low molecular weight. They can be reversibly separated from enzymes by dialysis.
4. They are heat-stable.
5. They are closely related to vitamins and are derivatives of vitamin B complex.
6. They function as catalysts and are essential for enzyme action. They accelerate the rate of reaction.
7. An essential component of most of coenzymes is phosphate in the form of nucleotide.
8. Coenzymes are usually not firmly attached to the enzyme protein. But they exist in the three state in the solution. They contact the enzyme protein only at the time of enzyme action.
9. The coenzymes, is repeatedly used to split many molecules of substrates both apoenzyme and coenzyme are regenerated at their original forms at the end of the reaction.

10. The coenzyme act as intermediate carriers of hydrogen atoms in the biological oxidation reduction reactions.

5.7.2 Role of coenzymes

- The function of coenzymes is to transport groups between enzymes.
- Chemical groups include hydride ions which are carried by coenzymes such as NAD.
- Phosphate groups are carried by coenzymes such as ATP.
- Acetyl groups are carried by coenzymes such as coenzyme A.
- Coenzymes which lose or gain these chemical groups in the course of the reaction are often reformed in the same metabolic pathway.

For example NAD^+ used in glycolysis and the citric acid is replaced in the electron transport chain.

5.7.3 Mechanism of Coenzyme Action

The coenzyme helps an enzyme to combine with the substrate and at the end it is cleaved from the substrate. One of the cleavage products is transferred to the coenzyme. Finally the coenzyme is released from the product and is ready for further enzyme action.

5.7.4 Classification of Coenzymes

Coenzymes are classified into three groups on the basis of their functions. They are:

1. Hydrogen transferring coenzymes
2. Group transferring coenzymes
3. Isomerase coenzymes

1. Hydrogen Transferring Coenzymes

Certain coenzymes transfer hydrogen atoms or electrons from one. These coenzymes are called hydrogen transferring enzymes. E.g. NAD, NADP, FMN, FAD, Ubiquinine (Q), etc.

2. Group Transferring Coenzymes

These coenzymes are involved in group transfer. Eg.ATP, CDP, UDP, CoA, TPP, etc.

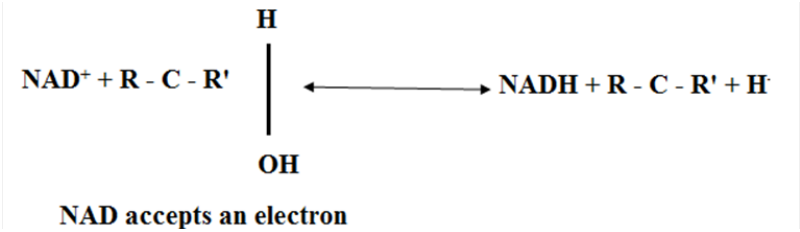
3. Isomerase Coenzymes

These coenzymes are responsible for the interconversion of isomers. E.g. UDP, TPP, etc.

5.7.5 Common Coenzymes

5.7.5.1 Nicotinamide Adenine Dinucleotide (NAD)

1. Nicotinamide Adenine Dinucleotide(NAD) is a coenzyme
2. It plays an important role in the oxidation of fuel molecules, such as glucose and fatty acids. The fuel molecules and their products during their oxidation do not transfer the electrons directly to O_2. Instead, these substrates transfer electrons to special carries which are either NAD or FAD.
3. Thus, NAD is an electro carriers of the electron transport chain located in the inner membrane of mitochondria.
4. NAD is a major electron acceptor in the electron transport system.
5. When NAD accepts electrons, NAD is reduced and the reduced form of NAD is called NADH.
6. It is a derivative of nicotinic acid, Vitamin B_5.
7. Chemically NAD is formed of four components, namely
   a. A nicotinamide
   b. An adenine
   c. Two pentose sugars, D-Ribose
   d. Two phosphate groups.
8. The coenzyme Nicotinamide Dinucleotide Phosphate (NADP) is another coenzyme related to NAD. It also functions as an electron acceptor. When it accepts electrons, it is reduced. The reduced NADP is called NADPH. The chemical structure of NAD is similar to that of NADP. But in NADP, a phosphate group (PO_3-*)* replaces the H of a ribose sugar.
9. In NAD and NADP, the Nicotinamide / nicotinamide ring is the most active part. In oxidation of a substrate, the Nicotinamide / nicotinamide ring accepts a hydrogen ion and two electrons which are equivalent to a hydride ion.
10. NAD^+ is the electron acceptor in many reactions of this type. In this dehydrogenation reaction one hydrogen atom of the substrate is directly transferred to NAD^+, where as the other appears in the solvent.
11. Both electrons lost by the substrate are transferred to the Nicotinamide ring.

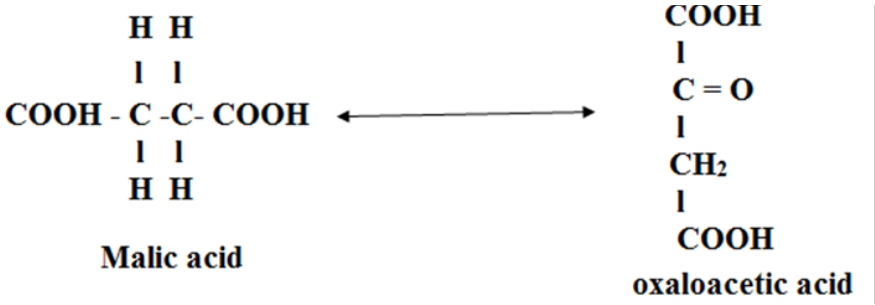


12. NAD and NADP are involved in most of the dehydrogenation reactions which occur in the oxidation of carbohydrates and fatty acids.
13. The dehydrogenation reactions are catalyzed by dehydrogenases. The coenzyme NAD or NADP is bound to the enzyme dehydrogenase transiently during the catalytic reaction and the hydride ion is removed from the substrate.

A very good example of such enzymatic reaction is that catalyzed by malic acid dehydrogenase which dehydrogenates malic acid to yield oxaloacetic acid, a step in Krebs' cycle.

$$\text{Malic acid} + \text{NAD} \xrightarrow[\text{dehydrogenase}]{\text{Malic acid}} \text{Oxaloacetic acid} + \text{NADH} + \text{H}^+$$

The dehydrogenase catalyzes the reversible transfer of a hydride ion from malic acid to NAD^+ to from NADH. The coenzyme NAD plays an important roles in glycolysis, Krebs' cycle, β oxidation, DNA splicing, etc.



5.7.5.2 Flavin Adenine Dinucleotide(FAD)

1. Flavin Adenine Dinucleotide (FAD) is a coenzyme.
2. It plays an important role in oxidation of carbohydrates and fatty acids. These fuel molecules and their products, during their oxidation, do not transfer the electrons directly to O_2. Instead, these substrates transfer electrons to special carriers which are either NAD or FAD.

3. Thus FAD is an electron acceptor of the electron transport chain located in the inner membrane of mitochondria.
4. When FAD accepts electrons, the FAD is reduced and the reduced form of FAD is called $FADH_2$.
5. FAD, like NAD^+ is a two-electron acceptor. However unlike NAD^+, FAD accepts both of the hydrogen atoms lost by the substrate.
6. FAD is a derivative of riboflavin, vitamin B_2.
7. Chemically, FAD consists of four components,

   They are,

   a. An isoalloxazine ring
   b. An adenine
   c. A ribose sugar
   d. Two phosphate groups
8. FMN, Flavin Adenine Mononucleotide is related coenzyme.

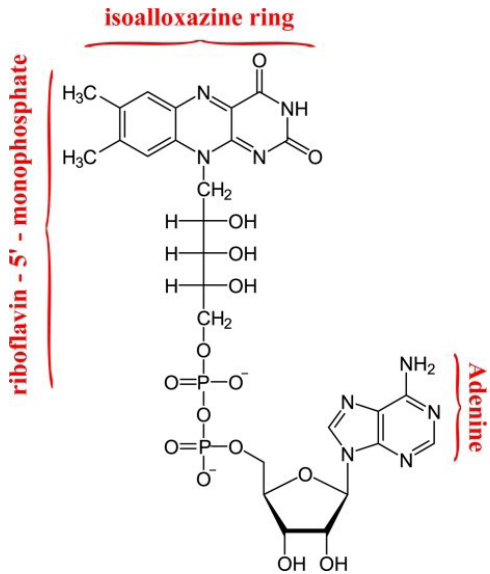
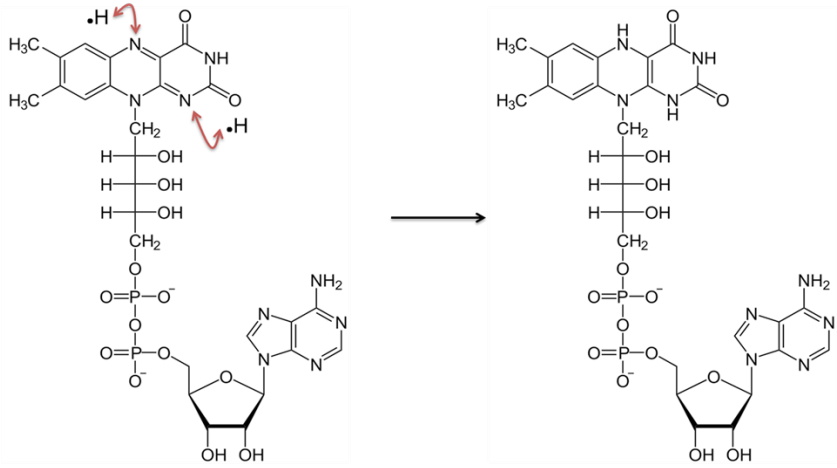


Fig 5.3 Flavin Adenine Dinucleotide (FAD)

9. In FAD and FMN, the isoalloxazine ring is the active site for accepting the hydrogen atoms.
10. They function as tightly bound prosthetic groups of a class of dehydrogenase called flavoproteins or flavin dehydrogenases. Succinic acid dehydrogenase is an example of a flavin dehydrogenase.



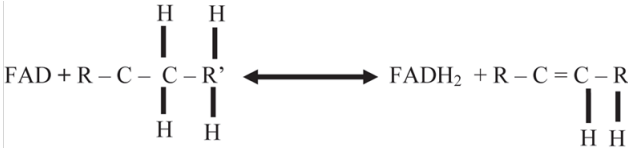
In FAD, isoalloxazine ring is the active part. It accepts two hydrogen atoms and becomes $FADH_2$.

It contains covalently bound protein prosthetic group of FAD and catalyzes the following reaction.

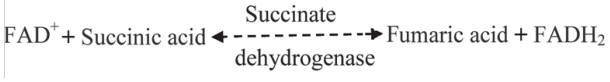
$$\text{Succinic acid} + \text{E-FAD} \longrightarrow \text{Fumaric acid} + \text{E} - FADH_2$$

In this reaction E-FAD refers to the succinic acid dehydrogenase molecule with its bound FAD.

11. FAD accepts electrons in the reactions of the following type.



12. A very good example, where FAD catalyzes the reaction is the conversion of succinic acid into fumaric acid in krebs' cycle.

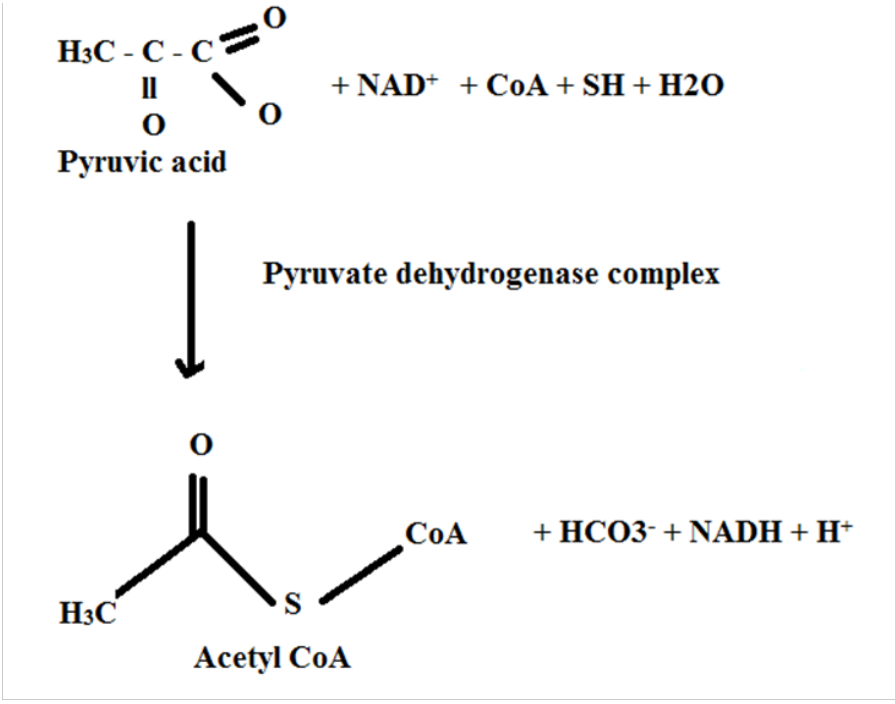


FAD accepts two hydrogen atoms from succinic acid and becomes $FADH_2$.

5.7.5.3 Coenzyme A (CoA)

1. Coenzyme A is an universal coenzyme.
2. It was discovered by Lipmann in 1945.

3. CoA is involved in the transfer of acyl and acetyl groups and hence the name CoA. 'A' stands for acetylation. It is a transient carrier of acyl groups.
4. It is a derivative of pantothenic acid.
5. The CoA is composed of four units, namely
   a) β – mercaptoethylamine
   b) Pantothenic acid
   c) Adenine
   d) Ribose 3' – phosphate
6. One end of CoA has a reactive group called thiol or sulfhydryl group (-SH). It is a very active group and hence CoA is abbreviated as CoA - SH.
7. During acyl group transfer the acyl group is covalently linked to the thiol group of CoA by a thioester bond. The resulting derivative is called an acyl CoA. An acyl group often linked to CoA is the acetyl unit. This derivative is called acetyl CoA. Acetyl CoA has a high acetyl group transfer potential.
8. CoA is a carrier of activated acetyl or other acyl group, just as ATP is a carrier of activated phosphoryl groups.
9. CoA plays important roles in carbohydrate and fat metabolism.



The CoA functions as a carrier of acetyl group. The acetyl group removed from pyruvic acid is accepted by CoA to become acetyl CoA. A very good example for the reaction where CoA involved is the conversion of pyruvic acid into acetyl CoA catalyzed by pyruvic acid dehydrogenase. In this reaction, the carboxyl group of pyruvic acid is removed as CO_2 and the acetyl group links with CoA to form acetyl CoA. This reaction is called oxidative decarboxylation. In this reaction, CoA functions as the carrier of acetyl group. In another reaction in Krebs' cycle, the acetyl CoA transfers its acetyl group to oxaloacetic acid which in turn is converted into citric acid and CoA is released for repeating the reactions.

Acetyl CoA + Oxalo acetic acid $\xrightarrow[\text{Synthetase}]{\text{Citric acid}}$ Citric acid + Coenzyme A

$+ H_2O$

The acetyl CoA transfer its CoA to Oxaloacetic acid and the CoA is released for repeating the reaction.

5.7.5.4 Adenosine Triphosphate (ATP)

1. ATP is the universal currency of free energy.
2. ATP is a nucleotide consisting of three units, namely
   a) Adenine
   b) Ribose sugar
   c) Triphosphate unit

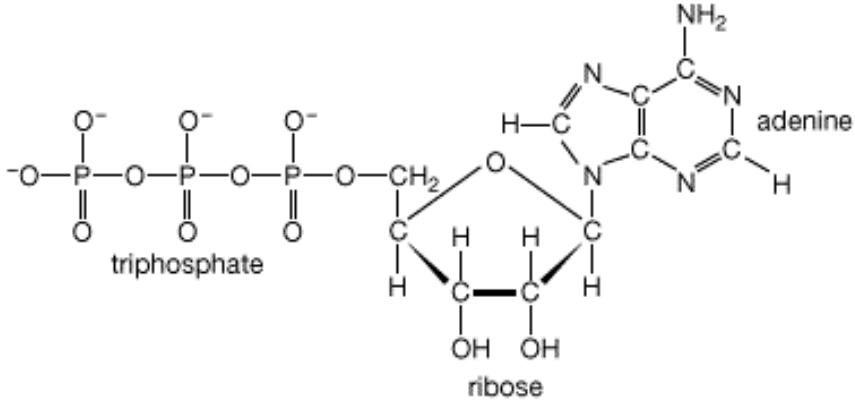


Fig 5.4 Adenosine Triphosphate (ATP)

3. ATP is an energy rich compound because its Triphosphate unit contains two phosphoanhydride bonds.
4. A large amount of free energy is released when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (Pi)

$$ATP + H_2O \longleftrightarrow ADP + Pi + H^+$$

or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PPi).

$$ATP + H_2O \longleftrightarrow AMP + PPi + H^+$$

5. It functions as immediate donor of free energy.
6. ATP has a higher phosphate group transfer potential.
7. As a coenzyme, ATP is involved in transphosphorylation reactions.
8. It can transfer its orthophosphate group or diphosphate group or adenosyl monophosphate group.
9. When it transfer orthophosphate group, it release ADP.

$$ATP \longleftrightarrow ADP + Pi$$

When ATP transfers adenosyl monophosphate, it releases pyrophosphate.

$$ATP \longleftrightarrow AMP + PPi$$

5.7.5.5 Thiamine pyrophosphate (TPP)

1. Thiamine pyrophosphate is a coenzyme.
2. It is derivative of *vitamin* B_1.
3. It is a *prosthetic* group because it is tightly bound to the enzyme protein.
4. Chemically TPP is formed of four units, namely
   a) A *pyrimidine* ring
   b) A *thiazole* ring
   c) A *pyrophosphate* unit

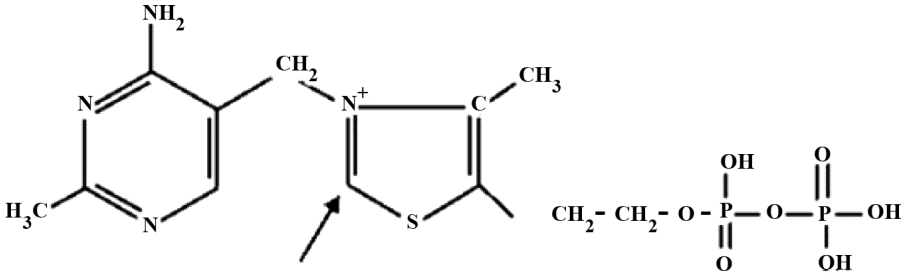
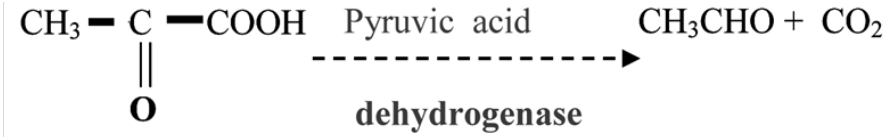


Fig 5.5 Thiamine pyrophosphate (TPP)

5. The thiazole ring is the active part of this coenzyme.
6. TPP functions as a coenzyme in several enzymatic reactions in which acetyl and aldehyde groups are transferred from a donor to an acceptor molecule. In such reactions, TPP serves as a transient intermediate carrier of the aldehyde group, which is covalently attached to the thiazole ring.
7. An example, in which TPP, involved is the reaction catalyzed by pyruvic acid decarboxylase, an important step in the fermentation of glucose.

In this reaction, the carboxyl group of pyruvic acid is lost as CO_2 and the rest of the pyruvic acid is called active acetaldehyde.



The active acetaldehyde is simultaneously transferred to the 2nd position (the carbon atom between the nitrogen and sulfur atoms) of the thiazole ring of the tightly bound TPP to yield is α-hydroxyethyl derivative.

Pyruvic acid + TPP – E ⟷ α- hydroxyethyl – TPP – E

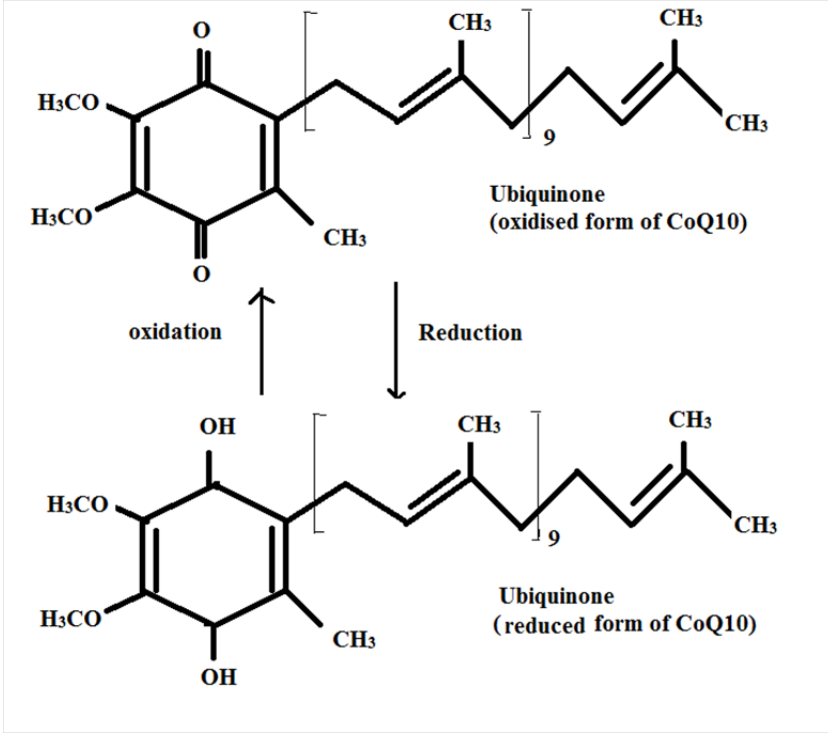
This intermediate exists only transiently, since the hydroxyethyl group is quickly cleaved from the coenzyme to yield free acetaldehyde.

α- hydroxyethyl – TPP – E ⟷ Acetaldehyde + TPP + E

8. TPP also serve as the coenzyme in pyruvic acid dehydrogenase and α-ketoglutaric acid dehydrogenase reactions taking place in citric acid cycle.

5.7.5.6 Ubiquinone or coenzyme Q

1. Ubiquinone is also called coenzyme Q. It is called Ubiquinone because it is ubiquitous in biological systems.
2. Co-Q is a quinone derivative with a long isoprenoid tail. The number of isoprene units in Q depends on the species.
3. The most forms in mammals contains10 isoprene units and so it is designated as Q_{10}.
4. Co-Q is the only electron carrier in the respiratory chain that is not tightly bound or covalently attached to a protein.
5. In fact, Q serves as a highly mobile carrier of electrons between the flavoproteins and the cytochromes of the electron- transport chain.



5.8 FUNCTION OF COENZYME

- The coenzyme is essential for the biological activity of the enzyme.
- A coenzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction.
- One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of enzyme.

5.9 FACTORS AFFECTING ENZYME ACTIVITY

The activity of enzyme is affected by a number of factors such as temperature, pH, enzyme and substrate concentration.

5.9.1 Effect of temperature on enzymes

- The rate of enzyme action increases with increase of temperature upto 40^0C.
- The temperature at which the enzyme action is maximum is called the optimum temperature.
- For most of the enzymes, the optimum temperature lies between 30^0C and 40^0C. At low temperature, the enzyme is ineffective.
- At high temperature, for example at 60^0C the enzyme becomes inactive. This is because the enzyme is denatured or destroyed by high temperature. Thus enzymes are said to be thermolabile.

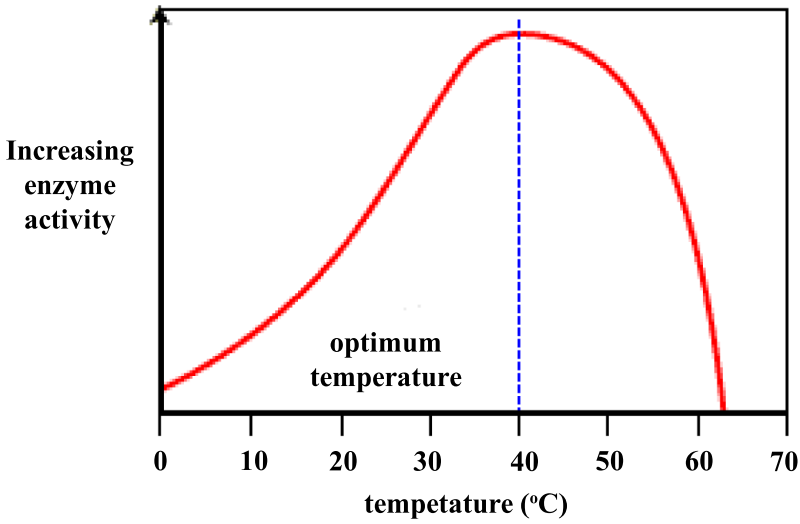


Fig 5.5 Effect of temperature on enzymes

5.9.2 Effect of pH on enzymes

- The rate of enzyme action increases with increase in the H^+ ion concentration up to a certain pH when the enzyme action is maximum. This pH is called the optimum pH.
- Increased H^+ ion concentration beyond this peak point will destroy the enzymes, because of their protein nature and bring down the enzyme action.
- Most of the enzymes act effectively in a pH range 5.0 to 9.0. But some enzymes like pepsin are active even at pH value between 1.2 and 1.8.

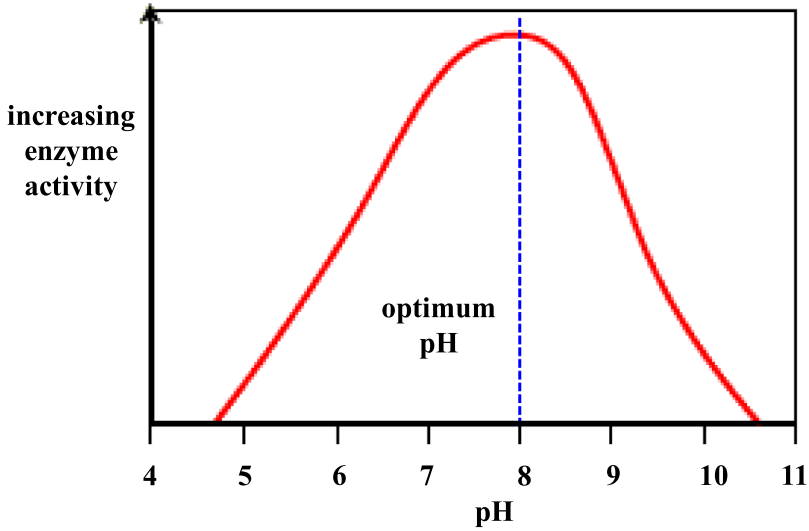


Fig 5.6 Effect of pH on enzymes

5.9.3 Effect of enzyme concentration

- An enzyme works even when it is present in low quantity. The velocity of the reaction increases with the increase in the concentration of enzymes.
- The velocity (V) of a reaction is proportional to the concentration of enzyme (E). When the enzyme concentration is doubled, the velocity is also doubled. This is because when concentration is doubled as much as twice, the active sites become available to combine with the substrate.

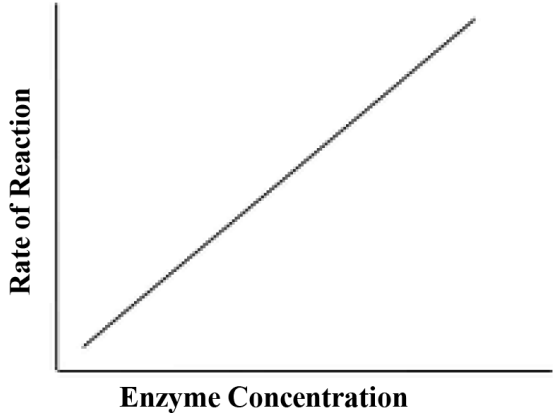


Fig 5.7 Effect of enzyme concentration

5.9.4 Effect of substrate concentration

- An increase of substrate concentration results in a very rapid increase in the velocity of reaction.
- As the substrate concentration continues to increase, the increase in the rate of reaction begins to slow down with a large substrate concentration. Thus up to a certain point the reaction rate is proportional to the substrate concentration.

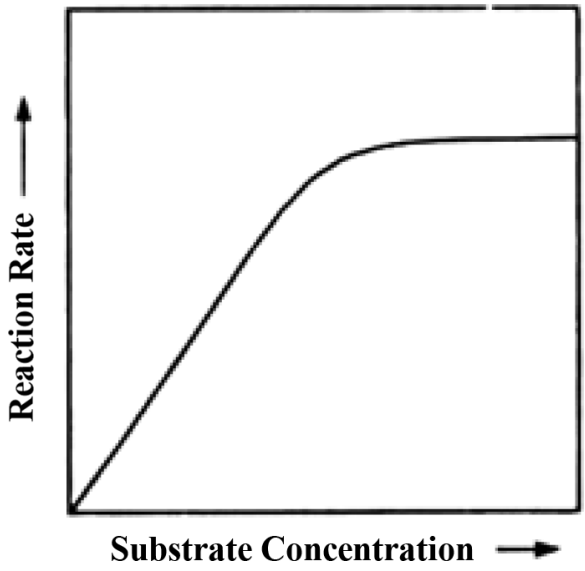


Fig 5.8 Effect of substrate concentration

UNIT VI

NUCLEIC ACIDS

Nucleic acids - Classification – DNA, Chemical composition, Nucleoside, Nucleotide, Polynucleotide, Watson and Crick Model Of DNA- RNA, Types - mRNA, tRNA, rRNA - Biological Function of Nucleic acids.

6 NUCLEIC ACIDS

- The nuclei acid was first isolated in 1868 by *Miescher* from the nuclei of pus cells on hospital bandages. Hence it was called Nuclein. Altmann (1889) gave the name nucleic acid.
- Nucleic acid is a macromolecule with acid property and it was isolated from the nucleus of cells and hence it is named as nucleic acid. It is made up of C, H, O, N and P.
- Nucleic acids are found in all organisms such as plants, animals, bacteria and viruses. They are found in the nucleus as well as in the cytoplasm.
- Nucleic acid molecule is a long chain polymer. It is composed of monomeric units, called nucleotides. Each nucleotide consists of a nucleoside and a phosphate group. Each nucleoside consists of pentose sugar and a nitrogenous base. The sugar is ribose in the case of RNA and deoxyribose in the case of DNA.
- The nitrogenous bases are of two types, namely purines and pyrimidines. There are two main purine bases, adenine and guanine. Similarly there are three main pyrimidine bases. They are cytosine, thymine and uracil. DNA contains all these bases except uracil. RNA contains these entire bases except thymine.

6.1 POLYNUCLEOTIDE OR NUCLEIC ACIDS

- A number of nucleotide units link with one another to form a polynucleotide chain or nucleic acid. Nucleotide is derived from a nucleoside by the addition of a molecule of phosphoric acid. A base combined with a sugar molecule is called a nucleoside.

6.1.1 Classification of Nucleic acids

Nucleic acids are broadly classified into two types based on the type of sugar present in them. They are:

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

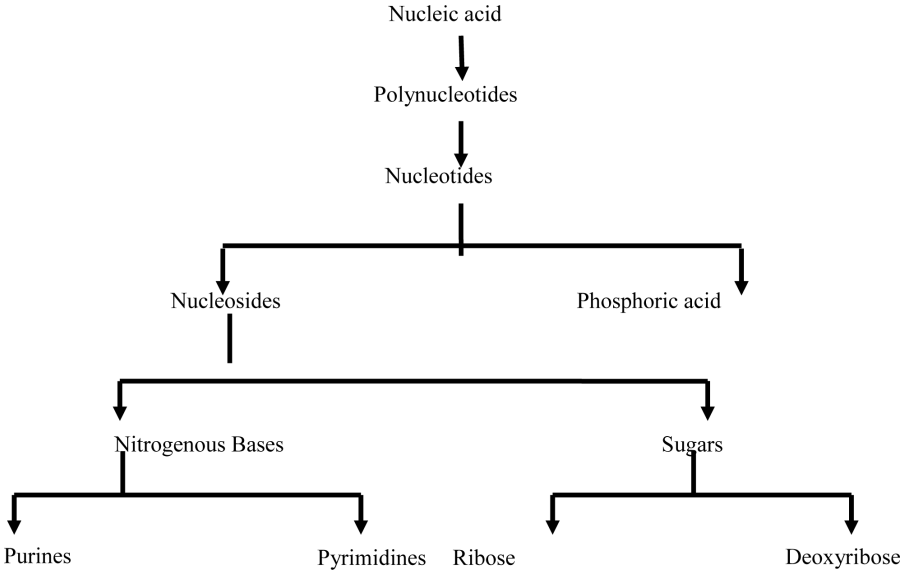


Fig 6.1 Composition of Nucleic acids

The ribonucleic acid is further divided into three types, namely

- Messenger RNA (mRNA)
- Transfer RNA (tRNA)
- Ribosomal RNA (rRNA)

6.2 DEOXYRIBONUCLEIC ACID (DNA)

- Deoxyribonucleic acid is the molecule of heredity. It is a nucleic acid containing deoxyribose sugar. It is made up of two polynucleotide chains. Each chain has a large number of deoxyribonucleotides.
- Deoxyribonucleotides are formed of deoxyribose sugar, nitrogenous bases and phosphoric acid. The sugar and phosphoric acid perform a structural role. The nitrogenous bases of DNA carry genetic information.
- DNA is present in all cells majority of plant viruses contain RNA except pure plant virus for example, cauliflower mosaic virus has double stranded DNA. In eukaryotic cells, DNA is present in the chromosomes of nucleus. In addition, the mitochondria and plastids also contain DNA.

- In eukaryotic nucleus, the DNA is in the form of double helix. In bacteria, mitochondria and plastids the DNA molecules are circular. In viruses and bacteriophages, they are coiled.
- The number of DNA molecules in eukaryotic cells corresponds to the number of chromosomes per cell.

6.2.1 Chemical Composition

- DNA is made up of three chemical components namely,
  1. Sugar
  2. Phosphoric acid and
  3. Nitrogenous bases.

Sugar

- The sugar present in the DNA is called deoxyribose. It is pentose sugar which contains five carbon atoms. It contains one O atom less than the ribose sugar. At carbon No. 2 of deoxyribose, H-C-H group is present. But in ribose sugar, the second carbon atom contains H-C-OH group.

Phosphoric acid

- Phosphoric acid links the consecutive nucleotides by joining their pentose sugars with a phosphate diester bond. This bond links carbon 5' in one nucleoside with carbon 3' in the next nucleoside.

Nitrogenous bases

- These are N_2 containing organic compounds. They are of two types namely purines and pyrimidines.
- Purines are two ringed nitrogen compounds. They are of two types namely adenine and guanine.
- Pyrimidines are single ringed nitrogen compounds. They are of two types namely thymine and cytosine.

6.2.2 Nucleosides

- A base combined with a sugar molecule is called a nucleoside. In DNA, four different nucleosides are present. They are

  Adenosine – Deoxyribose sugar + Adenine

  Guanosine – Deoxyribose sugar + Guanine

Cytidine – Deoxyribose sugar + Cytosine

Thymidine – Deoxyribose sugar + Thymine

- In a nucleoside, the first carbon of sugar is linked with 3rd position of pyrimidines or 9th position of purines.
- In RNA, deoxyribose sugar is replaced by ribose and the base thymine is replaced by uracil.

6.2.3 Nucleotides

- Nucleotides is derived from a nucleoside by the addition of a molecule of phosphoric acid. The phosphate molecule is linked with sugar molecules at 3rd carbon or 5th carbon.
- The nucleotides will be called 3'p5' OH nucleotide and 3'p5' OH nucleotide.
- The 3'p5' OH nucleotide mean it has a phosphate group at the 5' end and a hydroxyl group at the 3' end. The 3'p5' OH nucleotide means it has a phosphate group at the 3' end and a hydroxyl group at the 5' end.
- The DNA contains four different types of nucleotides. They are adenylic acid, guanylic acid, cytidylic acid and thymidylic acid.
- The RNA contains uridylic acid instead of thymidylic acid.

6.2.4 Polynucleotide

- A number of nucleotide units are linked with one another and form a polynucleotide chain. Nucleotides are linked with another by phosphodiester bond. A phosphodiester bond will be formed between any two adjacent nucleotides.

6.3 WATSON AND CRICK MODEL OF DNA

- Watson and Crick in 1953 proposed a model to explain the arrangement of molecules in DNA. The model is characterized by the following features.
- DNA is a nucleic acid. It is deoxyribonucleic acid.
- It is a macromolecule.
- Each DNA is formed of two polypeptide chains.
- The two chains are spirally coiled to form a double helix.
- Each chain is formed of many units called nucleotides. Nucleotides are the building blocks of DNA.

- A nucleotide is formed of three components namely a phosphoric acid, a deoxyribose sugar and a nitrogenous base. As there are four kinds of nitrogenous bases, there are four kinds of nucleotides, namely

  Adenylic acid = Phosphate group + Adenine

  Guanylic acid = Phosphate group + Guanine

  Cytidylic acid = Phosphate group + cytosine

  Thymidylic acid = Phosphate group + Thymine

- A nucleotide is formed of a nucleoside and a phosphoric acid. Thus a nucleoside is formed of a base and a deoxyribose sugar. As there are four types of nitrogenous bases, four types of nucleosides are present. They are

  Adenosine = Deoxyribose + Adenine

  Guanosine = Deoxyribose + Guanine

  Cytidine = Deoxyribose + Cytosine

  Thymidine = Deoxyribose + Thymine

- Adenine and guanine belongs to a group of compound called purines. Similarly, thymine and cytosine belong to another group called pyrimidines.
- In DNA molecules, purines are linked with pyrimidines.
- Adenine is linked with thymine and guanine is linked with cytosine.
- The adjacent chains are linked by hydrogen bonds. Adenine of one chain is linked with the thymine of another chain by two hydrogen bonds. Similarly guanine of one chain is linked with the cytosine of the second chain by three hydrogen bonds.
- The amount of adenine is equal to the amount of thymine and the amount of guanine is equal to the amount of cytosine.
- The two chains of a DNA are complementary to each other.
- One end of the polynucleotide chain is 3' and the other end is called 5'. In the 3' end, the third carbon of the sugar is free and it is not linked to any nucleotide. In the 5' end, the fifth carbon of the sugar is free and it is not linked to any nucleotide.
- A DNA molecule looks like a ladder. The sugar and phosphate form the back bones and base pairs from the horizontal rounds.
- A single DNA molecule may contain about 2000 base pairs or nucleotides.
- The two complementary chains are twisted around each other to form a double helix. One turn of helix measures about 34A°. It contains 10 paired nucleotides. Distance between two base pairs is 3.4A°.

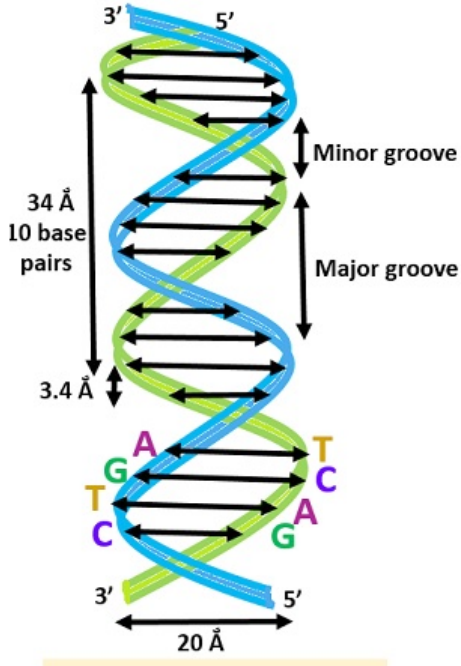


Fig 6.2 Structure of DNA by Watson and Crick Model

Structural variation in DNA

- In addition to the double helicular DNA, there are single stranded DNA and circular shaped DNA having the same chemical composition.
- **Single stranded DNA** - DNA of exists normally as single stranded molecules. Such a type of DNA was isolated by Sinshemer in 1959.
- **Circular DNA** - The circular DNA is characteristic of the cell organelle, mitochondria. Mitochondrial DNA differs from nuclear DNA in several aspects. The GC content is higher in mitochondrial DNA. The amount of genetic information carried by mitochondrial DNA is not sufficient to provide specifications for all the proteins and enzymes present in the organelles.

Fig 6.3 Single Stranded DNA

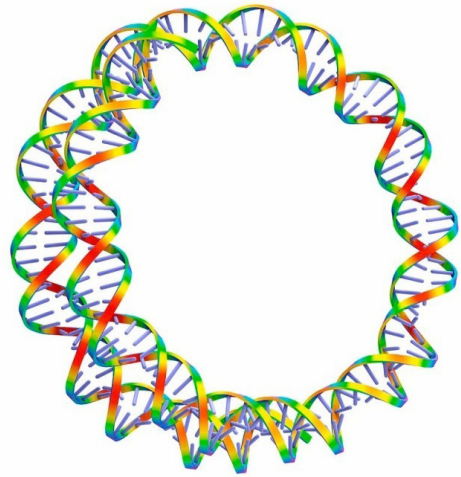


Fig 6.4 Circular DNA

6.4 RIBONUCLEIC ACID

- Ribonucleic acid is a nucleic acid containing ribose sugar. It is found in large amount in the cytoplasm and at a lesser amount in the nucleus. In the cytoplasm, it is mainly found in the ribosome and in the nucleus.
- RNA is formed of a single strand. It consists of several units called ribonucleotides. Hence each RNA molecule is formed of several nucleotides.
- Each nucleotide is formed of three different molecules, namely phosphate, ribose sugar and nitrogen base. The nitrogen bases are of two types, namely purines and pyrimidines. The purines present in the RNA is adenine and guanine. The pyrimidines present in RNA are cytosine and uracil.
- The RNA molecule is single stranded. Sometimes, the strand may be folded back upon it and this double strand may be coiled to form a helical structure like that of DNA.
- In RNA, the purines and pyrimidines are not present in equal amount.

6.4.1 Types of RNA

There are three types of RNA. They are the following

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA)

6.4.1.1 Messenger RNA (mRNA)

- Messenger RNA (mRNA) is a ribonucleic acid which carries genetic information for protein synthesis from the DNA to the cytoplasm. The term mRNA was coined by Jacob and Monad in 1961.
- The mRNA forms about 3 to 5% of the total cellular RNA. The mRNA is synthesized as a complementary strand upon the chromosomal DNA. The genetic message from DNA is transcribed to this mRNA. The mRNA carries the message in the form of triplet codes.
- The hybrid mRNA inside the nucleus is called heterogenous nuclear RNA (hnRNA). It is processed in the nucleus and enters the cytoplasm through nuclear membrane. In the cytoplasm mRNAs are deposited on some ribosomes. In the ribosomes, mRNA acts as a template for protein synthesis.
- The life span of mRNA in bacteria is about 2 minutes. In eukrayotes, it lives for few hours to a few days. In the animal eggs and plant seeds, the mRNA is stabilized for months or years. Protein synthesis must be carried out within this lifespan.

Structure of mRNA

- mRNA is the messenger RNA. It is a ribonucleic acid carrying information from the DNA to the cytoplasm.
- The mRNA is a single stranded polynucleotide chain.
- The mRNA contain phosphoric acid, ribose sugar, purines namely adenine and guanine and pyrimidines namely cytosine and uracil.
- The mRNA carriers genetic information from DNA. The genetic information carried by the mRNA is called genetic code. The genetic code is the sequence of nitrogen bases in mRNA. The genetic code is formed of several codons. Each codon is a sequence of three nitrogen basses which codes for one amino acid. As each codon is formed of three nitrogen bases, it is called triplet code.
- Among RNAs, mRNA is the longest one. Most of the mRNAs contain 900 to 15, 000 nucleotides.
- Each mRNA contains the codons from one polypeptide chain. If the mRNA contain 900 nucleotides the polypeptide chain synthesized by this mRNA will contain 300 amino acids.
- One end of mRNA is called 5’ end and the other end is called 3’ end.

- At the 5' end a cap is found in most eukrayotes and animal viruses. The cap formed by the condensation of a guanylate residue. The cap helps the mRNA to bind with ribosomes.
- The cap is followed by a non-coding region. It does not contain code for protein and hence it cannot translate protein. It is formed of 10 to 100 nucleotides and is rich in A and U residues.
- The non coding region is followed by the initiation codon. It is made up of AUG.
- The initiation codon is followed by the coding region which contains the code for protein. It has an average of 1.500 nucleotides.
- The coding region is followed by a termination codon. It completes the translation. It is made up of UAA or UAG or UGA in eukaryotes.
- The termination codon is followed by a non- coding region. It has a nucleotide sequence of AAUAAA.
- At the 3' end of mRNA there is a polyadenylate sequence.

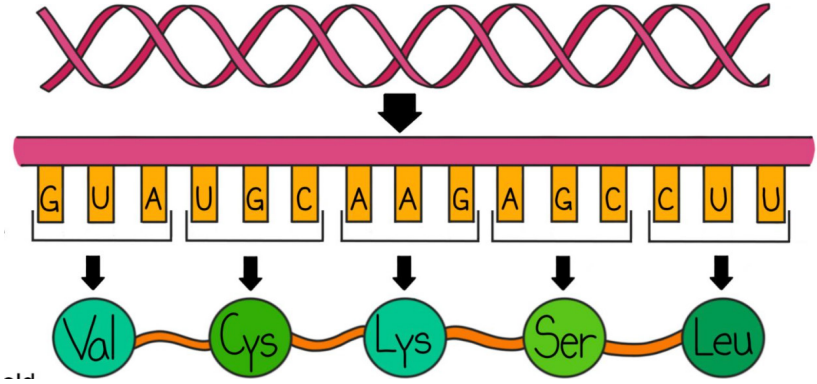


Fig 6.5 mRNA

6.4.1.2 Transfer RNA (tRNA)

- It is a single stranded RNA.
- It is smaller than mRNA.
- It transports amino acids to the site of protein synthesis.
- Each tRNA transport only one variety of amino acid.
- There are about 60 types of tRNA. As there are only 20 types of amino acids, certain amino acids are carried by more than one tRNA.
- The tRNA is formed of small units called nucleotides. There are about 75 to 80 nucleotides in a tRNA.

- The tRNA contains phosphoric acid, ribose sugar, purines and pyrimidines.
- The tRNA is folded on itself to form a clover leaf like structure. It has two terminal ends, three main loops and one mini loops.
- tRNA has four regions, namely
- Acceptor arm or amino acid binding site
- D arm or enzyme site
- TψC arm or ribosomal site
- Anticodon arm or recognition site
- Each amino acid is activated by a specific enzyme. The activating enzyme is attached to a main loop of tRNA. Hence the site is called enzyme site. It recognizes, catalyzes and picks up the appropriate amino acid from the cytoplasm.
- The tRNA is attached to the ribosome through one main loop. Hence this region of tRNA is called ribosome site.
- The tRNA contains certain rare and unusual nitrogen bases, namely pseudouridine, methylguanine, methylamino-purine, inosinic acid.
- Transfer RNA are more stable.
- They are synthesised from the DNA of chromosome.

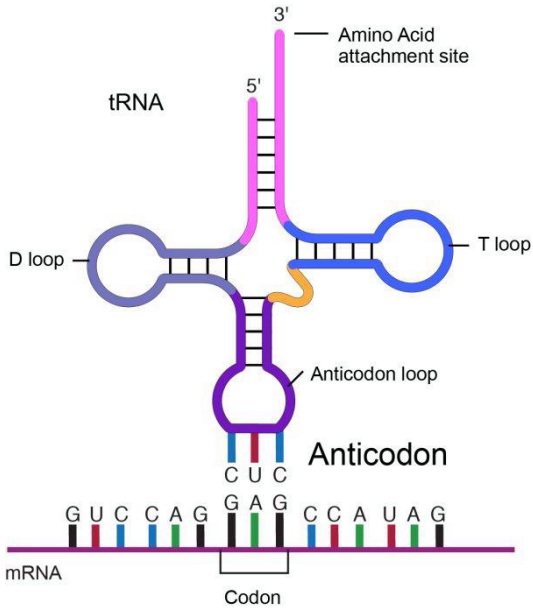


Fig 6.6 tRNA

6.4.1.3 Ribosomal RNA (rRNA)

- Ribosomal RNA is a ribonucleic acid present in the ribosome and hence it is called ribosomal RNA. It is also called insoluble RNA. It constitutes about 80% of the cellular RNA. It is the most stable form of RNA.
- The ribosomal RNA is formed of a single strand. It is polynucleotide chain. Each strand is formed of many nucleotide units. Each nucleotide is formed of three different molecules namely phosphate, a ribose sugar and a nitrogen base.
- The nitrogen bases are of two types namely purines and pyrimidines. The purines present in the rRNA are adenine and guanine. The pyrimidines present in rRNA are cytosine and uracil.
- In some regions, the single strand itself align upon itself to form a double helix. The helical regions are connected by intervening single stranded regions. In the helical regions most of the base pairs are complementary. They are joined by hydrogen bonds. In the unfolded single strand regions, the base pairs are not complementary. Because of this nature, in the rRNA the purines and pyrimidines bases have no equality.
- The rRNA are classified into 7 types according to their sedimentation co-efficient. They are the following.

  28S rRNA

  18 S rRNA

  5.8S rRNA

  5S rRNA

  23 S rRNA

  16 S rRNA

- Though the rRNA constitutes the main bulk of the cytoplasmic RNA its function is not clearly known. However it is believed that rRNA plays the major role in protein synthesis.
- In all organisms except virus, the RNA is doing non- genetic functions. Such RNAs are called non genetic RNAs.

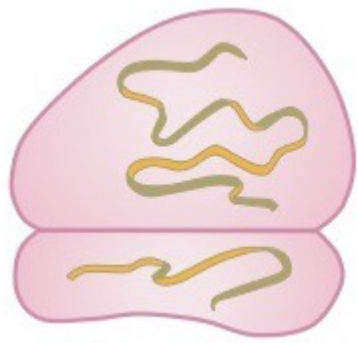


Fig 6.7 rRNA

6.5 BIOLOGICAL FUNCTION OF NUCLEIC ACIDS

Nucleic acids play vital roles in the life of organisms

1. Protein synthesis

Nucleic acid synthesize the various proteins of the protoplasm.

2. Memory

RNA is associated with memory storage functions in the brain.

3. Replication

During cell division, DNA divides and offers exact copies to the daughter cells.

4. Genetic information

DNA contains genetic information. DNA transfers characters from parents to offspring.

5. Mutation

DNA produces mutation resulting in new characters.

UNIT VII

VITAMINS

Introduction, Vitamins and it's classification - provitamins – biological functions - Properties of Vitamins – Classification of vitamins - fat soluble vitamins (Vitamin – A, D, E and K) and water soluble (vitamins - B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}) Vitamin C and their composition, structure, sources, properties, functions, deficiency symptoms and dietary requirements.

7 VITAMINS

Vitamins are required by the body to grow and develop. Different organisms have different vitamin needs. Some organisms do not require vitamins. Example: Bacteria A. Hope you have verified this information. I couldn't find any information regarding this fact since I'm not aware of 'bacteria A'.

Vitamin is an organic compound and an essential nutrient that an organism needs in small amounts. Vitamins are classified by both biological and chemical activity and not their structure.

Different kind of vitamins are required by different organisms. Vitamins must be included in the diet in optimum amount. Excess of vitamin or low level of vitamin causes disorders.

7.1 VITAMINS AND IT'S CLASSIFICATION

Vitamins are the group of organic compounds which are essential for normal growth and nutrition and are required in small quantities in the diet because they cannot be synthesized by the body.

Vitamins are classified into fat soluble vitamins and water soluble vitamins.

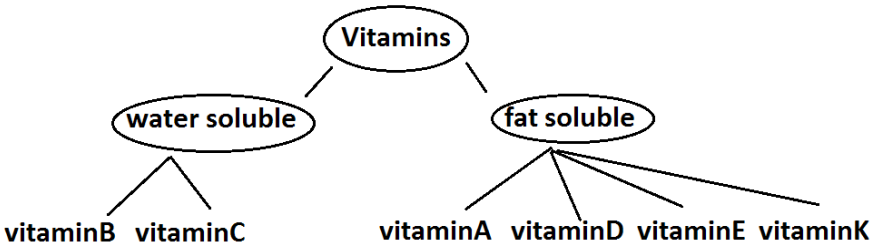


Fig 7.1 Classification of Vitamins

Totally there are 13 vitamins in which 4 is a fat soluble vitamin (A, D, E, K) and the remaining 9 is a water soluble vitamin (B and C).

7.2 PROVITAMINS

Provitamin is an inactive vitamin which can be converted into a vitamin. It is a precursor of vitamin. A precursor is converted by the body to the vitamin.

Examples: Carotene – provitamin for Vitamin A

Ergosterol – provitamin for Vitamin D

7.3 BIOLOGICAL FUNCTIONS

7.3.1 Water-soluble vitamins

The important roles of water soluble vitamins are

Table 7.1 Water-soluble vitamins- Functions and Sources

Nutrient	Function	Sources
Vitamin B_1 Thiamine	Nerve function, Required for energy metabolism.	whole-grain or enriched breads and cereals, legumes, Rice polishing's, liver, kidney, yeast, milk, groundnuts, eggs, green vegetables and dairy products (except butter)
Vitamin B_2 Riboflavin	Normal vision and skin health	Milk and milk products; leafy green vegetables. Yeast, egg white, liver, kidney, meat
Vitamin B_3 Niacin	Essential for nervous system, digestive system, and skin health	Meat, poultry, fish, whole-grain or enriched breads and cereals, leafy green vegetables, peanut butter
Vitamin B_5 Pantothenic acid	Required for energy metabolism	Plant and animal tissues, meat products like liver, meat and kidney, yeast, grain, cereals, pulses, groundnut and coffee
Vitamin B_6 Pyridoxine	Required for protein metabolism.	Meat, fish, poultry, vegetables, fruits, cereal grains, molasses and yeast
Vitamin B_7 Biotin	Part of an enzyme needed for energy metabolism	Widespread in foods; also produced in intestinal tract by bacteria
Vitamin B_9 Folic acid	Essential for blood cells.	Green leafy vegetables and fruits

Vitamin B_{12} Cobalamin	Part of an enzyme needed for making new cells; important to nerve function	Meat, poultry, fish, seafood, eggs, milk and milk products; not found in plant foods
Vitamin C Ascorbic acid	Essential for immune system	Found only in fruits and vegetables, especially citrus fruits, vegetables in the cabbage family, cantaloupe, strawberries, peppers, tomatoes, potatoes, lettuce, papayas, mangoes, kiwifruit

7.3.2 Fat-soluble vitamins

Table 7.2 Fat-soluble Vitamins – Functions and Sources

Nutrient	Function	Sources
Vitamin A	Needed for vision, healthy skin and mucous membranes, bone and tooth growth, immune system health	Leafy, dark green vegetables, dark orange fruits, fish oil, particularly in shark liver oil
Vitamin D	Bones development	Sunlight, egg yolks, liver, fatty fish, fortified milk
Vitamin E	Antioxidant; protects cell walls	Oils of soyabean, corn, cottonseed, safflower, leafy green vegetables, whole-grain products, liver egg yolks; nuts and seeds
Vitamin K	Needed for proper blood clotting	Leafy green vegetables

7.4 PROPERTIES OF VITAMINS

- Vitamins are organic compounds.
- Plants synthesize vitamins.
- Animals obtain the vitamins from the food stuffs.
- Man requires lesser vitamins. They are required in very small quantities.
- They do not provide any energy for the animals; but they regulate the physiological activities functioning as coenzymes.
- Vitamins are destroyed by high temperature and cooking for longer duration of time.

- When vitamins are deficient in the food, they produce a set of diseases called deficiency diseases. These diseases can be cured only by treatment with the particular vitamin deficient.

7.5 CLASSIFICATION OF VITAMINS

Vitamins are classified based on their solubility. They are classified as fat -soluble vitamins and water-soluble vitamins.Vitamins A, D, E and K are called fat soluble vitamins. B-complex vitamins and Vitamin C are called water soluble vitamins.

7.6 FAT SOLUBLE VITAMINS

7.6.1 Vitamin A: Retinol or Anti Xerophthalmic Vitamin

It is a fat soluble vitamin. Vitamin A is needed by the retina of the eye in the form of retinal rhodopsin, the light-absorbing molecule which was necessary for both low-light and color vision. It exists in two forms, Vitamin A_1 (Retinol$_1$) and Vitamin A_2 (Retinol$_2$). The empirical formula of A_1 is $C_{20}H_{29}OH$ and A_2 is $C_{20}H_{27}OH$.

Animals synthesize vitamin A from carotene. So carotene is called provitamin A. A single carotene is split into two molecules of Vitamin A.

Composition

- Vitamin A is an alcohol.
- The empirical formula is $C_{20}H_{29}OH$
- Vitamin A consists of an ionone ring and a hydrocarbon chain. It has 5 conjugated double bonds. The hydrocarbon chain ends in a terminal alcohol group.

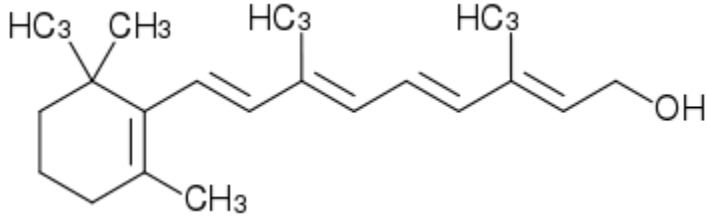


Fig 7.2 Structure of Vitamin A

Sources

Leafy, dark green vegetables; dark orange fruits Fish oil, particularly in shark liver oil

Properties of Vitamin A

- Vitamin A is soluble in fat and soluble in water.
- It is destroyed by light and oxidation.
- It is thermo labile.
- It forms esters.
- It can be oxidized to form aldehyde.

Functions

- Vitamin A is an important component of rhodopsin of retina. Hence it is essential for vision.
- Vitamin A is essential for cell growth, immune function, fetal development and vision.
- It promotes growth.
- It is essential for protein synthesis.
- It maintains the normal growth and shape of bones.
- It is essential for the synthesis of mucopolysaccharides.
- It promotes fertility.
- It has some specific functions on carbohydrate metabolism.
- It is essential to the normal structure and functions of epithelial tissues.
- It is essential for the metabolism of DNA.

Deficiency

- Deficiency of vitamin A retards growth in children.
- Vitamin A is essential for the synthesis of rhodopsin which was essential for vision in dim light. When vitamin A is deficient rhodopsin cannot be synthesized. This leads to a failure of vision in dim light. This type of eye defect is called night blindness or nyctalopia.
- Vitamin A deficiency leads to reddening, dryness and lusterless condition of the eye. This defect is called xerophthalmia. When the level of deficiency is more, the cornea becomes soft, disorganized and destroyed. This defect is called keratomalacia.
- Deficiency causes phrynoderma or toad skin. The skin becomes hard and horny. The skin appears scaly and rough.
- Degeneration of lachrymal gland.
- Sweat and sebaceous glands of skin degenerate.

- The glands present in the alimentary canal and the epithelial lining degenerate.
- The epithelium of the respiratory tract becomes stratified and degenerate.

7.6.2 Vitamin D or Calciferol or Antirachitic vitamin

- Vitamin D is also called Sunshine Vitamin. It exists in five forms D_1, D_2, D_3, D_4 and D_5.
- The skin of man contains large amount of cholesterol. This cholesterol is converted into vitamin D on exposure of skin to sunlight.

Composition

- Vitamin D is a sterol.
- The empirical formula of vitamin D_2 is $C_{28}H_{43}OH$ and it is called Ergocalciferol.

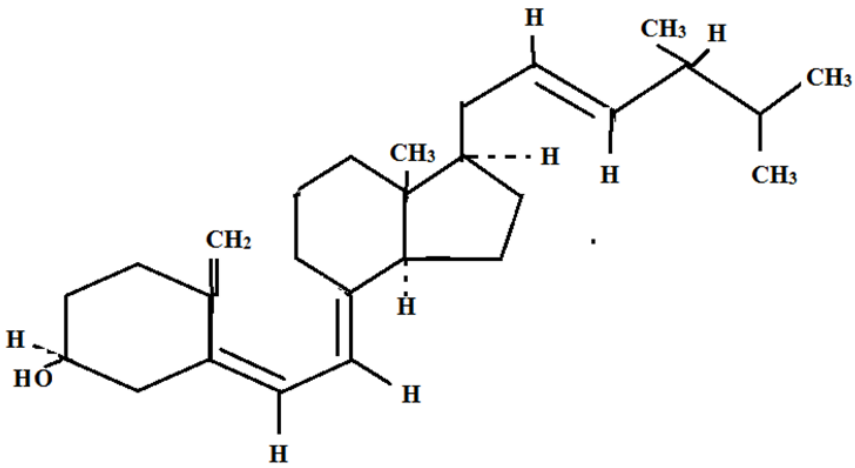


Fig 7.3 Structure of Vitamin D

Sources

Sunlight, Egg yolks, liver, fatty fish, fortified milk.

Properties

- Vitamin D acts both as hormone and a vitamin.
- It helps for the absorption of calcium.
- Vitamin D is involved in many biological processes.

Functions

1. It helps calcium absorption in the intestine.
2. It improves absorption of phosphate.
3. It is essential for calcium metabolism.
4. It helps in the normal development of bone and teeth.
5. It helps in the deposition of calcium and phosphates in the bones.
6. It maintains normal cellular growth and function.
7. It helps for immune function.

Deficiency

- Deficiency of this vitamin causes **rickets** in children and **osteomalacia** in adults. It is a disease of bones. It occurs in children.The long bones especially the leg bones tend to bend leading to the development of **bow legs.**
- The chest becomes protruded leading to pigeon chest**.**

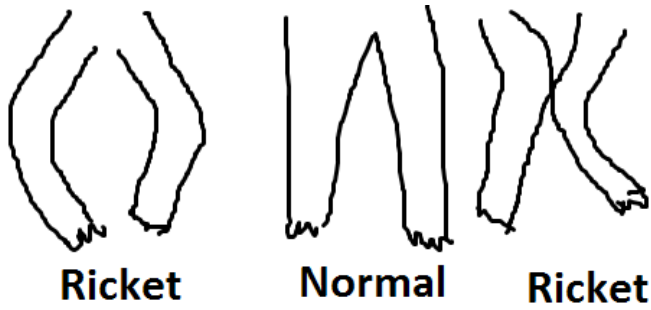


Fig 7.4 Rickets

7.6.3 Vitamin E or Tocopherol or Antisterlity vitamin

- It is a fat soluble vitamin.
- Tocopherol is a Greek word. Tokos means children and phero means to bear.
- It exists in 3 forms α, ß, γ – tocopherol.

Composition

- Vitamin E is a tocopherol which contains an unsaturated alcohol.
- The empirical formula of α – tocopherol is $C_{29}H_{50}O_2$

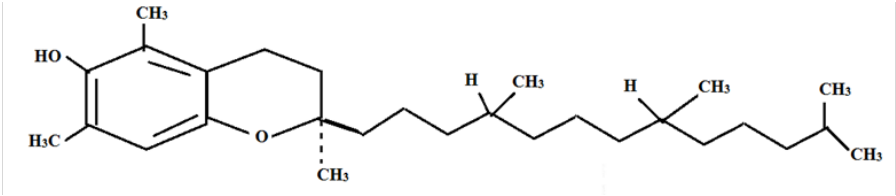


Fig 7.5 Structure of Vitamin E

Sources

- Oils of soyabean, corn, cotton seed, safflower, leafy green vegetables, whole-grain products, liver, egg yolks, nuts and seeds.

Properties

- Vitamin E has anti – oxidant properties.
- Vitamin E is essential for health and it has anti inflammatory properties.

Functions

- It functions as an antioxidant. It prevents oxidation of certain substances like vitamin A, fatty acids and sulphur containing amino acids.
- Vitamin E is very effective to reduce UV damage in skin.
- Essential for the normal reproduction of rats.
- Essential for the normal functioning of muscles.
- Essential for the biosynthesis of Co-enzyme Q.

Deficiency

- Vitamin E is essential for central nervous system. So the deficiency of vitamin E leads to muscle weakness.
- Deficiency causes Neurological and Neuromuscular problems.
- In rats, guinea pigs and rabbits, deficiency leads to degenerative changes in the muscles and paralysis. The defect is called nutritional muscular dystrophy.
- In chicks, the deficiency leads to the disintegration of blood vessels.
- Exudative diathesis occurs which shows the appearance of large patches of subcutaneous oedema on the breast, abdomen, neck and legs.
- Combined deficiency of Vitamin E and selenium causes hepatic necrosis.

7.6.4 Vitamin K or Anti haemorrhagic vitamin:

- It is a fat soluble vitamin.
- It is named K because it is essential for coagulation or blood clotting.

Composition

- Vitamin K is a naphthoquinone derivative and it exists in two forms naturally as K_1 $K_2$and K_3 is a manmade.

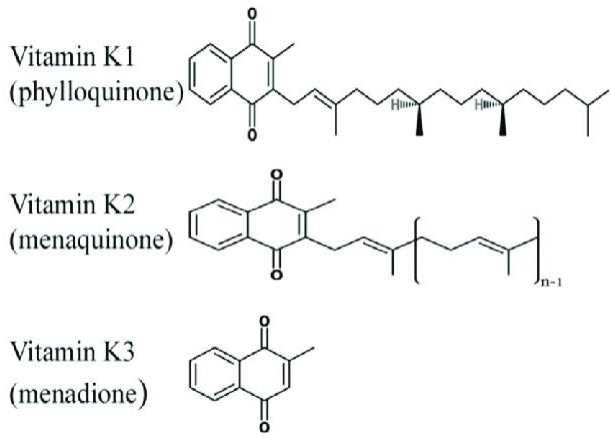


Fig 7.6 Structure of Vitamin K

Sources

Leafy green vegetables.

Properties

- Vitamin D helps in preventing mineralization which helps for maintaining blood pressure.

Functions

- It is essential for the synthesis of prothrombin in the liver. Prothrombin is essential for the coagulation of blood. Hence vitamin K is essential for the coagulation of blood. Hence it is called an antihaemorrhagic vitamin.
- Vitamin K is very essential for bone density.
- It also helps for the heart to pump the blood freely.
- It plays a key role in the respiratory chain mechanism and oxidative phosphorylation.

Deficiency symptoms

When vitamin K is deficient, coagulation is prevented; when there occurs an injury, generally bleeding is stopped within 5 to 8 minutes. This is due to coagulation of blood on the wound surface. When vitamin K is deficient coagulation cannot occur. This leads to continuous bleeding from the wound and the victim dies because of loss of blood.

7.7 WATER SOLUBLE VITAMINS

Vitamin B complex

It includes a set of water soluble vitamins. It is divided into four groups namely B_1, B_2, B_6 and B_{12}. The important B complex vitamins are

- B_1 - Thiamine
- B_2 - Riboflavin
- B_3 - Niacin
- B_5 - Pantothenic acid
- B_6 - Pyridoxine
- B_7 - Biotin
- B_9 - Folic acid
- B_{12} - Cobalamine

7.7.1 Vitamin B_1 or Thiamine:

It is soluble in water. Unpolished rice is the richest source.

Composition

Thiamine contains pyrimidine and thiazole molecule. The empirical formula of thiamine is $C_{12}H_{17}N_4OS$. Thiamine is 2,5-di methyl -6-amino pyrimidine bonded through a methylene linkage to 4-methyl-5-hydroxy ethyl thiazole.

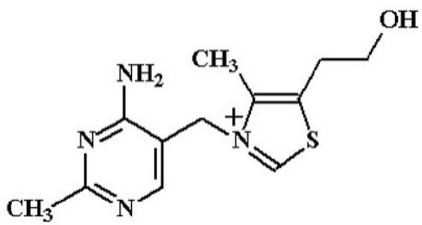


Fig 7.7 Structure of vitamin B_1

Structure of vitamin B1

Sources

Whole-grain or enriched breads and cereals, legumes, rice polishing's, liver, kidney, yeast, milk, groundnuts, eggs, green vegetables.

Properties

- It is an yellow coloured water soluble vitamin.
- It is stable in acid medium.
- It was unstable in alkaline medium.
- On improper cooking, vitamin B_1 gets destroyed.
- It is hygroscopic.

Functions

- It helps in maintaining the healthy nervous system.
- It helps for the digestion of food.
- It provides muscle strength.
- It is essential for heart functioning.

  Thiamine + ATP $\longrightarrow$ AMP + Thiamine pyrophosphate.

- The thiamine pyrophosphate acts as a coenzyme. It also acts as co-enzyme in many biological reactions.
- It activates carboxylase. Carboxylase is essential for the oxidative decarboxylation of pyruvic acid, keto-glutaric acid and other keto acids. It is an important step in the final oxidation of sugar in the tissues and brain.
- It also helps the enzyme system which is important in synthesis of fats.

Deficiency

Deficiency causes beriberi. Beriberi is characterized by oedema in legs.

7.7.2 Vitamin B_2 or Riboflavin

It is an orange-yellow compound containing D-ribose alcohol and flavin. Riboflavin is a flavin derivative. Riboflavin consists of a dimethyl isoalloxazine ring and a sugar alcohol D-ribitol attached at position number 9.

Composition

Vitamin B_2 is 7,8-dimethyl-10-(1Y-D-ribityl)isoalloxazine

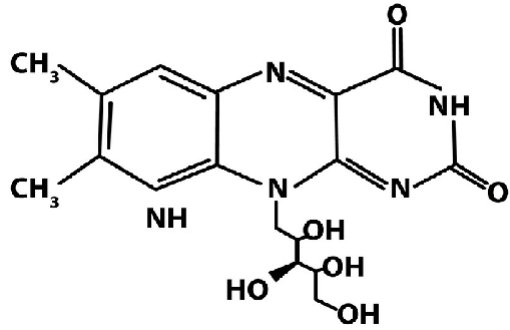


Fig 7.8 Structure of Vitamin B_2

Sources

Milk and milk products; leafy green vegetables. Yeast, egg white, liver, kidney, meat**.**

Properties

- The word flavin means yellow and it is a yellow orange coloured compound.
- It is unstable at high temperature.
- It is nontoxic.
- It is stable to heat in neutral and acid medium.
- It is sensitive to light.

Functions

- Riboflavin is very essential for maintaining good health of our body.
- Riboflavin is a component of two important co-enzymes, namely flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). They play major roles in various enzyme systems.
- It helps for breaking down the food components.
- It is essential for the metabolism of growth.
- It is an important component of acyl-CoA dehydrogenase.

Deficiency Symptoms

- Cheilosis: It is characterized by the development of fissures developing in the lips and at the corners of the mouth.

- Sore tongue.
- Deficiency leads to Night blindness.
- Seborrheic dermatitis affecting the face (ears, nose and forehead).
- Deficiency of riboflavin leads to avascularization of the cornea i.e eye becomes itchy, vision becomes poor in dim light
- The skin loses hair and it becomes dry and scaly.
- Growth is arrested.
- Deficiency of the vitamin also leads to anaemia.

7.7.3 Vitamin B_3 or Niacin

It is a pyridine derivative. It occurs in tissue as nicotinamide. Niacin is pyridine-3-carboxylic acid.

Composition

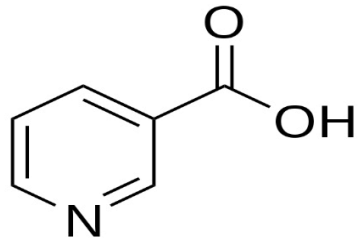


Fig 7.9 Structure of Vitamin B_3

Sources

Meat, poultry, fish, whole-grain or enriched breads and cereals, leafy green vegetables, peanut butter

Properties

- Niacin is an odourless, crystalline substance
- It is resistant to heat, oxidation and alkalis.
- It is important for the synthesis of protein and fats.

Functions

- Niacin is important for the release of energy.
- This vitamin is a component of coenzyme A.
- It is essential for several basic reactions in metabolism.

Deficiency symptoms

- Pellagra – Rough skin was formed. It is characterized by 3 symptoms namely dermatitis, diarrhea and dementia (organic loss of intenstinal function).

7.7.4 Vitamin B_5 or Pantothenic acid

Composition: It empirical formula is $C_9H_{17}O_5N$. Pantothenic acid is an amide of pantoic acid. It is compound of ß–alanine and α-γ-dihydroxy beta, beta dimethyl butyric acid. The empirical formula of Vitamin B_3 is $C_9H_{17}O_5N$.

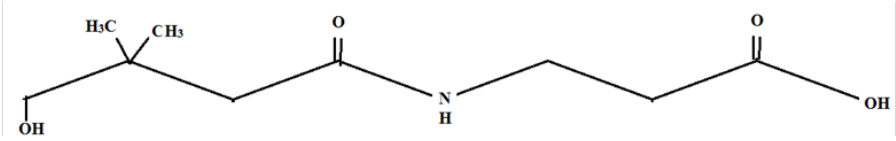


Fig 7.10 Structure of Vitamin B_5

Structure of Vitamin B_5

Sources

Plant and animal tissues, meat products like liver, meat and kidney, yeast, grain, cereals, pulses, groundnut and coffee.

Properties

- It is stable to heat.
- It is stable to oxidising and reducing agents.
- It is soluble in water.
- It is insoluble in chloroform.

Functions

- It is essential for growth.
- It plays an important role in oxidation and metabolism.
- It promotes the formation of fats from carbohydrates.
- It is an important component of two co-enzymes NADP and DPN.
- They control large number of reactions.

Deficiency

- Burning feet syndrome in man.
- It also causes respiratory infections.

7.7.5 Vitamin B_6 or Pyridoxine

Composition

- It is a pyridine derivative. Its empirical formula is $C_8H_{11}O_3N$.
- It occurs in 3 forms namely pyridoxine, pyridoxol and pyridoxamine.

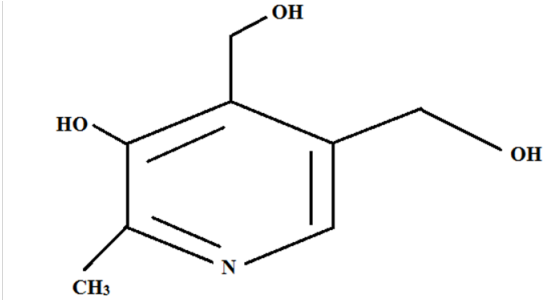


Fig 7.11 Structure of Vitamin B_6

Sources

Meat, fish, poultry, vegetables, fruits, Cereal grains, molasses and yeast

Properties

- It is sensitive to light and alkali.
- It exist as colourless crystals at room temperature.
- It has bitter taste.

Functions

- Vitamin helps for protein metabolism.
- Pyridoxol phosphate acts as a coenzyme.
- It helps in the synthesis of fats from carbohydrates and proteins.
- Premenstrual syndrome can be cured using vitamin B_6.
- It is involved in the active transport of aminoacids and certain metallic ions across cell membranes.
- It is linked with the metabolism of central nervous system.
- It helps to prevent the eye diseases.

Deficiency symptoms

- Deficiency leads to premenstrual tension.
- It is characterized by the scaliness, loss of hair, swelling, inhibition of growth.

- Deficiency leads to skin inflammation.
- Deficiency of the vitamin causes cardiovascular problems and anaemia.

7.7.6 Vitamin B_7: Anti-Egg-White injury factor

Composition

- The empirical formula is $C_{10}H_{16}O_3N_2$.
- It is a heterocyclic S-containing monocarboxylic acid containing two five – sided rings.

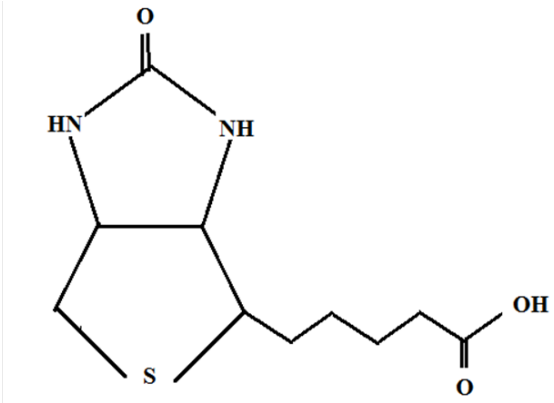


Fig 7.12 Structure of Vitamin B_7

Sources

Widespread in foods; also produced in intestinal tract by bacteria

Functions

- It is called co-enzyme R because it is a growth factor for the nitrogen fixing bacterium Rhizobium.
- It involves in the fixation of CO_2 and carboxylation.
- It involved in deamination of certain amino acids.
- It is essential for synthesis of lipids.
- It prevents dermatitis in dogs and rats.

Deficiency symptoms

- Deficiency causes muscle pains.
- Symptoms of thiamin deficiency.
- Blood cholesterol increases.

7.7.7 Vitamin B_9 or Folic acid

Vitamin B_9 is extracted from spinach leaf.

Composition

- A molecule of folic acid consists of 3 units, namely

1. Glutamic acid 2. Para amino benzoic acid 3. Pterin

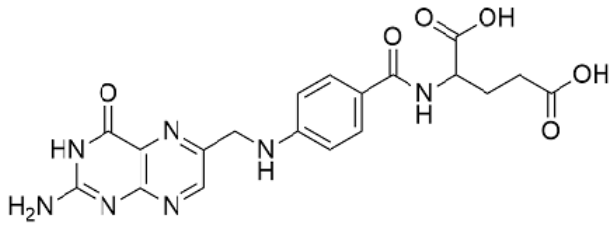


Fig 7.13 Structure of Vitamin B_9

Sources

Green leafy vegetables and fruits.

Properties

- It functions as a co-enzyme.

Functions

- It is essential for the synthesis of RNA.
- Its main role is in the formation and maturation of red cells.

Deficiency symptoms

- Deficiency causes anaemia.
- Deficiency leads to poor growth.
- Deficiency in man causes **megaloblasticanaemia** during pregnancy. It also causes **glossitis** (inflammation of the tongue) and gastrointestinal disorders.

7.7.8 Vitamin B_{12} or Cyanocobalamine:

Vitamin B_{12} is called Anti pernicious anaemia factor.

Composition

The empirical formula is $C_{63}H_{88}o_{14}N_{14}PCo$. The cobalt atom is centrally situated and it is surrounded by 4 reduced pyrrole rings collectively called corrin.

Sources

Meat, poultry, fish, seafood, eggs, milk and milk products. But it was not found in plant foods

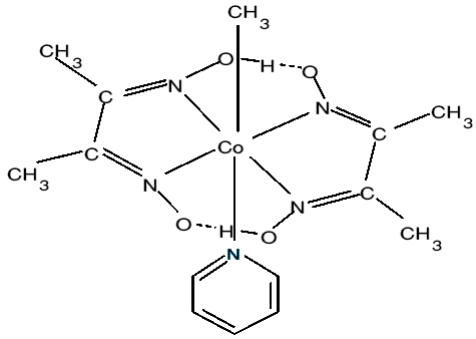


Fig 7.14 Structure of Vitamin B_{12}

Properties

- It is very stable at high temperature.
- Vitamin gets degraded in light.
- It is soluble in water, ethanol and methanol.
- Vitamin are deeply red coloured crystals.
- In the living cells B_{12} is converted into a co-enzyme called co-enzyme B_{12}. It is involved in a number of metabolic reactions.

Functions

- It is essential for the formation and maturation of RBC.
- It is involved in the synthesis of nucleic acids.
- It stimulates bone marrow to produce WBC and platelets.
- It helps the growth of micro-organisms.
- It synthesizes lipids from carbohydrates.
- It prevents hyperglycaemia.
- It prevents pernicious anaemia.

Deficiency symptoms

- Deficiency of B_{12} causes pernicious anaemia. It is characterized by a drastic decrease in blood cell count. RBC becomes abnormally larger.
- Another deficiency sign is hyperglycaemia.

7.7.9 Vitamin C

Composition

- Vitamin C has the chemical formula $C_6H_8O_6$
- Vitamin C is L-enantiomer of ascorbate

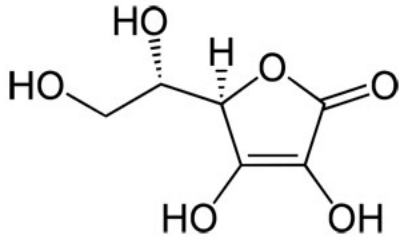


Fig 7.15 Structure of Vitamin C

Sources

- Oranges, Broccoli, Brussels sprouts and cauliflower, Spinach, cabbage and other leafy greens, Sweet and white potatoes, Tomatoes.

Properties

- It is a water soluble vitamin
- It increases the absorption of non haem iron
- It is an anti oxidant.

Function

- Vitamin C is a vital nutrient for health.
- Vitamin C may include protection against immune system, cardiovascular disease, eye disease and even skin wrinkling.
- Vitamin C may also help prevent acute respiratory infections.
- It helps to maintain bones, skin and blood vessels.

Deficiency

- Deficiency of vitamin C causes Scurvy.
- Poor wound healing occurs and immune functioning becomes weak due to vitamin C deficiency.

Vitamins	Daily Dietary Requirements
Vitamin A	Daily requirement of Vitamin A for adults is 750 µg. The requirement for infants and young children is 300 µg. Women during pregnancy and lactation require 1200 µg per day.
Vitamin D	Infants : 400 to 800 I.U. daily Children and adolescents : 400 I.U. daily During pregnancy and lactation : 400 to 800 I.U. daily Exposure to sunlight produces adequate amount of vitamin D in the skin.
Vitamin E	A daily allowance of 20 - 25 mg of vitamin E has been recommended for man.
Vitamin K	Sufficient quantity of the vitamin is synthesized by intestinal bacteria under normal conditions. However, exogenous supply is necessary only in conditions of potential vitamin K deficiency.
Vitamin B_1 (Thiamine)	For each 1000 calories, 0.5 mg is recommended for an average man.
Vitamin B_2 (Riboflavin)	Adults : 1.5 to 1.8 mg Pregnant, lactating women and children : 2 to 2.5 mg daily
Vitamin B_3 (Pantothenic acid)	Recommended daily requirement is 5 to 12 mg for adults.
Vitamin B_5 (Niacin)	Adults : 17 to 21 mg
Vitamin B_6 (Pyridoxine)	A daily intake of 2 mg for adults is recommended.
Vitamin B_7 (Biotin)	It is abundantly produced by intestinal bacteria
Vitamin B_9 (Folic acid)	No definite requirement for normal human.
Vitamin B_{12} (Cyanocobalamine)	Daily requirement of vitamin B_{12} is 2 to 4 µg.
Vitamin C	Recommended daily requirement is 75 to 100 mg.

UNIT VIII

NUTRITIONAL BIOCHEMISTRY

Nutritive value of Milk – Egg – Meat - Fish – Vegetable food (Cereals, Pulses, Nuts, Roots and Tubers, Green leafy vegetables) – Fruits – Tea – Coffee – Cocoa – Alcohol – Principles in balancing a diet - Energy yielding, Body building and Protective foods - Bioavailability – absorption – interactions between diet and drug ingredients – gastric emptying and drug absorption – drug excretion – low protein diets – dehydration and starvation – effect of drugs on growth, vitamins and minerals

8 NUTRITIONAL BIOCHEMISTRY

8.1 NUTRITIVE VALUE OF FOODS

Nutrition is the study of nutrients in food. The study of nutrients is very important to know about the requirements of micronutrients and macronutrients in our body. The study of nutrition tells the relation between diet, health and disease. Eating wrong type of food leads to the deficiency of nutrition. Malnutrition occurs when the intake of nutrients is too high or low.

8.2 MILK

Milk is the first food for humans and it is the richest nutritional food. Milk is obtained from cow, goat, buffalo, cattle and camels. Cow's milk is the most widely used.

The different types of milk used are fresh milk, dried milk, long –term milk, domestic condensed milk. The milk products derived from milk used are butter, ice cream, yoghurt, hard White and creamy cheese.

- Milk is a perfect and complete food. It is rich in calcium, potassium, sodium, chlorine and phosphorus.
- It contains not only protein, fat and carbohydrate but also minerals and vitamins.
- The carbohydrate of milk is lactose which is less sweet than sucrose.
- The chief proteins are caseinogen and lactalbumin. Caseinogen is a phosphoprotein. Caseinogen is associated with calcium as calcium caseinogenate. Small amounts of other proteins are also present.
- Milk fat is in the form of a very fine emulsion. It contains all saturated fatty acids as well as unsaturated fatty acids.
- It is rich in vitamin A and vitamin B_2 and good in vitamin B_1 and nicotinic acid but poor in C and D.
- Milk kept on unsterilized becomes sour due to formation of lactic acid from lactose by bacteria present in milk.

- When milk comes in contact with rennin or proteolytic enzymes, it becomes clotted.
- The protein of milk neutralizes gastric HCl to allow clotting.
- Condensed milk is prepared by the removal of water by evaporation vacuum. Vitamins A and B are preserved.
- Dried milk is prepared by passing a film of milk over hot rollers or spraying milk into hot to evaporate water.

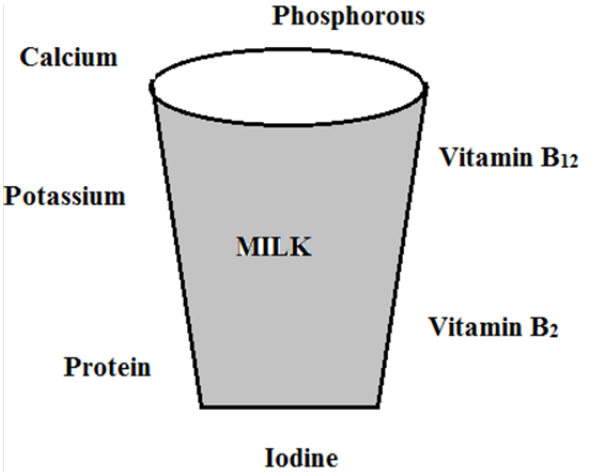


Fig 8.1 Composition of Milk

Health Benefits of Milk

- Milk helps to provide the body with energy and it helps for the growth.
- Milk helps to maintain the bone and teeth.
- Prevents cardiac diseases.
- Keeps the blood pressure at a normal rate.
- It improve the performance of the nervous system.
- It improves the digestion process.
- It protects the eyesight and maintain the skin, hair and delicate membranes.
- It helps to treat the dehydration.

Milk is very essential for our health maintenance. Taking beverages and food containing caffeine such as fizzy beverages, tea, coffee and chocolate reduces the absorption of calcium. Also, vitamin C rich fruits such as citrus must be taken in plenty, as it is instrumental in absorbing the calcium from the milk.

8.3 EGG

Eggs are very essential for a healthy diet and it is an inexpensive healthy food. It contains several vitamins and minerals. Eggs are also a source of vitamins A,B,E and K.

- Eggs store significant amounts of protein and choline.
- The hen's egg contains about 30% yolk, 59% white and 11% shell. The chemical composition of duck's egg is similar to that of hen's egg.
- The white part of egg contain proteins and salts. The greater part of the protein is ovalbumin. The other proteins are conalbumin, ovoglobulin and ovomucoid (a glycoprotein).
- The pale yellow colour is due to riboflavin. The white contains 10.7% protein, 0.1% fat and 1.4% carbohydrate.
- The yolk contains 51% water, 15% protein, 33% fat of which 4% cholesterol and 1% mineral. The proteins are vitellin (a phosphoprotein) and livetin (a globulin). The minerals are calcium, iron and phosphate.
- The yolk is rich in vitamins A_1, B_1, B_2 and D but not C. The other B group vitamins and E are also present.
- The egg is a powerhouse of disease-fighting nutrients.
- Eggs are rich sources of selenium, vitamin D, B_6, B_{12} and minerals such as zinc, iron and copper. Egg yolks contain more calories and fat than the whites.

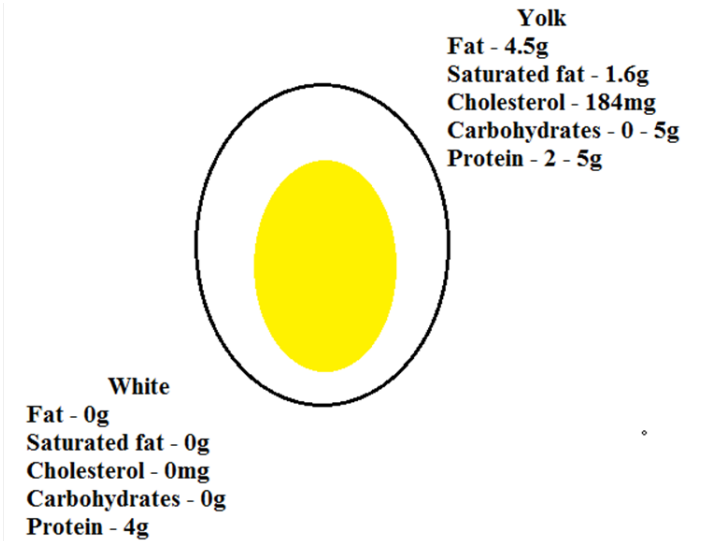


Fig 8.2 Composition of Egg

Health benefits of Egg

- Egg contains the essential body nutrients.
- The protein present in egg helps to maintain the muscle.
- Egg helps to maintain the nervous system.

8.4 MEAT

- Meat is the flesh of animals.
- Meat contains about 22% protein and B group vitamins but does not contain vitamins A, C or D.
- In boiling, there is a loss of salts, gelatin and extractives. During roasting, this loss is reduced.
- Protein obtained from meat is needed by the body for a variety of functions, such as wound healing, muscle building and immune function.
- Red meat is a good source of iron, which is needed for red blood cell production.
- Some meats are high in saturated fat and cholesterol. Eating too much fat and cholesterol can lead to higher risk for heart disease.
- The flavor of meat is due to the organic substances extracted from meat by boiling water.
- Meat has benefits for muscle and bone health, metabolism and iron absorption.

8.5 FISH

- Fish is a nutritious food containing protein, vitamins and minerals which are essential for the health.
- The three types of fish available are oily fish, white fish and shellfish.
- Oily fish contain vitamins A, D and E. They are also rich in essential omega-3 fatty acids which are essential for healthy brain, eye and nerve development in babies and children.
- Fish oils in fatty fish are the richest source of a type of fat that is vital to normal brain development in unborn babies and infants.

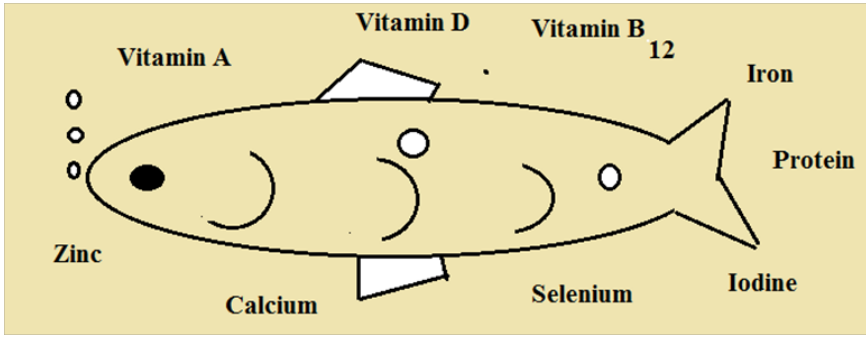


Fig 8.3 Composition of Fish

- Fish is free from carbohydrate just like meat. The fat content ranges from a trace to 5%. It is fair source of B group vitamins. Fatty fish contains some vitamins A and D.
- Large fish is rich in phosphorous but deficient in calcium. Small fish eaten with bones are good sources of calcium.
- It contains 22% protein, 1.4% fat, 75% water. The dried fish may contain about 5% carbohydrate because of the use of flour.

8.6 VEGETABLE FOODS

8.6.1 Cereals

Cereals can be defined as a grain or edible seed of the grass family. Cereals are very essential for a healthy diet. They are good sources of fibre, carbohydrates, protein and a wide range of vitamins and minerals. For a child's normal growth and development, grains are very essential. The crude cereals (wheat, rice, barley, maize, oats) contain 11% protein, 70% carbohydrate, 0.5 to 8% fat, 11% water and 2% minerals. Oatmeal is the richest in protein and fat, rice is the poorest.

- The main proteins of cereals are glutelins and gliadins. Small amounts of albumins and globulins are generally present.
- The carbohydrate is almost completely starch in the form of grains covered by a thin membrane of cellulose. Small amounts of sugar are also often present in cereals.

- The abundant mineral elements are calcium and phosphate. The phosphate is partly in the form of phytic acid (inositol hexaphosphate) which interferes the absorption of calcium.
- There is a great loss of vitamins B and E and mineral elements, especially Ca, P and Fe in the roller milled white flour. Carotene and riboflavin are lost by chemical blenching.
- Brown rice contains bleaching a fair amount of fiber (1.8%), while white rice is very low in fiber (0.3%).

8.6.2 Pulses

Pulses are globally important due to the higher content of protein. The term "pulses" includes a range of grams and peas. They are important foods as they are easily available and inexpensive.

- Pulses include grams and peas. Pulses are good sources of important vitamins and minerals like iron, potassium and folate, calcium and phosphorous.
- Pulses are 20 to 25 per cent protein by weight which is double the protein content of wheat and three times that of rice.
- The dried pulses (peas, beans, lentils etc), contain large amounts of protein. The main protein is a globulin called legumin. The dried pulses contain 20-25% protein, 11% water, less than 2% fat.

Fig 8.4 Pulses

- Due to their high soluble-fibre content, legumes are believed to reduce blood cholesterol.
- The soya bean contains high protein, high fat and low carbohydrate. Soya bean oil used for frying "fish and chips".

- The dried pulses are the good sources of many B group vitamins and minerals but deficient in vitamins A, D, B_{12} and C.

8.6.3 Nuts

Nuts are the storehouse of health.

- Nuts contain vitamins and are rich in minerals like manganese, potassium, calcium, iron, magnesium, zinc, fluoride and selenium.
- Nuts contain high protein and fat but low carbohydrate. The protein content is slightly lower than that of dried pulses.
- Nuts are the good sources of Vitamin-E (a powerful lipid soluble antioxidant). Vitamin-E is required for maintaining the integrity of cell membrane of mucosa and skin which is helpful for protecting from harmful oxygen-free radicals.
- Nuts also contain important B-complex groups of vitamins such as riboflavin, niacin, thiamin, pantothenic acid, Vitamin B_{-6} and folates which are very important for health.

Fig 8.5 Nuts

- They can be used for the preparation of milk substances which can be fed to infants over 6 months of age and to young children of some areas where milk is not available in sufficient amounts.
- Nuts reduced the risk of cardio vascular diseases and diabetes.

8.6.4 Roots and Tubers

Root and tuber crops consist of root crops, such as beets and carrots and tuber crops, such as potatoes and sweet potatoes, and the leaves of root crops.

- Roots and tubers primarily contains fibre content. These are the chief sources of starch. These can substitute sugar and cereals and are the valuable sources of iron and vitamin C.
- The common roots (carrots, beetroots, turnips) are almost free from starch. Their caloric value is completely due to sugars (sucrose, fructose and glucose). Carrots, the richest in sugars, are the sources of carotene.
- Roots contain valuable salts but negligible protein and fat.
- The main nutrient supplied by roots and tubers is carbohydrates. The protein content is low.
- Cassava, sweet potato, potato and yam contain some vitamin C and yellow varieties of sweet potato, yam and cassava contain beta-carotene or provitamin A.
- Roots and tubers are deficient in most other vitamins and minerals but contain significant amounts of dietary fibre.
- Taro is a good source of potassium. Leaves of taro are cooked and eaten as a vegetable. They contain betacarotene, iron and folic acid, which protects against anaemia.

Fig 8.6 Roots and Tubers

8.6.5 Green Leafy Vegetable

Dark green leafy vegetables contain vitamins and minerals which are essential for our health and it may prevent certain types of cancers and promote heart health.

- Green leafy vegetables are rich in carotenes. They are good sources of calcium, riboflavin, folic acid and vitamin C. Yellow pumpkin is a fair source of carotene.
- Green leafy vegetables are the cheapest among the protective foods.
- Green leafy vegetables gives beautiful skin and hair. Antioxidants such as vitamin C, lutein and zeaxanthin in greens reduce the risk of cataracts and muscular degeneration.
- Eating a diet rich in leafy greens can offer numerous health benefits including reduced risk of obesity, heart disease, high blood pressure.

Fig 8.7 Green Leafy Vegetables

8.7 FRUITS

Fresh fruits are the protective foods. Their energy value is due to sugars and starch. Many fruits contain pentoses and pectins.

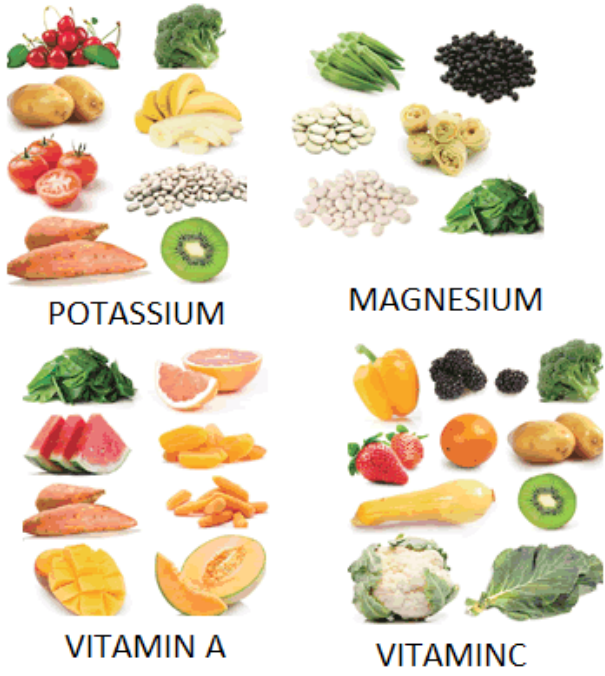


Fig 8.8 Fruits

- Fruits are naturally low in fat and have no cholesterol.
- Fruits are sources of many essential nutrients such as potassium, dietary fiber, vitamin C and folate (folic acid).
- Fruit sources of potassium help to maintain healthy blood pressure. Potassium containing fruits are bananas, prunes and prune juice, dried peaches and apricots.
- Vitamin C present in fruits is important for growth and repair of all body tissues, helps heal cuts and wounds and keeps teeth and gums healthy.
- Dietary fibre from fruits reduce blood cholesterol levels and may lower risk of heart disease.
- Cooking destroys vitamin C and makes fruits more digestible by softening the cellulose.
- Some fruits, notably the banana, contain starch as well as sugar. It has high protein content.

8.8 TEA, COFFEE, COCOA

- Tea contains no calories and it is a rich source of phytochemicals as well as a specific group of chemicals called methylxanthines.
- The black tea sold consists of the leaves of young shoots of the tea plant which are fermented and dried by heat. It has negligible caloric value. It has a stimulant and a diuretic effect. Strong tea disturbs gastric digestion due to tannic acid.
- Tea increases lipid oxidation and improves blood vessel function. Tea boost up the immune system.
- Coffee is the roasted seed of *Coffea arabica*. The odour is due to an oil, caffeol, formed when the beans are roasted. It has little caloric value. It contains caffeine and tannic acid.
- Caffeine in coffee make you more alert and actually improve your concentration.
- The seeds are obtained from the pods of the cocoa tree after fermentation and roasting. They contain 50% fat.
- Cocoa as beverage is of little importance. It becomes nourishing when taken with milk and sugar. Chocolate consists of ground cocoa-nibs mixed with sugar. Starch and flavouring are frequently added.

8.9 ALCOHOL

Alcohols have no nutritional values.

- Alcohol has an energy value of 7 calories per gram. It is consumed in the form of wines, spirits or liquors.
- Beers are the fermented product of malt and contain 4-8% alcohol. It has small amount of protein and some sugar. The process of making beer is known as brewing.
- Wines are the products of fermentation of fruit juices (usually grapes). The alcohol content is 10-20% and sugar 0.1-4%. They contain large amount of organic acids (tartaric, malic, succinic etc). Yeast consumes the sugar in the grapes and converts it to ethanol, carbon dioxide and heat. Different wines are produced from different varieties of grapes.

- Spirits are the distillation product of many fermented products. Whisky is a distilled beer and brandy is a distilled wine. They are practically free from sugar. The alcoholic content is 30-50%.
- Liquors are alcohol sweetened with cane sugar and flavoured with essences. The sugar content is 30% and alcohol is 35-55%.

8.10 PRINCIPLES IN PLANNING A BALANCED DIET

A balanced diet is a healthy diet.

In planning a balanced diet it is to be aimed that the diet must contain various groups of foodstuffs such as energy yielding foods, body building foods and protective foods in the correct proportions. The constituents of balanced diet differ according to age, sex, physical activity, economic status and the physiological condition.

A Nutritious food include

- Eating a variety of foods, including vegetables, fruits and whole-grain products.
- Eating lean meats, poultry, fish, beans and low-fat dairy products.
- Limiting consumption of salt, sugar, alcohol, saturated fats and trans fats.
- Reading food labels to ensure a healthy diet.

The food that we eat must contain all the nutrients such as proteins, carbohydrates, fats, vitamins and minerals. The WHO (World Health Organization) has given the basis of nutrition as

- Eat roughly the same amount of calories that your body uses.
- Healthy body weight = “calories in”- “calories out”.
- Eat a lot of plant foods such as vegetables, legumes, whole grains, fruits and nuts.
- Limit your intake of fats, preferring the healthier unsaturated fats to saturated fats and trans fats.
- Limit your intake of granulated sugar, ideally less than 10g/day.
- Limit salt / sodium consumption from all sources

For a balanced diet, children, elderly people need a little bit more protein and calcium for growth, maintenance or repairing. Also, eggs, fish, white

meat, legumes and dairy products are essential for a healthy diet. Eating fresh fruits and vegetables not only acts as healthy diet, but it also gives the diet as pleasure .

8.10.1 Energy yielding foods

1. The group contains high carbohydrates and also pure fats and carbohydrates. They are divided into two groups. (a) cereals, roots and tubers and (b) pure carbohydrates, fats. Because energy yielding foods provide body with energy that is measured in calories.
2. Cereals provide proteins, certain minerals and vitamins in addition to energy in the diets of the low-income groups.
3. Roots and tubers provide some amount of proteins, minerals and vitamins.
4. Pure carbohydrates and fats provide only energy.

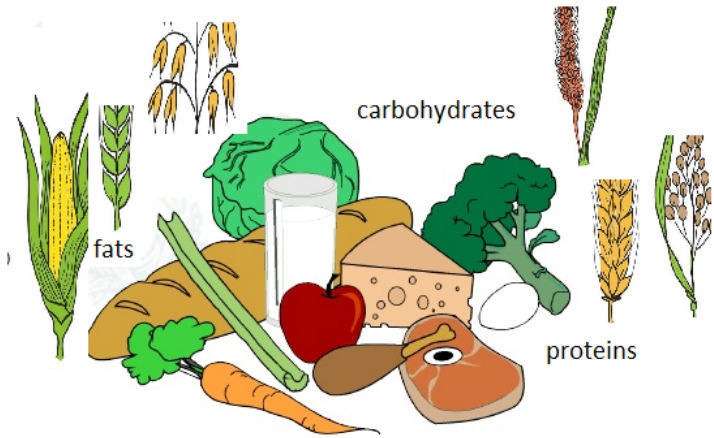


Fig 8.9 Energy yielding foods

8.10.2 Body building foods

Body building is defined as the developing of the body through exercise and diet. Our body needs protein to develop muscles.

Body building foods contain high protein and are divided into two groups:

(a) Milk, egg, meat and fish.

(b) Pulses oilseeds and nuts.

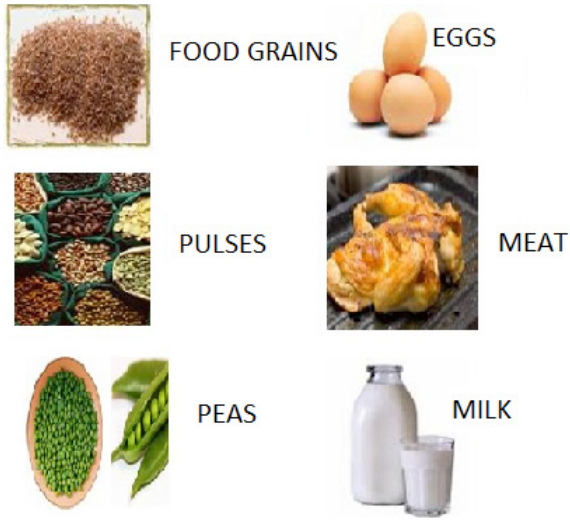


Fig 8.10 Body Building Foods

8.10.3 Protective foods

The protective foods are rich in proteins, vitamins and minerals.These are classified into two groups:

a. Foods rich in vitamins, minerals and proteins of high biologic value Example: milk, eggs, fish and liver.

b. Foods rich in certain vitamins and minerals only E.g. green leafy vegetables and some fruits.

Fig 8.11 Protective Foods

Vitamins and minerals are important because they help to keep the body healthy by regulating body processes. Vitamins and minerals help the body to produce substances that fight disease causing agents. Hence they are called protective foods.

8.11 BIOAVAILABILITY

The term bioavailability refers to the extent to which a drug reaches its site of pharmacologic action. It is the degree and rate at which an administered drug is absorbed by the body's circulatory system, the systemic circulation.

The bioavailability of a drug depends directly on the extent to which the drug is absorbed and distributed to the site of action and depends inversely on the extent to which it is metabolized and excreted prior to arriving at the site of action. The factors affecting bioavailability of the drug are

- Physical properties of the drug such as hydrophobicity, pKa, solubility
- The drug formulation
- Gastric emptying rate
- Interactions with other drugs/foods such as antacids, alcohol and fruit juices
- Health of the gastrointestinal tract
- Age
- Enzyme induction/inhibition by other drugs/foods:

  Enzyme induction which increases the rate of metabolism E.g. Phenytoin

  Enzyme inhibition which decreases the rate of metabolism E.g. grapefruit juice

8.12 ABSORPTION

- The term absorption refers to the rate at which and the extent to which, a drug leaves its site of administration. It is the movement of a drug from the site of administration to bloodstream.

  The factors that affect absorption include

- the route and site of drug administration
- the site and area of absorption
- the concentration of the drug at that site
- drug's solubility and therefore its ability to reach a site of entry into the bloodstream or cerebrospinal fluid and finally, the circulation at the site of absorption

- Molecular size of the drug.
- Degree of ionisation of drugs.
- Dosage forms affect the rate and extent of absorption.
- Absorption involves several phases. First, the drug needs to be introduced via some route of administration and in a specific dosage form such as a tablet, capsule, solution and so on. Absorption depends upon the route of administration.
- Absorption is influenced by physico-chemical processes.
- Food components affect drug absorption and bioavailability through three general mechanisms: Physico-chemical interactions between drug and food components in the gut lumen.

8.13 INTERACTIONS BETWEEN DIET AND DRUG INGREDIENTS

The action or side effects of a drug caused by taking with a food, beverage, supplement or another drug is called drug interaction.

- After taking a drug, the process involved are absorption, complex formation, precipitation and the effects of one interactant on another.
- Presence of food and nutrients in intestinal tract may affect absorption of drug.
- Absorption of iron from supplements reduces to 50% when taken with food.
- Antacid medications can result in reduced acidity in the stomach.
- Certain drugs chelate minerals and render both the drug and the mineral unavailable for absorption, Ex. Doxycycline chelates with calcium, magnesium, iron or zinc.
- Other drugs like ciprofloxacin, penicillamine and thyroxine form stable complexes with iron.

8.14 GASTRIC EMPTYING AND DRUG ABSORPTION

- A drug leaves the stomach at a rate that depends on gastric emptying. Gastric emptying, in turn, depends on the general functioning of the stomach and gastrointestinal tract.

- The rate of gastric emptying affects all drugs. This is because of the fact that the small intestine has a significantly larger surface area and contributes the most to drug absorption.
- In the fasting state or when little food is in the stomach, drugs usually leave the stomach rapidly and thus reach the small intestine.
- There must be a enough time between the meals and snacks so that the clearance of drug may takes place easily.
- If the drugs are given in multiple doses throughout the day, these drugs may accumulate in the stomach and be dumped into the duodenum at night when the stomach finally empties. This can be prevented by taking the drug on an empty stomach.
- Drugs must be taken one hour before or one hour after eating. Consequently, the presence of food in the digestive tract may reduce absorption of a drug.

The most common method for drugs to cross the cell membrane is by passive diffusion. To be absorbed, drugs must be transported across the lipid membrane of the mucosal barrier, either by diffusion or by a specific carrier. Most drugs are absorbed in the small intestine and by simple diffusion that depends on the concentration gradient of the drug across the intestinal mucosa.

Good food gives us good health. Poor eating habits such as insufficient intake or high intake both will leads us to obesity, high blood pressure, high cholesterol, heart disease and stroke, type-2 diabetes, osteoporosis etc. When a drug acts on processes that involve dietary components, that dietary component may affect that drug's functions.

- Consumption of vitamin K enriched foods or supplements may counteract the effects of the anticoagulants such as Warfarin, Coumadin, Dicumerol. It is more important to maintain a steady, consistent intake of vitamin K containing foods while receiving anticoagulants.
- Certain herbal leaves contain natural coumarins that can augment the effects of coumarins and should be avoided.
- Caffeine and other methyl xanthines have numerous effects on the function of other drugs.

8.15 DRUG EXCRETION

Drug excretion is a process of elimination of drugs from the body. Drugs are primarily excreted via the kidneys and the gastro intestinal tract.

- Many drugs are excreted by the kidneys in urine. Hence the drug dosing depends on the kidney function.
- Many drugs are excreted into breast milk and non-electrolytes (Ex. Ethanol) reach the same concentration in breast milk as they do in plasma.
- The half-life of a drug in circulation is a direct function of the volume of distribution of the drug and an inverse function of its clearance.
- The clearance of a drug depends on the amount of drug delivered to the organ of excretion and the extent to which the drug is extracted from the blood for excretion (the extraction ratio).
- In the case of renal excretion, clearance of the drug depends on the concentration of free drug in the plasma and on the amount of drug secreted and/or reabsorbed in the tubules per unit rate.

8.16 LOW PROTEIN DIETS AND HYPOALBUMINEMIA

- Hypoalbuminemia occurs when albumin levels in the blood are very low. Since albumin is a blood protein it is the liquid portion of the blood that maintains the proteins and blood cells.
- Albumin is very important for maintaining pressure in the blood vessels, hormones and medications.When the level of albumin is low, blood may not be able to transport essential materials effectively.
- The concentration of free drug in the plasma depends on the chemical characteristics of the drug.First, hypoalbuminaemia leads to an increase in total body water.
- Chronic low protein consumption with hypoalbuminaemia leads to an increase in free drug available for excretion.
- Another effect of a low protein diet, is to increase net renal excretion of base. The increased excretion of base is due to decreased production of acid residues (phosphate, sulphate and chloride) from protein metabolism.

- For the patients receiving basic drugs such as antacids, urinary pH should be monitored and if the pH is increasing, the drug dose should be reduced or an alternative drug considered.
- The common symptoms of hypoalbuminemia are jaundice, weakness, rapid heart beat, vomiting, diarrhea, nausea, dry and itchy skin.

8.17 DEHYDRATION AND STARVATION

- The loss of body fluids, mostly water is called dehydration. Dehydration occurs when the intake of water is low. The symptoms include thirst and urine becomes darker.
- Starvation leads to deficiency of calorific values. It leads to several organ failures.
- People who are lean but not starved have a higher proportion of body water per kilogram than nonlean people, so the half-life of drugs that are primarily distributed in the aqueous compartment is prolonged in lean persons.

8.18 ACTION OF DRUGS ON GROWTH

- Drug is defined as a substance used in the diagnosis, treatment, or prevention of a disease, or component of a medication. Drug-nutrient interactions involve changes to a drug caused by a nutrient, or changes to a nutrient as a result of the drug. Drugs can alter food intake by causing either a loss of appetite or an increase in appetite.
- Drugs may also cause a dry or sore mouth and make eating difficult or painful which leads to avoidance of food intake. Dry mouth may be caused by drugs that decrease salivary flow.
- Drugs can alter the taste of saliva or food by excretion in the saliva, often leading to a metallic waste.
- Many drugs cause gastric irritation that leads to a loss of appetite, e.g. aspirin
- Some of the drugs E.g. erythromycin , increase the rate of gastric emptying and the overall motility of the GI tract.
- Food aversions develop in more than half of all patients who undergo cancer chemotherapy and usually affect consumption of two to four foods.

- Beozars may cause feelings of gastric fullness, pain, nausea, vomiting and gastric outlet obstruction.

8.19 EFFECT OF DRUGS ON VITAMINS AND MINERALS

Drugs can affect vitamin and mineral status by interfering with absorption, metabolism and function.

Antacids

By increasing stomach pH, antacids decrease the bioavailability of vitamin A, folate, thiamine and phosphate.

- Thiamine is unstable at high pH and hence is inactivated when taken with antacids.
- When the pH of the upper part of the jejunum is increased after ingestion of sodium bicarbonate, folate absorption is reduced.
- Sodium overload with development of congestive heart failure can occur from intake of excessive sodium.
- Dietary phosphate combines with aluminium and magnesium hydroxide to form insoluble aluminium and magnesium phosphates, which are excreted through gastrointestinal tract. Hence a phosphate depletion syndrome may develop due to the low phosphate diet. Effect of phosphate depletion include muscle weakness, paresthesia in the limbs, anorexia, haemolytic anaemia convulsions and myocardial depression.
- Milk alkali syndrome occurs due to an excessive intake of milk and alkali. Use of calcium salt supplements cause this syndrome.
- Magnesium containing antacids can develop magnesium intoxication, symptoms of magnesium overload are nausea, vomiting, flushing, impaired respiratory function and partial or complete heart block.
- Absorption of iron occurs when it is in ferrous state, at low pH. The elevated pH induced by antacids may lead to iron aggregate formation and conversion of iron to its ferric form. In addition, aluminium hydroxide gels bind iron and thereby decrease its absorption.

Antituberculous agents

- The drugs used in the treatment of tuberculosis, rifampin and isoniazid, affect vitamin D metabolism because of their effect on vitamin D hydroxylase.
- Isoniazid can also cause a secondary niacin deficiency. This results due to an inhibition of the enzyme kynureninase.

Anticonvulsants

- The anticonvulsant drugs phenytoin and phenobarbital can cause hypocalcaemia, rickets in children and high turnover osteoporosis in adults.
- High doses of folic acid may counteract the anticonvulsant effects of phenobarbital, phenytoin and primidone.
- Phenytoin and phenobarbital decrease production of vitamin K dependent proteins.
- Drugs that bind bile acids (Ex. Cholestyramine and aluminium containing antacids) may lead to malabsorption of fats and hence to malabsorption of fat soluble vitamins, particularly vitamin A and E.
- Diuretics act by altering renal excretion of sodium and potassium, they may lead to the development of electrolyte imbalance. Common drugs that cause potassium deficiency include thiazide, laxatives, etc.

Nonnutritive foods increase the flavor and texture of food. Nutritive sweeteners provide the body with calories, while nonnutritive sweeteners are very low in calories or contain no calories at all. They can both be added to food and beverages.

Monosodium glutamate (MSG): This is used as a flavor enhancer in many foods, especially chinese foods. MSG can cause mental retardation in infants and intestinal discomfort in adults.

Tartrazine: It is a colour additive used in both foods and drugs which causes allergy.

Sulphites: These are added to foods, beverages and drugs as preservatives because of their antioxidant properties. They can cause severe allergic reactions.

UNIT IX

ANTIBIOTICS

Introduction – Role of Antibiotics- administration- Side effects – Types – Antibiotics affecting cell wall synthesis Penicillin, Cephalosporin, Cycloserine, Vancomycin, Bacitracin – Antibiotics affecting cytoplasmic membrane – Antibiotics interfering with Nucleic acid function – Antibiotics inhibiting protein synthesis Rifamycin, Streptomycin, Tetracylines, Chloroamphenicol, Erythromycin, Neomycin) – Antibiotics affecting enzyme systems – Drug resistance.

9 ANTIBIOTICS

Antibiotic substances are produced by certain members of the plant kingdom chiefly by micro organisms and green plants. Antibiotics are also produced by the kingdom fungi and animalia. Antibiotics are the substances that destroy or slow down the growth of bacteria. They can stop bacteria from reproducing or destroy them. Tomato plants, raddish seeds and other plants are the sources of antibacterial substances.

9.1 ROLE OF ANTIBIOTICS

An antibiotic is a chemical substance produced by a living organism. It is powerful medicines that fight bacterial functions. They either kill bacteria or keep them from reproducing.

- Antibiotics treat infections caused by bacteria and certain parasites but not viruses.
- Antibiotics are not effective against viruses such as the common cold or influenza.
- Antibiotics also lead to the side effects such as diarrhea, stomach problems, nausea, vomiting and rashes.
- The different types of antibiotics used are bactericidal antibiotic and bacteriostatic antibiotic. Bactericidal antibiotic kills the bacteria by interfering with the cell wall or cell components. Ex: penicillin. Bacteriostatic stops bacteria from multiplying.

9.2 ADMINISTRATION OF ANTIBIOTICS

For treating infections using antibiotics, different routes are used.

- Antibiotics are also available as creams, ointments or lotion to apply to the skin to treat certain skin infection.

- Antibiotics are also used in soaps and disinfectants.
- Antibiotics are usually taken as tablets and syrup.
- For severe infections, antibiotics can be given as injection.

9.3 SIDE EFFECTS

Antibiotics will cause side effects such as

- Vomiting
- Diarrhoea
- Stomach upset
- Allergies
- White patches on tongue

9.4 TYPES OF ANTIBIOTICS

The most commonly used antibiotics are

- Penicillins - phenoxymethylpenicillin, flucloxacillin and amoxicillin.
- Cephalosporins - cefaclor, cefadroxil and cefalexin.
- Tetracyclines - tetracycline, doxycycline and lymecycline.
- Aminoglycosides - gentamicin and tobramycin.

9.5 ANTIBIOTICS AFFECTING CELL WALL SYNTHESIS

- Antibiotics commonly target bacterial cell wall formation.
- Any substance that destroys the cell wall or prevents the synthesis of cell wall leads to the formation of sensitive cells.
- The peptidoglycan layer is important for cell wall structural integrity. Peptidoglycan consists of polysaccharide chains composed of alternating units of N-acetyl-glucosamine and N-acetylmuramic acid.
- Antibiotics prevent the bacteria from synthesizing peptidoglycan.
- Antibiotics that affect the bacterial cell wall are

  a. Penicillin

  b. Cephalosporin

  c. Cycloserine

d. Vancomycin

e. Bacitracin

β-lactam antibiotics are a broad class of antibiotics that includes penicillin derivatives and cephalosporin.

9.5.1 Penicillin

- Penicillin in the most widely used antibiotic.
- Penicillin is produced by Penicillium notatum, Penicillium chrysogenum and other species of molds.
- Penicillin's are a class of β-lactam antibiotics of related structure with slightly different properties and activities.
- All penicillin's have a common basic nucleus, a β-lactam thiazolidine ring with different side chains.

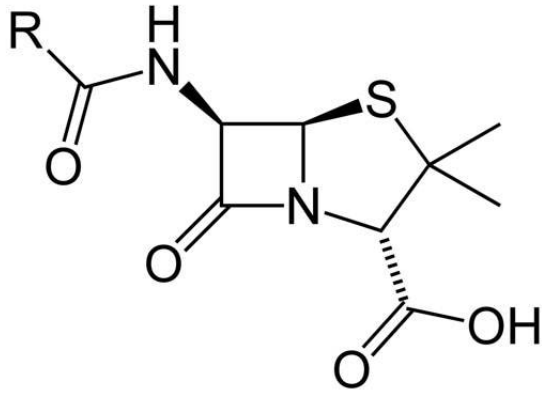


Fig 9.1 Structure of Penicillin

- It is rarely toxic to humans and may cause some skin reactions.
- Penicillin is used to treat many kinds of infections.
- Benzyl penicillin known as penicillin G is the most useful natural penicillin.
- Phenethicillin, semi synthetic penicillin is effective as penicillin G.
- Ampicillin, a semisynthetic penicillin acts against bacteria.
- Ampicillin is strongly bactericidal and it has no toxicity.
- Penicillin is used to prevent and control infections caused by gram-positive organisms.

- Penicillin is ineffective against gram-negative infections such as typhoid, fever and dysentry.
- The side effects of using penicillin are skin rashes, diarrhoea, irregular breathing, joint pain.

9.5.2 Cephalosporins

- Cephalosporin is a group of β-lactam antibiotics derived from fungi.
- They are used for the treatment of infections caused by bacteria.
- Cephalosporin nucleus has been modified to gain different properties.
- Cephalosporin's are effective against gram-positive and gram-negative bacteria.
- Cephalosporin's have anti-bacterial properties similar to semi-synthetic penicillin's.

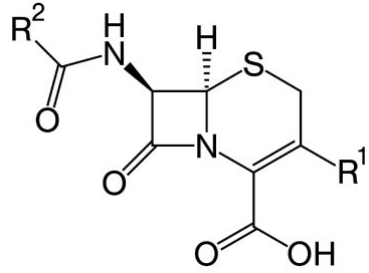


Fig 9.1 Structure of Cephalosporin

- They have a low toxicity.
- Some adverse effect of the drug reactions are diarrhoea, rash, vomiting, headache, dizziness and fever.
- They inhibit enzymes in the cell wall of susceptible bacteria, disrupting cell synthesis.
- The adverse effects of the drug leads to diarrhea, rashes, electrolytic disturbances.

9.5.3 Cycloserine

- Cycloserine is used to treat tuberculosis bacteria and also for urinary problems
- Cycloserine is stable under basic conditions.
- The side effects include allergies and sleepiness.

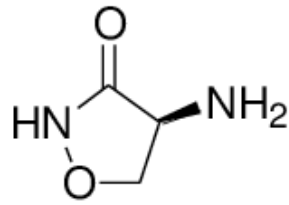


Fig 9.2 Structure of Cycloserine

9.5.4 Vancomycin

- Vancomycin is used to treat the bacterial infections caused in skin, blood stream, bone and joints.
- The side effects of this drug mainly causes allergic reactions.

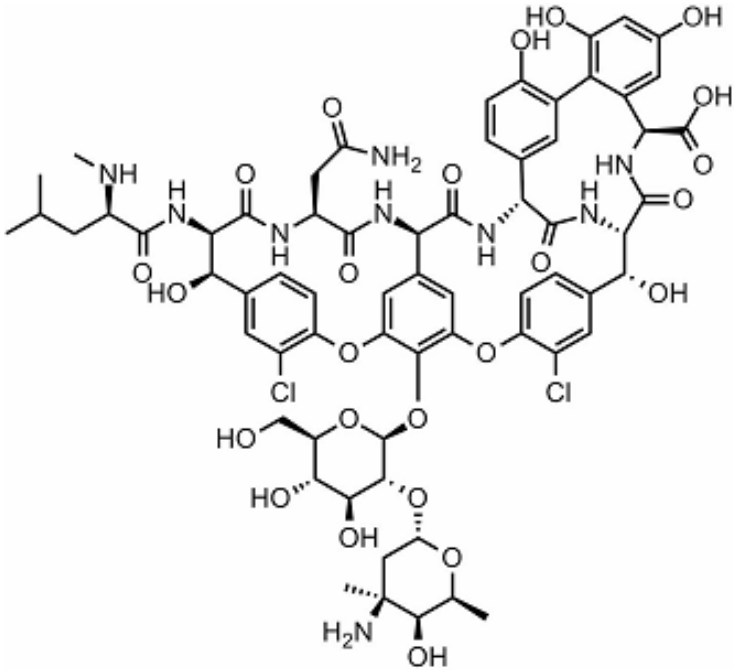


Fig 9.3 Structure of Vancomycin

9.5.5 Bacitracin

- Bacitracin is a mixture of cyclic peptides. It works by stopping the growth of bacteria. It is used to treat the infections caused by burns and cuts.
- It is also used in plant tissue culture to reduce the bacterial infections.

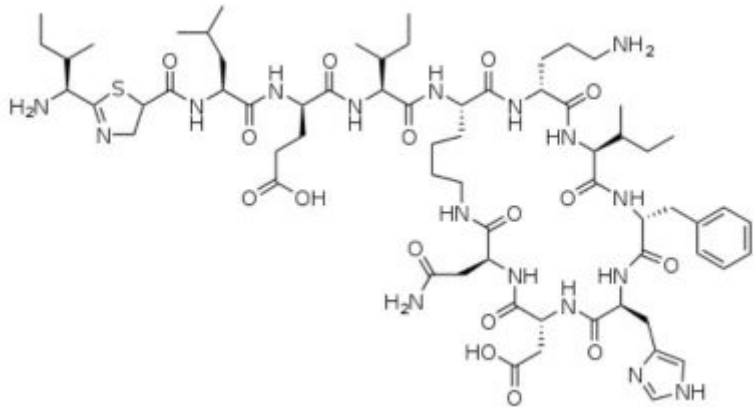


Fig 9.4 Structure of Bacitracin

9.6 ANTIBIOTICS AFFECTING CYTOPLASMIC MEMBRANE

- The cell membrane plays a vital role in the cell.
- Several polypeptide antibiotics produced by *Bacillus sp*. have the ability to damage cell membrane structure.
- Polymyxins, gramicidin's and polyene tyrocidines are antibiotics which damage the cytoplasmic membrane.

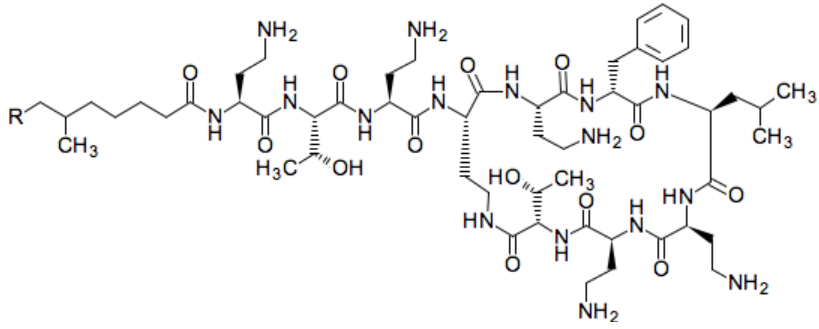


Fig 9.5 Structure of Polymyxin

- Polymyxins are particularly effective against gram-negative organisms. They work mostly by breaking up the bacterial cell membrane. Polymyxins bind specifically to cell membrane.
- Gramicidin are linear peptides with fifteen aminoacids.
- Tyrocidines and gramicidin's are more effective against gram-positive organisms.
- Nystatin, amphotericin act upon fungi and animal cells but do not affect bacteria. They increase the cell permeability.

9.7 ANTIBIOTICS INTERFERING WITH NUCLEIC ACID FUNCTION

- Certain group of antibiotics interfere with the DNA synthesis.
- Example - quinolones

9.8 ANTIBIOTICS INHIBITING PROTEIN SYNTHESIS

- A protein synthesis inhibitor is a substance that stops the growth of cells.
- A number of antibiotics interfere with the metabolism of proteins. Some important antibiotics are
  1. Rifamycin
  2. Streptomycin
  3. Tetracyclines
  4. Chloramphenicol
  5. Erythromycin
  6. Neomycin

9.8.1 Rifamycin

- Rifamycins are synthesized by the bacterium *Amycolatopsis rifamycinica*.
- Rifamycin is used to treat diarrhoea.
- This antibiotics can treat the bacterial infections by stopping the growth of bacteria.

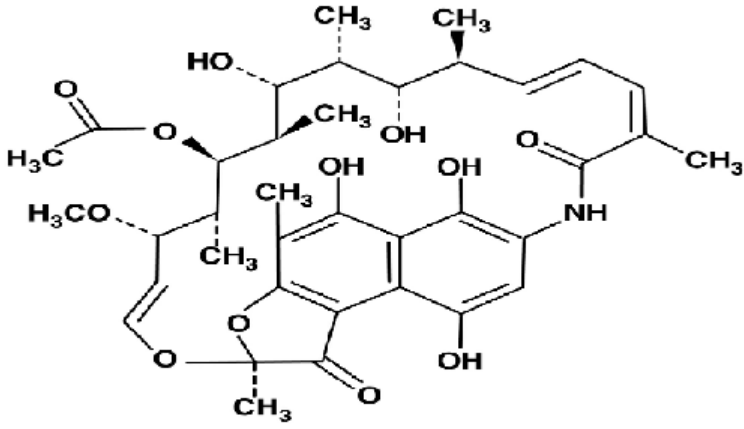


Fig 9.6 Structure of Rifamycin

9.8.2 Streptomycin

- Streptomycin is an antibiotic used to treat a number of bacterial infections.
- Streptomycin is produced by *Streptomyces griseus*.
- Streptomycin is an amino glycoside antibiotic.
- Highly purified streptomycin is non-toxic to humans and other animals when given in small doses.
- Streptomycin inhibits protein synthesis by combining irreversibly with 30S subunit mRNA. So it disturbs the normal sequence.
- Streptomycin is inhibitory particularly for tuberculosis. It is also used for the treatment of plague.
- In veterinary medicine, it is used against gram negative bacteria in large animals such as horses, sheep and cattle.

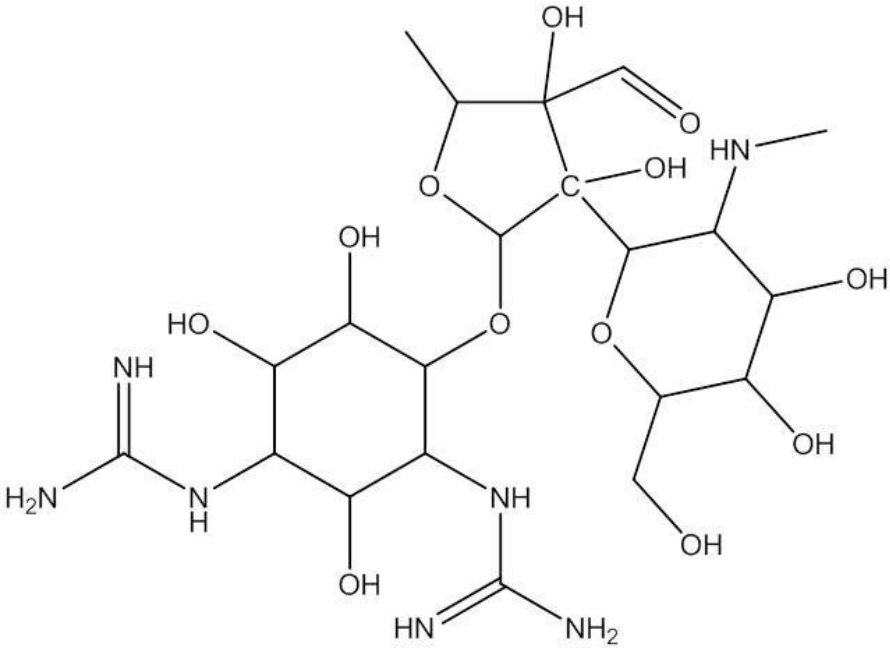


Fig 9.7 Structure of Streptomycin

- Streptomycin is used as pesticide and it used to control bacterial diseases of certain fruit, vegetables.
- It also causes side effects such as vomiting, fever and rash.

9.8.3 Tetracylines

- Many type of bacterial infections can be cured by tetracycline.
- Tetracylines are mainly used to treat urinary infections.
- The common side effects of tetracylines are nausea, vomiting, diarrhoea, vagina itching.
- For childrens, tooth damage may happens.
- Tetracyclines are used as biomarker.

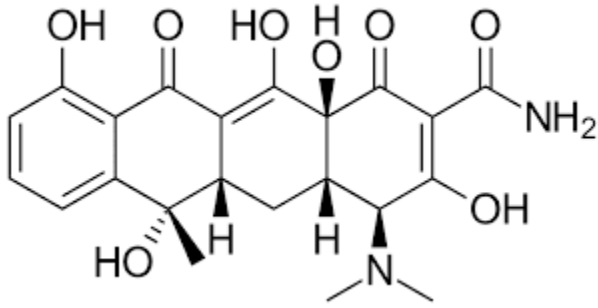


Fig 9.8 Structure of Tetracylines

9.8.4 Chloramphenicol

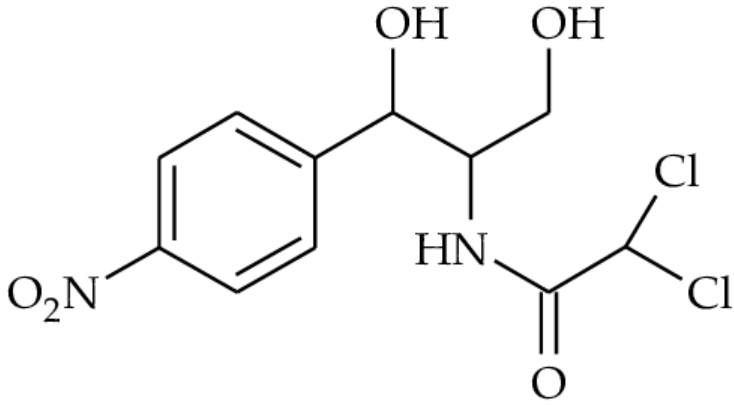


Fig 9.9 Structure of Chloramphenicol

Chloramphenicol was discovered after being isolated from Streptomyces venezuelae and it is used to treat many infections.

Chloramphenicol can be taken as ointments, injections.

9.8.5 Erythromycin

- Erythromycin is an antibiotic useful for the treatment of a number of bacterial infections.
- Erythromycin is produced by a strain of Streptomyces erythraeus.
- Erythromycin is active against the gram-positive bacteria and with some gram-negative bacteria.
- It resembles penicillin but it is active against organisms which are resistant to penicillin and streptomycin.
- It contains large lactone ring linked with amino sugars through glycosidic bonds.
- It belongs to the chemical class of antibiotics known as macrolides.

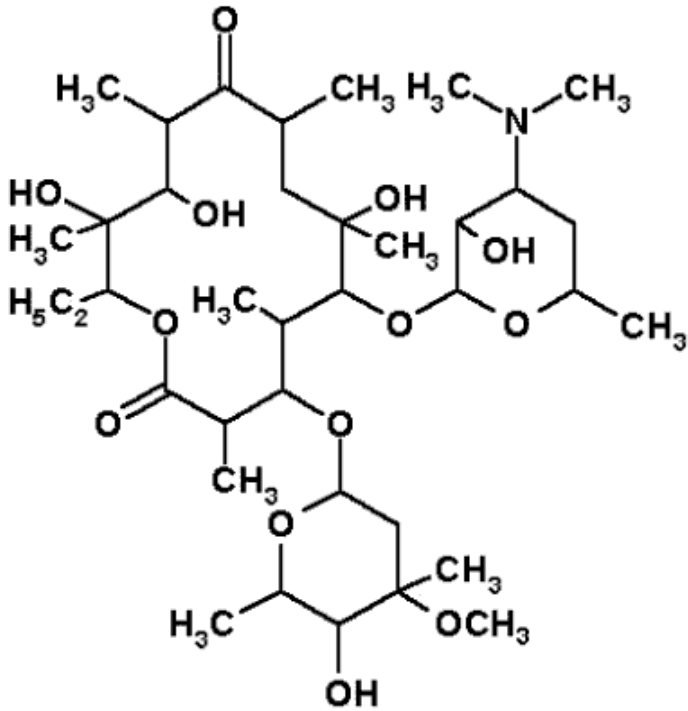


Fig 9.10 Structure of Erythromycin

- Erythromycin inhibits protein synthesis because of binding on 50s subunit ribosome. So, it leads to the blocking of transpeptidation and translocation in protein synthesis.

- Some side effects are skin infections and respiratory tract infections, vomiting, diarrhoea and abdominal pain.

9.8.6 Neomycin

- Neomycin is bactericidal.
- It is an aminoglycoside antibiotic.
- They can be used as creams, ointments and eye drops.

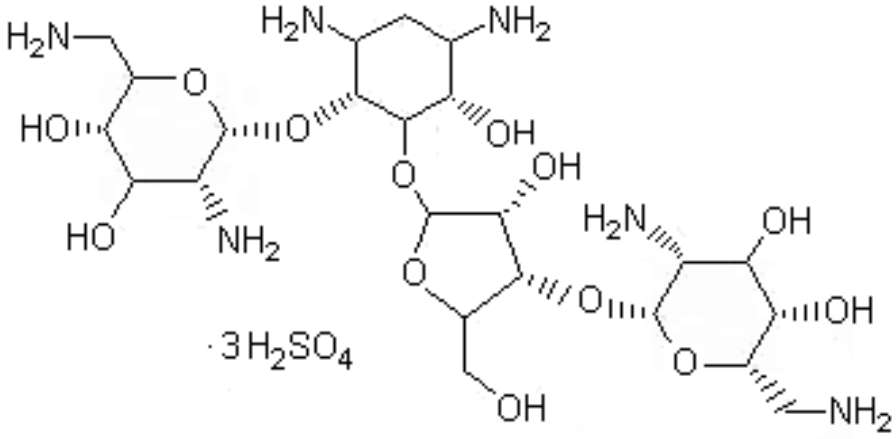


Fig 9.11 Structure of Neomycin

- It is not given as injection since it causes kidney damage.

9.9 ANTIBIOTICS AFFECTING ENZYME SYSTEM

- Antibiotics bind to the target and destroy the enzyme.

9.10 DRUG RESISTANCE

- Drug resistance is the resistance which causes the reduction in effectiveness of medication in curing a disease.
- Drug resistance is the major problem in controlling the micro-organism.
- Drug resistance may be due to competitive inhibition between an essential metabolite and a metabolic drug , development of an alternative metabolic pathway, inability of the drug to penetrate the cell, alteration of ribosomal protein structure.

- Development of resistance can be minimized by correct dosage, using combinations of antibiotics, using different antibiotic and avoiding antibiotics for unnecessary needs.
- The main causes of antibiotic resistance are
  - ♦ Over-prescription of antibiotics.
  - ♦ Patients not finishing the entire antibiotics.
  - ♦ Over use of antibiotics in livestock and fish farming.
  - ♦ Poor infection control in health care.
  - ♦ Poor hygiene and sanitation.

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GLOSSARY

Absorption	The process by which one thing absorbs or is absorbed by another.
Acetyl-CoA	Acetyl-CoA (acetyl coenzyme A) is a molecule that participates in many biochemical reactions in protein, carbohydrate and lipid metabolism
Acidic amino acids	They have a carboxyl group in the side chain.
Aromatic amino acids	They have a benzene ring in the side chain
Agar - Agar	Agar or agar-agar also known as “China grass” is a jelly-like substance, obtained from red algae. Agar is a mixture of two components: the linear polysaccharide agarose and a heterogeneous mixture of smaller molecules called agaropectin.
Agriculture	It is the science and art of cultivating plants and livestock.
Allergies	A condition in which the immune system reacts abnormally to a foreign substance.
Amino acids	They are organic compounds that combine to form proteins. Amino acids and proteins are the building blocks of life
Aminoglycosides	Aminoglycosides are a class of antibiotics used mainly in the treatment of aerobic gram-negative bacilli infections, although they are also effective against other bacteria including Staphylococci and Mycobacterium tuberculosis.
Amphipathic lipids	The lipid molecules in cell membranes are amphipathic (or amphiphilic) - that is, they have a hydrophilic (“water-loving”) or polar end and a hydrophobic (“water-fearing”) or nonpolar end.

Antacids	Antacids are a class of medicines that neutralize acid in the stomach. They contain ingredients such as aluminum, calcium, magnesium, or sodium bicarbonate which act as bases (alkalis) to counteract stomach acid and make its pH more neutral.
Antibiotics	An antibiotic is a type of antimicrobial substance active against bacteria and is the most important type of antibacterial agent for fighting bacterial infections.
Anticonvulsant	Anticonvulsants (also commonly known as antiepileptic drugs or as antiseizure drugs) are a diverse group of pharmacological agents used in the treatment of epileptic seizures.
Atom	An atom is the smallest constituent unit of ordinary matter that constitutes a chemical element.
Atomic number	The atomic number or proton number of a chemical element is the number of protons found in the nucleus of every atom of that element.
Atomic theory	Atomic theory is a scientific theory of the nature of matter, which states that matter is composed of discrete units called atoms.
Atomic weight	The total weight of an atom is called the atomic weight. It is approximately equal to the number of protons and neutrons.
ATP	ATP (adenosine 5′-triphosphate) is the main energy currency in living cells. It undergoes a type of reaction called hydrolysis where one or two of the terminal phosphate groups are released.
Amylose	Amylose is a polysaccharide made of α-D-glucose units, bonded to each other through α glycosidic bonds. It is one of the two components of starch, making up approximately 20-30%.
Amylopectin	Amylopectin is a water-insoluble polysaccharide and highly branched polymer of α-glucose units found in plants.

Apoenzyme	An apoenzyme is an inactive enzyme, activation of the enzyme occurs upon binding of an organic or inorganic cofactor.
Bacitracin	Bacitracin is a mixture of related cyclic peptides produced by organisms of the licheniformis group of Bacillus subtilis var Tracy, first isolated in 1945. These peptides disrupt Gram-positive bacteria by interfering with cell wall and peptidoglycan synthesis. Bacitracin is primarily used as a topical preparation.
Basic amino acids	They contains an amino group in the side chain
β pleated sheet	The β-sheet is a common motif of regular secondary structure in proteins. Beta sheets consist of beta strands connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted, pleated sheet.
Bioavailability	The proportion of a drug or other substance which enters the circulation when introduced into the body and so is able to have an active effect.
Biochemistry	It is the branch of science that explores the chemical processes within and related to living organisms.
Biomolecule	A molecule that is produced by a living organism.
Biological buffers	A biological buffer is an organic substance that has a neutralizing effect on hydrogen ions.
Bicarbonate buffer system	The bicarbonate buffer system is an acid-base homeostatic mechanism involving the balance of carbonic acid, bicarbonate ion and carbon dioxide in order to maintain pH
Buffer	A buffer solution is an aqueous solution consisting of a mixture of a weak acid and its conjugate base, or vice versa.
Carbohydrates	A carbohydrate is a biomolecule consisting of carbon, hydrogen and oxygen atoms, usually with a hydrogen–oxygen atom ratio of 2:1.
Carboxypeptidases	A carboxypeptidase is a protease enzyme that hydrolyzes a peptide bond at the carboxy-terminal end of a protein or peptide.

Caramelization	It is the browning of sugar, a process used extensively in cooking for the resulting sweet nutty flavour and brown colour.
Catalyst	A catalyst is a substance that can be added to a reaction to increase the reaction rate without getting consumed in the process.
Carotenoids	Carotenoids, also called tetraterpenoids, are yellow, orange, and red organic pigments that are produced by plants and algae, as well as several bacteria, and fungi.
Cell	It is the fundamental unit of life
Cellulose	Cellulose is a polysaccharide consisting of a linear chain of several hundred to many thousands of β linked D-glucose units. Cellulose is an important structural component of the primary cell wall of green plants.
Cephalosporins	The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as "Cephalosporium".
Chemical bonds	A chemical bond is a lasting attraction between atoms, ions or molecules that enables the formation of chemical compounds.
Chitin	A fibrous substance consisting of polysaccharides, which is the major constituent in the exoskeleton of arthropods and the cell walls of fungi.
Chloramphenicol	Chloramphenicol is an antibiotic useful for the treatment of a number of bacterial infections.
Cholesterol	Cholesterol is an organic molecule. It is a sterol, a type of lipid. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes.
Chondroitin	Chondroitin is a substance that occurs naturally in the connective tissues of people and animals.
Compound lipids	Compound lipids are also called heterolipids and they are esters of fatty acids with alcohol.

Co-enzyme	A substance that enhances the action of an enzyme. Coenzymes are small molecules. They cannot by themselves catalyze a reaction but they can help enzymes to do so.
Conjugated protein	A conjugated protein is a protein that functions in interaction with other chemical groups attached by covalent bonding or weak interactions.
Cyclic fatty acids	Cyclic fatty acids are an unusual class of minor fatty acids generally produced by bacteria and less frequently by plants.
Cycloserine	Cycloserine, sold under the brand name Seromycin, is an antibiotic used to treat tuberculosis.
Diglycerides	A diglyceride, or diacylglycerol (DAG), is a glyceride consisting of two fatty acid chains covalently bonded to a glycerol molecule through ester linkages.
Decarboxylation	Decarboxylation is a chemical reaction that removes a carboxyl group and releases carbon dioxide.
Dehydrogenases	Enzymes that catalyze the removal of hydrogen from one substrate and pass it on to second substrate.
Deoxyribose	A sugar derived from ribose by replacement of a hydroxyl group by hydrogen.
Derived lipids	Derived lipids are the substances derived from simple and compound lipids by hydrolysis.
Derived proteins	These are proteins derived by partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes.
Dextrorotation	It refers to clockwise or right-handed rotation
Diarrhea	Loose, watery bowel movements that may occur frequently and with a sense of urgency.
Disaccharide	A disaccharide is the sugar formed when two monosaccharides are joined by glycosidic linkage.

Disulfide bond	A disulfide bond, also called an S-S bond, or disulfide bridge, is a covalent bond derived from two thiol groups.
DNA	Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses.
Drugs	A drug is any substance that causes a change in an organism's physiology or psychology when consumed.
Enzyme	An enzyme is a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.
Essential amino acids	An essential amino acid, or indispensable amino acid, is an amino acid that cannot be synthesized by the organism at a rate commensurate with its demand and thus must be supplied in its diet.
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.
Esterases	An esterase is a hydrolase enzyme that splits esters into an acid and an alcohol in a chemical reaction with water called hydrolysis.
Ergosterol	Ergosterol is a sterol that resides on the cell membranes of fungi and acts to maintain cell membrane integrity, similar to mammalian cholesterol.
Erythrocyte	A red blood cell, which (in humans) is typically a biconcave disc without a nucleus.
Erythromycin	Erythromycin is an antibiotic used for the treatment of a number of bacterial infections.
Epimers	An epimer is each of two isomers with different configurations of atoms about one of several asymmetric carbon atoms present.

Extracellular water	Extracellular water is the water located outside your cells. The water in your blood falls into this category.
FAD	Flavin adenine dinucleotide is a redox-active coenzyme associated with various proteins, which is involved with several important enzymatic reactions in metabolism
$FADH_2$	Flavin adenine dinucleotide or FADH2, is a redox cofactor that is created during the Krebs cycle and utilized during the last part of respiration, the electron transport chain.
Fats	Fat molecules consist of primarily carbon and hydrogen atoms and are therefore hydrophobic and are soluble in organic solvents and insoluble in water.
Fat soluble vitamins	Fat-soluble vitamins are absorbed along with fats in the diet and can be stored in the body's fatty tissue. They come from plant and animal foods or dietary supplements. Vitamins A, D, E, and K are fat-soluble.
Fatty acid	A fatty acid is a carboxylic acid with a long aliphatic chain which is either saturated or unsaturated.
Fermentation	It is a metabolic process that produces chemical changes in organic substrates through the action of enzymes.
Fibrous protein	A fibrous protein is a protein with an elongated shape. Fibrous proteins provide structural support for cells and tissues.
FMN	Flavin mononucleotide, is a biomolecule produced from riboflavin by the enzyme riboflavin kinase and functions as the prosthetic group of various oxidoreductases,
Fructose	Fructose, or fruit sugar, is a simple ketonic monosaccharide found in many plants, where it is often bonded to glucose to form the disaccharide sucrose.

Genetic Engineering	Genetic engineering is the manipulation of genes or the direct manipulation of an organism's genes using biotechnology.
Galactose	Galactose, is a monosaccharide sugar that is about as sweet as glucose, and about 65% as sweet as sucrose.
Globular proteins	Globular proteins or spheroproteins are spherical ("globe-like") proteins
Glucose	Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$.Glucose is mainly made by plants and most algae during photosynthesis from water and carbon dioxide, using energy from sunlight, where it is used to make cellulose in cell walls, which is the most abundant carbohydrate.
Glycogen	Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in animals, fungi and bacteria. The polysaccharide structure represents the main storage form of glucose in the body.
Glycolipids	Glycolipids are lipids with a carbohydrate attached by a glycosidic bond. Their role is to maintain the stability of the cell membrane.
Glycosidic linkage	A glycosidic bond or glycosidic linkage is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.
Haemoglobin	Haemoglobin (Hb) is a protein found in the red blood cells that carries oxygen in your body and gives blood its red colour.
Haworth projection	A Haworth projection is a common way of writing a structural formula to represent the cyclic structure of monosaccharides with a simple three-dimensional perspective.
Henderson–Hasselbalch equation	The Henderson–Hasselbalch equation relates the pH of a solution containing a mixture of the two components to the acid dissociation constant, K_a, and the concentrations of the species in solution.

Heterocyclic amino acids	The amino acids have a ring in their side chain
Heteropolysaccharides	Heteropolysaccharides (heteroglycans) contain two or more different monosaccharide units.
Holoenzyme	Holoenzymes are the active forms of enzymes.
Homopolysaccharides	Homopolysaccharides are polysaccharides composed of a single type of sugar monomer.
Hormone	A hormone is any member of a class of signaling molecules, produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behavior.
Hyaluronic acid	Hyaluronic acid, also called hyaluronan, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues.
Hydrogen bonds	The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule.
Hydrolases	Hydrolases are enzymes that catalyze the cleavage of a covalent bond using water.
Hydrolytic rancidity	Hydrolytic rancidity refers to the odor that develops when triglycerides are hydrolyzed and free fatty acids are released.
Hydrophobic bonds	Hydrophobic bonds in proteins arise as a consequence of the interaction of their hydrophobic (i.e.,"water-disliking") amino acids with the polar solvent, water.
Hydroxy amino acids	They contain a hydroxyl group in their side chain
Hydroxy fatty acids	Any fatty acid carrying one or more hydroxy substituents.
Hypoalbuminemia	Hypoalbuminemia (or hypoalbuminaemia) is a medical sign in which the level of albumin in the blood is low.
Intracellular water	Intracellular water is the water inside cells that bathes all the necessary biological molecules including the proteins and nucleic acids.

Ionic bonds	Ionic bonding is a type of chemical bonding that involves the electrostatic attraction between oppositely charged ions.
Isoelectric point	The isoelectric point, is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean.
Isomerases	Isomerases are a general class of enzymes that convert a molecule from one isomer to another.
Isomers	Isomers are each of two or more compounds with the same formula but a different arrangement of atoms in the molecule and different properties.
Isomerism	Isomerism is the phenomenon in which more than one compounds have the same chemical formula but different chemical structures.
Isoniazid	Isoniazid, also known as isonicotinic acid hydrazide (INH), is an antibiotic used for the treatment of tuberculosis.
Lactose	Lactose is a disaccharide. It is a sugar composed of galactose and glucose subunits. Lactose makes up around 2–8% of milk.
Lanosterol	Lanosterol is a tetracyclic triterpenoid and is the compound from which all animal and fungal steroids are derived.
Levorotation	It refers to counterclockwise or left-handed rotation.
Ligases	A ligase is an enzyme that can catalyze the joining of two large molecules by forming a new chemical bond, usually with accompanying hydrolysis of a small pendant chemical group on one.
Lipids	A lipid is a biomolecule that is insoluble in water and soluble in nonpolar solvents. It is also known as fats.
Lyases	A lyase is an enzyme that catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure.

Maltose	Maltose, also known as maltobiose or malt sugar, is a disaccharide formed from two units of glucose joined with an α bond.
Medicine	It is the science and practice of establishing the diagnosis, prognosis, treatment, and prevention of disease.
Messenger RNA	Messenger RNA (mRNA) is a single-stranded RNA molecule that is complementary to one of the DNA strands of a gene.
Monoglycerides	Monoglycerides are a class of glycerides which are composed of a molecule of glycerol linked to a fatty acid via an ester bond.
Monosaccharide	Monosaccharides, also called simple sugar are the simplest form of sugar and the most basic units of carbohydrates.
Monosodium glutamate	Monosodium glutamate, also known as sodium glutamate, is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids.
Molecule	A molecule is a particle made up of two or more atoms that are chemically bonded together.
Mucopolysaccharides	Mucopolysaccharides are long chains of sugar molecules that are found throughout the body, often in mucus and in fluid around the joints. They are more commonly called glycosaminoglycans.
Mutarotation	Mutarotation is the change in the optical rotation because of the change in the equilibrium between two anomers, when the corresponding stereocenters interconvert.
Mutation	A mutation is a change that occurs in our DNA sequence, either due to mistakes when the DNA is copied or as the result of environmental factors such as UV light and cigarette smoke.
Nicotinamide Adenine Dinucleotide (NAD)	Nicotinamide adenine dinucleotide (NAD) is a cofactor that is central to metabolism. NAD is called a dinucleotide because it consists of two nucleotides joined through their phosphate groups.

NicotinamideAdenine DinucleotidePhosphate (NADP)	Nicotinamide adenine dinucleotide phosphate, is a cofactor used in anabolic reactions, such as the Calvin cycle and lipid and nucleic acid synthesis, which require NADPH as a reducing agent.
NADPH	NADPH is the reduced form of NADP+; used in anabolic reactions, such as lipid and nucleic acid synthesis.
Neomycin	Neomycin is an antibiotic that fights bacteria in the body.
Neutral sugars	The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, with the types and proportions of neutral sugars varying with the origin of pectin.
Nitrogenous bases	A molecule that contains nitrogen and has the chemical properties of a base. The nitrogenous bases in DNA are adenine (A), guanine (G), thymine (T), and cytosine (C). The nitrogenous bases in RNA are the same, with one exception: adenine (A), guanine (G), uracil (U), and cytosine (C).
Non- essential amino acids	An amino acid that can be made by humans and so is not essential to the human diet.
Non-essential fatty acids	Non essential fatty acids are which is not required by the body instead may cause harm.
Non-polar molecules	Nonpolar molecules occur when electrons are shared equal between atoms of a diatomic molecule or when polar bonds in a larger molecule cancel each other out.
Non – Reducing sugar	Non-reducing sugars do not have an OH group attached to the anomeric carbon so they cannot reduce other compounds.
Nucleic acids	Nucleic acids are the biopolymers or small biomolecules, essential to all known forms of life. The term nucleic acid is the overall name for DNA and RNA.

Nucleosides	Nucleosides are glycosylamines that can be thought of as nucleotides without a phosphate group. A nucleoside consists simply of a nucleobase and a five-carbon sugar ribose whereas a nucleotide is composed of a nucleobase, a five-carbon sugar, and one or more phosphate groups.
Nucleotides	Nucleotides are molecules consisting of a nucleoside and a phosphate group. They are the basic building blocks of DNA and RNA.
Nutrition	It is the science that interprets the nutrients and other substances in food in relation to maintenance, growth, reproduction, health and disease of an organism.
Oligosaccharides	An oligosaccharide is a saccharide polymer containing a small number of monosaccharides.
Osazone	Osazones are a class of carbohydrate derivatives found in organic chemistry formed when sugars are reacted with excess of phenylhydrazine.
Oxidation	Oxidation is the loss of electrons during a reaction by a molecule, atom or ion.
Oxidative rancidity	Oxidative rancidity is associated with the degradation by oxygen in the air.
Oxidoreductases	An oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant, also called the electron donor, to another, the oxidant, also called the electron acceptor.
Oximes	Oxime, any of a class of nitrogen-containing organic compounds usually prepared from hydroxylamine and an aldehyde, a ketone, or a quinone.
Penicillins	Penicillins are a type of antibiotic derived from Penicillium fungi. An antibiotic is a type of medicine that inhibits the growth of or kills bacteria.

Peptidases	Peptidases are enzymes that hydrolyse peptide bonds and constitute a structurally and functionally diverse set of proteins.
Peptide bond	A peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H2O).
pH	pH is a scale used to specify how acidic or basic a water-based solution is.
Phenobarbital	Phenobarbital is a barbiturate. Phenobarbital slows the activity of your brain and nervous system. Phenobarbital is used to treat or prevent seizures. Phenobarbital is also used short-term as a sedative to help you relax.
Phenytoin	Phenytoin is an anti-epileptic drug, also called an anticonvulsant. Phenytoin works by slowing down impulses in the brain that cause seizures.
Phosphate buffer system	Phosphate buffer system operates in the internal fluids of all cells. It consists of dihydrogen phosphate ions as the hydrogen ion donor (acid) and hydrogen phosphate ion as the ion acceptor (base).
Phospholipids	Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic.
Phosphoric acid	Phosphoric acid is a colorless, odorless phosphorus-containing inorganic acid. Phosphoric acid is a sequestering agent which binds many divalent cations, including Fe++, Cu++, Ca++, and Mg++.
Physiology	It is the scientific study of functions and mechanisms in a living system.
Polar molecules	Polar molecules occur when there is an electronegativity difference between the bonded atoms.

Polymer	A polymer is a large molecule, or macromolecule, composed of many repeated subunits.
Polynucleotide	A polynucleotide molecule is a biopolymer composed of 13 or more nucleotide monomers covalently bonded in a chain. DNA and RNA are examples of polynucleotides with distinct biological function.
Polysaccharides	Polysaccharides are long chains of carbohydrate molecules, specifically polymeric carbohydrates composed of monosaccharide units bound together by glycosidic linkages.
Prosthetic group	A non-protein group forming part of or combined with a protein.
Proteases	A protease is an enzyme that catalyzes proteolysis, the breakdown of proteins into smaller polypeptides or single amino acids.
Proteins	Proteins are large biomolecules or macromolecules, consisting of one or more long chains of amino acid residues.
Protein synthesis	Protein synthesis is the process in which cells make proteins.
Provitamin	A substance which is converted into a vitamin within an organism.
Purines	A purine is an aromatic heterocycle composed of carbon and nitrogen. Purines include adenine and guanine, which participate in DNA and RNA formation.
Pyrimidines	Pyrimidine is one of two classes of heterocyclic nitrogenous bases found in the nucleic acids DNA and RNA. In DNA the pyrimidines are cytosine and thymine, in RNA uracil replaces thymine.
Ramachandran plot	The Ramachandran plot is a plot of the torsional angles - phi (φ)and psi (ψ) - of the residues (amino acids) contained in a peptide.
Rancidity	Rancidification is the process of complete or incomplete oxidation or hydrolysis of fats and oils when exposed to air, light, or moisture or by bacterial action, resulting in unpleasant taste and odor.

Reducing sugar	A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group.
Reduction	Reduction is a chemical reaction that involves the gaining of electrons by one of the atoms involved in the reaction between two chemicals.
Replication	Replication is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules.
Ribose	Ribose is a simple sugar and carbohydrate. The naturally-occurring form, d-ribose, is a component of the ribonucleotides from which RNA is built.
Ribosomal RNA	Ribosomal RNA (rRNA), molecule in cells that forms part of the protein-synthesizing organelle known as a ribosome and that is exported to the cytoplasm to help translate the information in messenger RNA (mRNA) into protein.
Rifamycin	Rifamycin is a nonabsorbable rifampin-like antibacterial agent that is used as treatment of travelers' diarrhea. Rifamycin has minimal oral absorption and has not been implicated in causing liver test abnormalities or clinically apparent liver injury.
Rifampin	Rifampin is an antibiotic that is used to treat or prevent tuberculosis (TB). Rifampin may also be used to reduce certain bacteria in your nose and throat that could cause meningitis or other infections.
RNA	Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.
Saturated fatty acids	Saturated fatty acids are compounds that consist of a hydrocarbon chain and a carboxylic acid group (-COOH) at the end of the chain.
Simple amino acids	They have no functional groups in the side chain

Simple lipids	A simple lipid is a fatty acid ester of different alcohols and carries no other substance.
Simple proteins	On hydrolysis they yield only the amino acids and occasional small carbohydrate compounds.
Starch	Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as energy storage.
Starvation	Suffering or death caused by lack of food.
Steroids	A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration.
Stereo isomerism	Stereoisomerism, or spatial isomerism, is a form of isomerism in which molecules have the same molecular formula and sequence of bonded atoms (constitution), but differ in the three-dimensional orientations of their atoms in space.
Streptomycin	Streptomycin is an aminoglycoside antibiotic produced by the soil actinomycete Streptomyces griseus. It acts by binding to the 30S ribosomal subunit of susceptible organisms and disrupting the initiation and elongation steps in protein synthesis.
Substrate	A substrate is typically the chemical species being observed in a chemical reaction which reacts with a reagent to generate a product.
Sucrose	Sucrose is common sugar. It is a disaccharide, a molecule composed of two monosaccharides: glucose and fructose.
Sulfur-containing amino acids	They contain a sulfur atom in the side chain
Sulphites	Sulphites are substances that are naturally found in some foods. They are used as an additive to maintain food colour, shelf-life and prevent the growth of fungi or bacteria.

Tartrazine	Tartrazine is a synthetic lemon yellow azo dye primarily used as a food coloring.
Terpenes	Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, and by some insects.
Tetracyclines	Tetracycline is an antibiotic that fights infection caused by bacteria.
Thermoregulation	Thermoregulation is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different.
Thiamine pyrophosphate (TPP)	Thiamine pyrophosphate or thiamine diphosphate or cocarboxylase is a thiamine derivative which is produced by the enzyme thiamine diphosphokinase.
Transaminase	Transaminases or aminotransferases are enzymes that catalyze a transamination reaction between an amino acid and an α-keto acid.
Transferases	A transferase is any one of a class of enzymes that enact the transfer of specific functional groups from one molecule to another.
Transfer RNA	Transfer ribonucleic acid (tRNA) is a type of RNA molecule that helps decode a messenger RNA (mRNA) sequence into a protein.
Triglyceride	A triglyceride is an ester derived from glycerol and three fatty acids.
Ubiquinone	Ubiquinone (coenzyme Q) is a lipophilic metabolite that functions in the electron transport chain in the plasma membranes of prokaryotes and the inner mitochondrial membranes of eukaryotes, apart from its roles as an antioxidant and in the regeneration of tocopherols.
Unsaturated fatty acids	An unsaturated fat is a fat or fatty acid in which there is one or more double bond in the fatty acid chain.
Vancomycin	Vancomycin is an antibiotic used to treat a number of bacterial infections..

Vitamin	A vitamin is an organic molecule that is an essential micronutrient that an organism needs in small quantities for the proper functioning of its metabolism.
Vitamin A	Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, retinoic acid, and several provitamin A carotenoids (most notably beta-carotene).
Vitamin B_1	Thiamine, also known as thiamin or vitamin B_1, is a vitamin found in food, and manufactured as a dietary supplement and medication.
Vitamin B_2	Riboflavin, also known as vitamin B_2, is a vitamin found in food and used as a dietary supplement.
Vitamin B_3	Niacin, also known as nicotinic acid, is an organic compound and a form of vitamin B3, an essential human nutrient.
Vitamin B_5	Pantothenic acid, also called vitamin B_5, is a water-soluble vitamin. Pantothenic acid is an essential nutrient. Animals require pantothenic acid in order to synthesize coenzyme-A.
Vitamin B_6	Vitamin B_6 refers to a group of chemically similar compounds which can be interconverted in biological systems.
Vitamin B_7	Biotin also called vitamin H, vitamin B_7 is a water-soluble B vitamin. It is involved in a wide range of metabolic processes, both in humans and in other organisms.
Vitamin B_9	Folate, also known as vitamin B_9 and folacin, is one of the B vitamins used as a dietary supplement and in food fortification as it is more stable during processing and storage.
Vitamin B_{12}	Vitamin B_{12}, also known as cobalamin, is an important water-soluble vitamin. It plays an essential role in the production of your red blood cells and DNA.

Vitamin C	Vitamin C, also known as ascorbic acid and ascorbate, is a vitamin found in various foods and sold as a dietary supplement. It is used to prevent and treat scurvy.
Vitamin D	Vitamin D is a nutrient found in some foods that is needed for health and to maintain strong bones.
Vitamin E	Vitamin E is a group of fat soluble compounds that include four tocopherols and four tocotrienols. Vitamin E can cause nerve problems.
Vitamin K	Vitamin K is a group of structurally similar, fat-soluble vitamins found in foods and in dietary supplements. The human body requires vitamin K for complete synthesis of certain proteins that are needed for blood coagulation
Water	Water is an inorganic, transparent, tasteless, odorless, and nearly colorless chemical substance
Water soluble vitamins	Water-soluble vitamins are carried to the body's tissues but are not stored in the body. Vitamin C and members of the vitamin B complex are water-soluble.
Wax	A wax is a simple lipid which is an ester of a long-chain alcohol and a fatty acid.
Xanthophylls	Xanthophylls are yellow pigments that occur widely in nature and form one of two major divisions of the carotenoid group; the other division is formed by the carotenes.
Van der Waals bond	Van der Waals forces include attraction and repulsions between atoms, molecules and surfaces as well as other intermolecular forces.
Zwitter ion	A molecule or ion having separate positively and negatively charged groups.

Biochemistry

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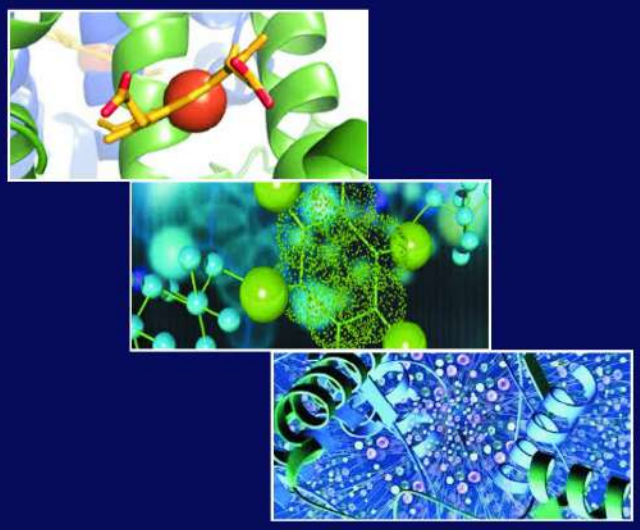

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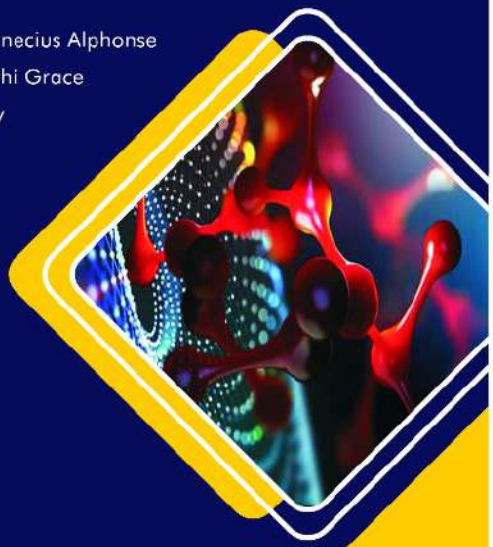

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PREFACE

We feel pleasure to publish this book "***Biochemistry***" **Volume -II** as a continuation of our first volume to the Undergraduate and Postgraduate students of Science. This book includes the metabolism and mechanism of important biomolecules, Hormones, Biochemical Techniques, Cellular Respiration and Biological Oxidation. Comments and suggestions for this book from faculty and students for the improvement of this book are always welcome. We request the readers to send your valuable suggestions and comments to smcjasbooks@gmail.com

Authors

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Dr. P. Subavathy

Biochemistry - Volume II

Syllabus

Unit I Bioenergetics

Introduction to bioenergetics – Thermodynamic principles - System and it's types - Biological reactions – Exergonic reaction – Endergonic reaction – Energy and its forms - Energy Poor compounds Energy Rich compounds – Adenosine triphosphate – Guanosine triphosphate – Uridine triphosphate – Cytidine triphosphate – Acyl phosphate - Energy coupling.

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Unit III Metabolism of Proteins and Amino Acids

Protein Metabolism – Catabolic pathway - Deamination - Transamination - Decarboxylation and Anabolic pathway - Protein synthesis. Amino acid Metabolism - Essential and Non-essential amino acids - Ornithine Cycle –Catabolism of Phenylalanine and Tyrosine – Catabolism of Tryptophan.

Unit IV Metabolism of Lipids

Introduction – Catabolic pathway - Oxidation of glycerol - Oxidation of fatty acids - β oxidation and ketogenesis – Energetics of fatty oxidation - Anabolic pathway - Biosynthesis of fatty acids - Synthesis of triglycerides - Disorders of fat metabolism (Hypercholestrolemia, Hyperlipoproteinemia and Atherosclerosis).

Unit V Metabolism of Nucleic Acids

Biosynthesis of Purine Ribonucleotides – Inhibitors of purine synthesis – Formation of purine nucleoside diphosphates and triphosphates – Salvage pathway for purines – Degradation of purine metabolism – Disorders of purine metabolism. Biosynthesis of Pyrimidine Ribonucleotides – Regulation of pyrimidine synthesis - Degradation of pyrimidine nucleotides - Disorders of pyrimidine metabolism.

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Unit VII Cell Respiration and Biological Oxidations

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Unit VIII Biochemical Techniques

Introduction – Microscopy - Optical and Electron Microscope – Centrifuge – Hand Centrifuge - Desktop Centrifuge - Continuous Flow Centrifuge - High Speed Centrifuge - Gas Centrifuge - Hematocrit Centrifuge -Ultra Centrifuge - Micro centrifuge- Refrigerated Centrifuge - Vacuum Centrifuge - Advantages - Applications – pH meter – Principle, Electrodes used, Applications – Electrophoresis – Zone (Paper, Gel, Thin Layer, Cellulose acetate Electrophoresis) - Moving Boundary Electrophoresis - Applications.- Colorimeter - Principle and Applications.

Unit IX Minerals

Introduction- Macrominerals- Microminerals- Role of Minerals in Human Life: Macrominerals - Calcium, Sodium, Potassium, Phosphorous, Magnesium, Chloride, Sulphur, Copper, Iodine. Microminerals - Iron, Boron, Zinc, Selenium, chromium, Manganese, Molybdenum.

Unit X General Biochemical Procedures

Basics of Analysis : Qualitative and Quantitative analysis- Solution, Solvent, Solute, Strength Normality, Molarity, Molality standard Solution and Percent solution – Buffer - Qualitative analysis of biomolecules : Test for carbohydrates, monosaccharides, proteins, aminoacids. Estimation of Aminoacids (Glycine) by Formal Titration- Estimation of Protein by Biuret Method- Estimation of Carbohydrate by Anthrone Method- Techniques for sample preparation : Ultra filtration, Lyophilisation. Quantitative Estimation of Lipid - Determination of Iodine Number, quantitative estimation of fatty acid.

CONTENTS

CHAPTER

1 BIO–ENERGETICS

1.1 Introduction

Bioenergetics deals with energy flow in living organisms. Photosynthesis, Cellular respiration are bioenergetics process. Exergonic and Endergonic reactions are the types of bioenergetics reaction. Photosynthesis is the major biological process. The major source of energy for all the living beings is Sun. We need energy to do work. Some examples of biological work are nerve impulse, cellular functions, membrane functions, cellular growth and maintenance, muscle contraction. Bioenergetics tells us how to acquire and transform energy in living organism.

Example: Glycolysis, Gluconeogenesis, Citric acid cycle, ketosis, Oxidative phosphorylation, Photosynthesis.

Energy and work can be measured quantitatively in calorie. A calorie is the amount of heat required to raise the temperature of 1gram of water by °C

1 calorie = 4.18 Joules

1.2 Thermodynamic Principles

Thermodynamics is the study of the relations between heat, work, temperature, and energy. By learning the laws of thermodynamics, we can understand how the energy in a system changes and whether the system can perform useful work on its surroundings.

1.2.1 First Law of Thermodynamics

"Energy of the universe remains constant" Energy may be converted from one form into another but can never be created nor destroyed.

Examples:

- In photosynthesis, the radiant energy of light is transformed into the chemical energy.

- In muscles and nerves, chemical potential energy stored is transformed into kinetic and electric energy.

1.2.2 Second Law of Thermodynamics

"Matter tends to move disorderliness rather than orderliness, because orderliness in matter is always accompanied by a high degree of potential energy".

Laws of thermodynamics helps to understand the flow of energy through living systems. They help us to determine whether a biochemical reaction is possible or not.

1.3 System

The system consists of those molecules which are reacting. In biology, there are three types of systems namely

1. Isolated system
2. Closed system
3. Open system.

1.3.1 Isolated System

An isolated system exchanges neither matter nor energy with its surroundings.

Example: Earth system.

1.3.2 Closed System

A closed system exchanges only energy and not matter with its surroundings.

Example: Chlorophyll system.

1.3.3 Open System

An open system exchanges both energy and matter with its surroundings.

Example: Cell system.

Let us consider the following system

1.4 Biologic Reactions

Biological reactions takes place inside the living organism. In biologic system, there are two kinds of reactions.

1. Exergonic reactions
2. Endergonic reactions.

1.4.1 Exergonic Reactions

Exergonic reaction is a chemical reaction which involves the release of free energy and the free energy change is negative. The reaction is spontaneous and it provides energy for performing some work.

$$\Delta G = \text{-ve}$$

Example: Adenosine triphosphate (ATP) is hydrolysed to form Adenosine diphosphate (ADP) and Phosphoric acid.

$$ATP + H_2O \rightarrow ADP + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

This spontaneous process provides 7300 calories/mole of free energy at pH 7. This energy is utilized to bring about various biochemical reactions.

1.4.2 Endergonic Reactions

Endergonic reaction is a chemical reaction in which the free energy is absorbed. In endergonic reaction, the free energy change is positive. The reaction is not spontaneous. Energy has to be supplied from outside.

$$\Delta G = + \text{ ve}$$

Example: Glucose is phosphorylated to form glucose 6-phosphate and water.

$$\text{Glucose} + H_3PO_4 \rightarrow \text{Glucose 6-phosphate} + H2O$$

$$\Delta G = +5500 \text{ calories/mole}$$

5500 calories has to be supplied from outside to get one mole of glucose 6-phosphate.

1.5 Energy and its Forms

Energy is the capacity to do work. Energy occurs in many forms. The important type of energy are

1. Potential energy
2. Kinetic energy
3. Internal energy

1.5.1 Potential Energy

Potential energy is the energy due to position The following are examples of potential energy.

1.5.2 Kinetic Energy

Kinetic energy is the energy associated due to its motion.

$$\text{Kinetic energy} = \tfrac{1}{2}\, mv^2$$

where, m = mass; v = velocity

When a body is moving, potential energy is converted in to kinetic energy. The conversion of potential energy into kinetic energy always involves the production of heat. So there is always some wastage of potential energy.

All living organism need energy to perform its activities. In biochemistry there are two types of energy containing compounds. They are Energy poor and energy rich compounds.

1.6 Energy-Poor Compounds

In energy-poor compounds, the phosphorous is attached to the compound by means of an ordinary co-valent bond. The structure may be represented as R-P. Removal of the phosphorous P from R-P releases a small amount of free energy. For example, glucose 6-phosphate upon hydrolysis produces glucose and phosphoric acid. Removal of phosphoric acid from glucose 6-phosphate releases about 3300 calories/mole of free energy at pH7.

$$\text{Glucose 6-phosphate} + H_2O \rightarrow \text{Glucose} + H_3PO_4$$

$$\Delta G = -3300 \text{ calories/mole}$$

1.7 Energy-Rich Compounds

Phosphate containing compounds are high energy compounds. In energy-rich compounds, the phosphorous is attached to the compound by means of a special high energy bond. Lipmann introduced the symbol "~" to indicate a high energy bond which possesses high levels of chemical energy. There is nothing special about the bonds themselves. They are high-energy bonds in the sense that much free energy is released when they are hydrolyzed. The structure may be represented as R~P. Removal of the phosphorous from R~P releases 7000-14000 calories/mole of free energy. These compounds are specialized for storage and transfer of free energy. Some high energy compounds found in cells with their standard free energy changes (ΔG).

1.7.1 Adenosine Triphosphate (ATP)

- ATP occurs in all living cells. ATP transports within the cells for metabolism.

ATP plays the role of energy currency in all living creature. ATP contains three phosphate groups.

- The main functions of ATP are transporting organic substances, synthesizing chemical compounds and supplying energy for cellular activities.

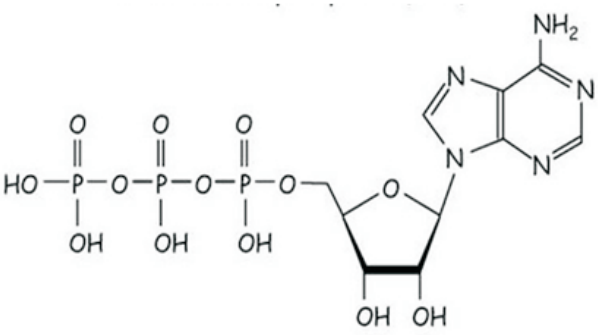


Figure 1.1 Structure of ATP

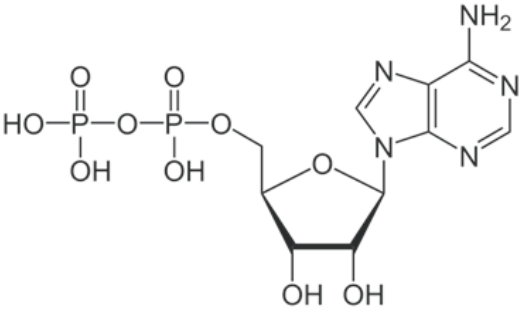


Figure 1.2 Structure of ADP

- The structure of ATP may be represented as A ~ P ~ P ~ P where A represents the nucleoside, adenosine which has adenine and ribose and P represents Phosphoric acid group. The terminal and second phosphoric acid groups are attached to the compound by means of high-energy bond. So, ATP has two high energy bonds.

$$A \sim P \sim P \sim P + H_2O \rightarrow A \sim P \sim P + H_3PO_4$$

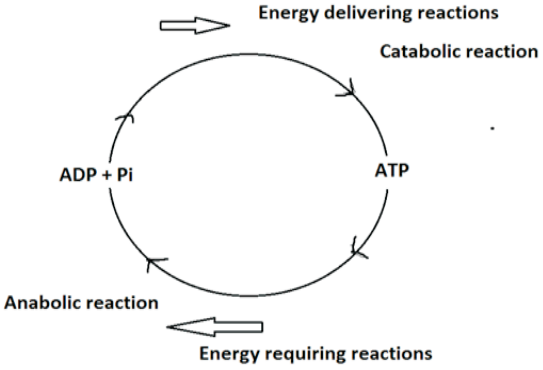
$$\Delta G = -7300 \text{ calories/mole}$$

$$A \sim P \sim P + H_2O \rightarrow A \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

ADP has one high energy bond. The second phosphoric acid group can be removed by hydrolysis. The ADP becomes adenosine monophosphate (AMP) and phosphoric acid. The process release 6500 calories/mole of free energy at pH 7.

The hydrolysis of AMP to adenosine releases 2200 calories/mole of free energy at pH 7. ATP is the energy bank of the cell as it lends and also conserves the energy released by a system.



1.7.2 Guanosine Triphosphate (GTP)

- Guanosine is a purine nucleoside triphosphate. GTP acts as an activator in metabolic reactions. GTP is mainly employed in protein synthesis and gluconeogenesis.
- GTP is mainly involved in the energy transfer within the cell.GTP is also used in genetic translation and mitochondrial function.
- The structure of GTP may be represented as G ~ P ~ P ~ P. G represents guanosine which contains guanine and ribose. P stands for phosphoric acid group. GTP has two high-energy bonds. Upon hydrolysis, GTP becomes guanosine diphosphate (GDP), and phosphoric acid. The process releases 7300 calories/mole of free energy at pH 7.

$$G \sim P \sim P \sim P + H_2O \rightarrow G \sim P \sim P + H_3PO_4$$

$\Delta G = -7300$ calories/mole

$$G \sim P \sim P + H_2O \rightarrow G \sim P + H_3PO_4$$

$\Delta G = -6300$ calories/mole

GDP has a single high-energy bond. Upon hydrolysis it becomes guanosine monophosphate (GMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

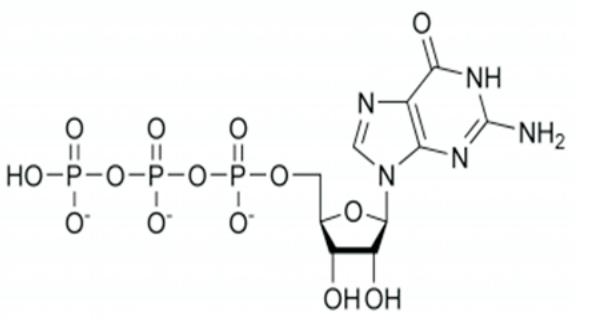


Figure 1.3 Structure of GTP

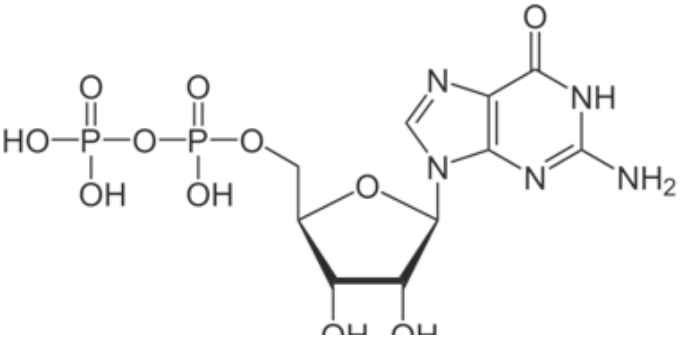


Figure 1.4 Structure of GDP

1.7.3 Uridine Triphosphate (UTP)

- Uridine triphosphate is a pyrimidine nucleoside triphosphate UTP is primarily used for the synthesis of polysaccharides. UTP and UDP are important metabolites in glycogenesis.

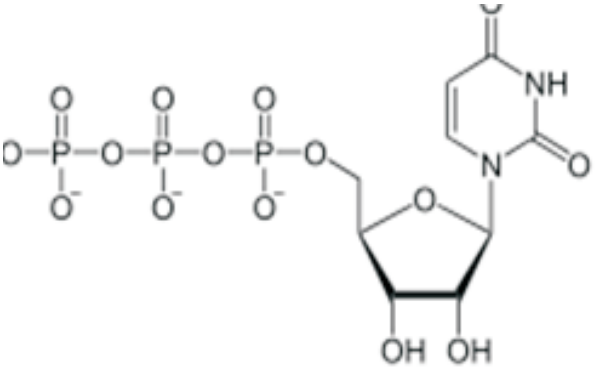


Figure 1.5 Structure of UTP

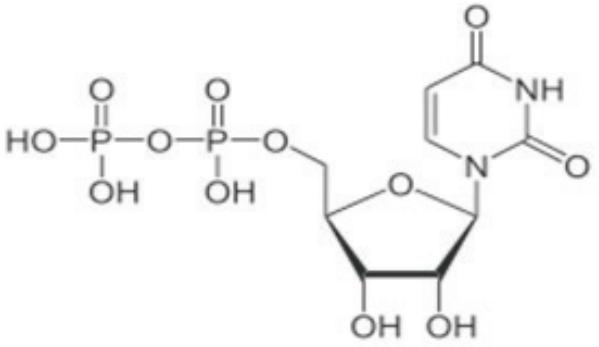


Figure 1.6 Structure of UDP

- The structure of UTP may be represented as U ~ P ~ P ~ P. G represents uridine which contains uracil and ribose. P stands for phosphoric acid group. It has two high-energy bonds.
- Upon hydrolysis UTP becomes uridinediphosphate (UDP) and phosphoric acid. The process releases a free energy of 7300 calories/mole at pH 7. UDP has a single high-energy bond upon hydrolysis it becomes uridine monophosphate (UMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

$$U \sim P \sim P \sim P + H_2O \rightarrow U \sim P \sim P + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

$$U \sim P \sim P + H_2O \rightarrow U \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

1.7.4 Cytidine Triphosphate (CTP)

- CTP is a high energy molecule similar to ATP. CTP is primarily employed in lipid synthesis.
- CTP is a coenzyme in metabolic reactions. CTP also acts as the inhibitor of enzyme aspartate carbomyl transferase.
- The structure may be represented as C ~ P ~ P ~ P. C represents nucleoside cytidine which contains cytosine and ribose. P stands for phosphoric acid group. It has two high-energy bonds.
- Upon hydrolysis CTP becomes cytidine diphosphate (CDP) and phosphoric acid. The process releases a free energy of 7300 calories/mole at pH 7.

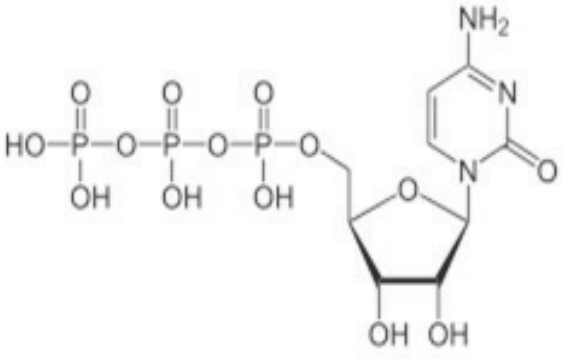


Figure 1.7 Structure of CTP

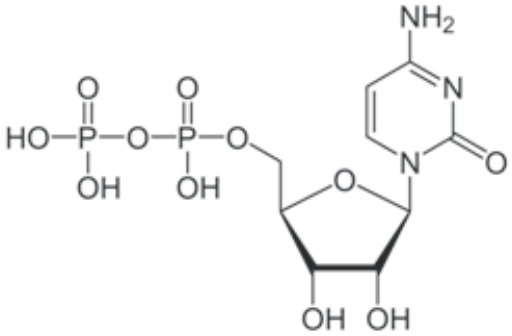


Figure 1.8 Structure of CDP

- CDP has a single high-energy bond upon hydrolysis it becomes cytidine monophosphate (CMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

$$C \sim P \sim P \sim P + H_2O \rightarrow C \sim P \sim P + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

$$C \sim P \sim P + H_2O \rightarrow C \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

1.7.5 Acyl Phosphate

1,3 Diphosphoglyceric acid is an example of acyl phosphate. It has one high-energy phosphate bond. Upon hydrolysis it becomes 3-phosphoglyceric acid and phosphoric acid. The process releases free energy of 11800 calories/mole at pH 7.

1.8 Energy Coupling

Energy coupling occurs in an biological system. An exergonic reaction provides large amount of free energy. An endergonic reaction, requires energy from outside. Both exergonic and endergonic reaction can be combined in a suitable manner. This is known as coupling of chemical reactions.

The energy released by the exergonic reaction can be used to carry out the endergonic reaction. Consider, the following equations of pH 7.

Exergonic reaction

$$ATP + H_2O \rightarrow ADP + H_3PO_4 \qquad \Delta G = -7500 \text{ calories/mole}$$

The hydrolysis of ATP provides 7500 calories/mole of free energy.

Endergonic reaction

$$\text{Glucose} + H_3PO_4 \rightarrow \text{Glucose 6-phosphate} + H_2O$$

$$\Delta G = +5500 \text{ calories/mole}$$

About 5500 calories of free energy has to be supplied from outside to get one mole of glucose 6-phosphate. So, these two reactions can be coupled together. The free energy released by the first reaction is used by the second reaction.

Adding the above reactions,

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

$$\Delta G = -2000 \text{ calories/mole}$$

So the overall reaction is exergonic so, the reaction moves in forward direction.

Hence ATP plays a very important role in biological systems. So, the coupling of reactions using ATP is very important in bio-energetics.

CHAPTER

2 METABOLISM OF CARBOHYDRATES

2.1 Introduction

2.1.1 Metabolism

- Metabolism is defined as the enzymatic reaction taking place in the cell. It covers all the changes from the event of entry of a substance into the organisms to the discharge of the final end products into the outside environment.
- Through the metabolic process, energy obtained is utilized for the proper growth, maintenance of life and the performance of biological functions.

2.2 Carbohydrate Metabolism

Carbohydrate metabolism is a fundamental biochemical process that ensures a constant supply of energy to living cells. Carbohydrates form the major bulk of human diet and also one of the chief sources of energy.

During digestion, carbohydrates are broken down into simple, soluble sugars that can be transported across the intestinal wall into the circulatory system to be transported throughout the body.

Carbohydrates are mainly in the form of polysaccharides and disaccharides which are hydrolyzed into monosaccharide such as glucose, fructose and galactose by the enzymes of the digestive tract.

In the liver all the other hexoses are converted to glucose by the respective Isomerases. The glucose thus formed take-up any one of the following routes in the liver depending upon the necessity of the body.

1. ***Oxidation:*** At the time of physiological demand for energy, glucose is oxidized to carbon dioxide, water and energy in all the tissues.
2. ***Storage:*** In the absence of physiological demand for energy, excess glucose may be converted to glycogen and is stored in the liver, muscle and other tissues.

3. ***Conversion of fat:*** As the amount of glucose stores in the liver is limited, excess of this is converted to fatty acids and glycerol and are stored as triglycerides in the fat depots.
4. ***Conversion to other carbohydrates:*** A part of glucose may be converted to ribose and deoxyribose which are required for the synthesis of nucleic acids.

   Glucose may also form glucuronic acid which is involved in the formation of mucopolysaccharides and also in the detoxification reactions.

   It may also form galactose which is a component of glycolipids and lactose.

   It also forms mannose, glucosamine and galactosamine which form the components of mucopolysaccharides and glycoproteins.
5. ***Conversion to amino acids:*** Animals do not depend upon their diet for the non-essential amino acids and these amino acids are synthesized within the body itself either from glucose or their metabolites. Such amino acids can also be converted back into glucose, hence these amino acids are said to be glycogenic.
6. ***Glycolysis:*** Under special circumstances such as severe muscular concentration, glucose undergoes partial degradation namely glycolysis resulting in the formation of lactic acid which is largely disposed off by the liver.

2.3 Anabolic and Catabolic Pathways

The major metabolic pathways for carbohydrates may be as follows:

2.3.1 Anabolic Pathways

Anabolic pathways are those involved in the synthesis of the compounds which contains the body's structure and machinery. The free energy required for these processes comes from catabolism.

Ex - Glycogenesis

2.3.2 Catabolic Pathways

Catabolic pathways involve oxidative processes that release free energy, usually in the form of high-energy phosphate or reducing equivalents.

Ex - Glycogenolysis, Glycolysis and the oxidation of pyruvate, Citric acid cycle and Pentose Phosphate Pathway.

2.4 Intermediary Metabolism of Carbohydrates

The metabolism of carbohydrates occurs through the following pathways:

- Glycogenesis
- Glycogenolysis
- Glycolysis
- Citric acid cycle
- Hexose Monophosphate Shunt and
- Gluconeogenesis

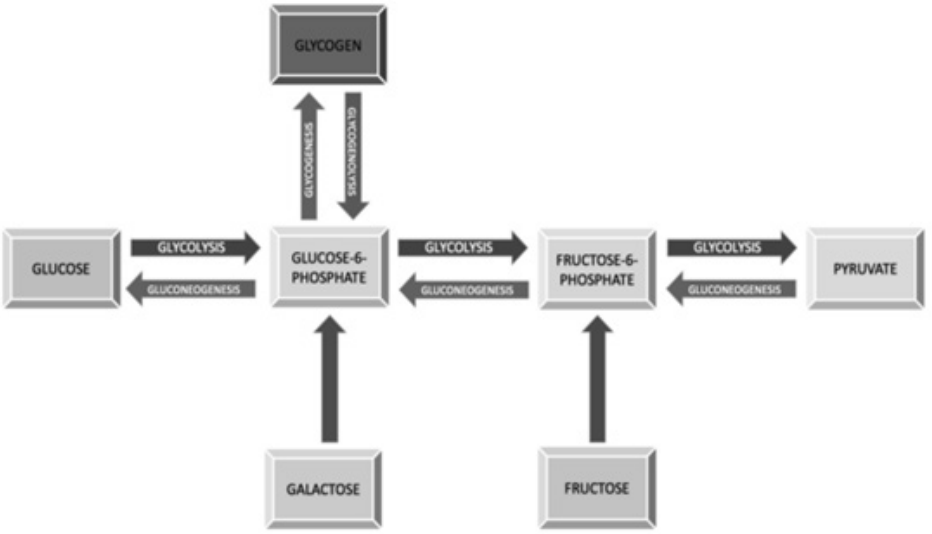


Figure 2.1 Metabolism of Carbohydrate

2.4.1 Glycogenesis

Glycogenesis is the process of glycogen synthesis from glucose. Glycogenesis takes place in the cytosol and requires ATP and UTP, besides glucose.

It occurs in all the tissues of the body but the major sites are liver and muscles. A considerable amount is synthesized in kidney also.

Glycogenesis is a very essential process. The excess glucose is converted and stored up as glycogen which could be utilized at the time of requirement. If this process does not take place the tissues are exposed to excess of glucose immediately after a meal.

The following are the various reactions of glycogenesis:

Step 1: Synthesis of UDP glucose – The enzymes hexokinase (in muscle) and glucokinase (in liver) convert glucose to glucose 6-phosphate. Glucose is first phosphorylated in the presence of ATP and the activation of Mg++ takes place.

Step 2: Glucose 6-phosphate is then reversibly converted to glucose-1-phosphate by the enzyme phosphoglucomutase in the presence of Mg++. Glucose 1,6-diphosphate act as coenzyme in this reaction.

Step 3: Hydrolysis of uridine triphosphate activates the glucose 1-phosphate in the presence of enzyme uridine diphosphate glucose pyrophosphorylase. It results in the formation of uridine diphosphoglucose. The inorganic pyrophosphate is eliminated in this reaction.

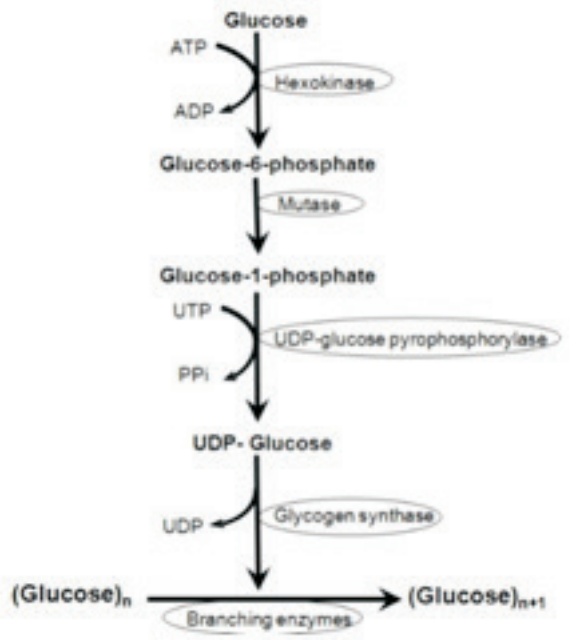


Figure 2.2 Glycogenesis

Step 4: In the end of an already existing glycogen chain the UDPG transfer the glucose molecule. The carbon 1(C1) of the activated glucose of UDPG forms a glycosidic bond with the carbon 4 (C4) of the terminal glucose residue of glycogen liberating UDP by the enzyme glycogen synthetase. As glucose molecules are added to the pre-existing glycogen chain by the successive α-1,4 linkages the chain becomes elongated.

Step 5: When the chain has become long with more than 8 glucose residues, a second enzyme namely the branching enzyme (amylo 1,4- 1,6 transglycosylase) acts on the glycogen and helps in joining of 1, 4 glycogen chain with a similar neighboring chain to form α-1, 6 linkage thus forming a branching point in the molecule.

This leads to the formation of a new non-reducing end, besides the existing one. Glycogen is further elongated and branched, by the enzymes glycogen synthase and glucosyl 4-6 transferase.

The glycogen thus formed may be stored in tissues mainly in liver and muscles and to some extend in the kidney also.

2.4.2 Glycogenolysis

Glycogenolysis means break down of glycogen into glucose (i.e. glucose-6-phosphate).

Glycogenolysis is the process of breaking down stored glycogen in the liver so that glucose may be produced for use in energy metabolism.

Glycogen may be broken down to glucose in liver and kidney or it may be broken down to glucose 6-phosphate in the muscles.

The following are the various reaction of glycogenolysis:

Step 1: The first step is the breakdown of glycogen catalyzed by two enzymes which act independently. Two key enzymes in glycogenolysis are glycogen phosphorylase and debranching enzyme.

The cleavage of terminal α-1, 4 bond of glycogen is catalyzed by the first enzyme namely glycogen phosphorylase with inorganic phosphate. It produces glycogen with one glucose molecule less and a molecule of glucose 1-phosphate.

The removal of glucose residues as glucose 1-phosphate continues until about 4 glucose residues remain on either side of the α-1,6 branch. Glycogen acts upon phosphorylase alone it results in the formation of a glycogen molecule. It contains each branch having 4 glucose units and this is called the limit dextrin.

The enzyme phosphorylase cannot cleave α-1, 6 linkages. This is carried out by another enzyme called the debranching enzyme which hydrolyses these bonds and thus make more α-1, 4 linkages accessible to the action of glycogen phosphorylase.

The combined action of glycogen phosphorylase and the debranching enzyme converts glycogen into glucose 1-phosphate.

The enzymes phosphorylase involved in the process of glycogenolysis, occurs in the liver and muscles tissues. In the liver, phosphorylase exists in two forms, namely an inactive form known as dephosphophosphorylase and an active form known as phosphosphorylase. The inactive forms can be converted into the active form in the presence of ATP and an enzyme dephosphophosphorylase kinase.

Muscle phosphorylase also exist in two distinct forms, namely phosphorylase a and phosphorylase b. Phosphorylase b can be converted into active phosphorylase a with the presence of the enzyme phosphorylase b kinase and ATP.

Step 2: The glucose 1-phosphatae is then reversibly converted to glucose 6-phosphate by the action of the enzyme phosphoglucomutase.

Step 3: The conversion of glucose 6-phosphate to glucose takes place in the liver and kidney by the action of the enzyme glucose 6-phosphatase. This enzyme removes phosphate from glucose 6-phosphate enabling the free glucose to diffuse from the cell into the extracellular spaces including the blood.

The reaction does not occur in the muscles because muscles lack the enzyme glucose 6-phosphate.

Glycogenolysis occurs in the hepatocytes and also in myocytes.

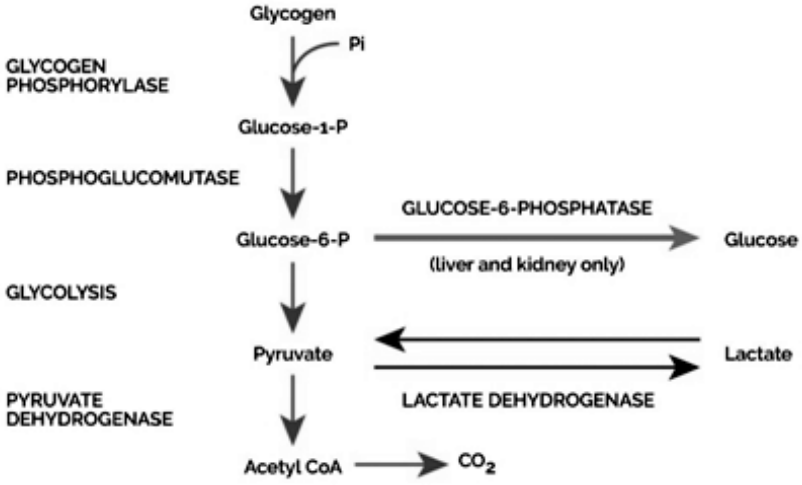


Figure 2.2 Glycogenolysis

2.4.3 Glycolysis

Glycolysis is the process of breaking down of a molecule of glucose to two molecules of pyruvic acid, two molecules of ATP, two molecules of NADH and two molecules of water.

The term glycolysis is derived from two Greek word "glycos" means sugar and "lysis" means dissolution and the term "glycolysis" means splitting of sugar.Glycolysis takes place in the cytoplasm. Glycolysis can occur with or without oxygen.

It is also known as the Embden – Meyerhof pathway.

Glycolysis is a primitive metabolic pathway since it operates in even the simplest and archaic cells. Glucose 6-phosphate is a principal compound in the metabolism of glucose.

There are 10 enzymes involved in the breaking down of sugar, where a molecule of glucose yields two molecules of pyruvic acid.

The enzyme involved in glycolysis are found in the extra mitochondrial soluble fraction of the cells and hence glycolysis occurs in the cytoplasm of the cells outside the mitochondria.

Step 1: Phosphorylation - Glucose is converted to glucose 6-phosphate with the presence of hexokinase. Glucose undergoes phosphorylation to produce glucose 6-phosphate. This is an irreversible reaction which requires ATP and Mg^{++}. ATP is the phosphate group donor and reacts as the Mg-ATP complex. It donates one high energy phosphate group and converted into ADP.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{} \text{Glucose 6-phosphate} + \text{ADP}$$

Step 2: Isomerisation - Glucose 6-phosphate is converted to its isomeric form fructose 6-phosphate in the presence of the enzyme phosphohexose isomerase. It involves an aldose-ketose isomerisation.

$$\text{D-Glucose 6-phosphate} \xleftrightarrow{\text{Phosphohexose isomerase}} \text{D-Fructose 6-phosphate}$$

Step 3: SecondPhosphorylation -The second phosphorylation reaction is followed by the isomerization. Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-diphosphate and this reaction is catalyzed by the enzyme phosphofructokinase.

$$\text{D-fructose6-phosphate} + \text{ATP} \xleftrightarrow{\text{Phosphofructokinase}} \text{D-fructose 1,6-diphosphate}$$

(Mg^{++})

Step 4: Cleavage -Fructose 1,6-diphosphate is split by aldolase into two triose phosphates (3 C sugars), glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The splitting occurs between carbon atoms 3 and 4.

$$\text{D-fructose1,6-diphosphate} \xleftrightarrow{\text{Aldolase}} \text{D-glyceraldehyde 3-phosphate} + \text{Dihydroxyacetone phosphate}$$

Step 5: Isomerization - Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate undergo inter conversion (isomerisation) in the presence of phosphotriose isomerase. Thus two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-diphosphate.

$$\text{D-Glyceraldehyde 3-phosphate} \xleftrightarrow{\text{Phospho triose isomerase}} \text{Dihydroxyacetone phosphate}$$

Step 6: Phosphorylation and Oxidative Dehydrogenation - Glyceraldehyde 3-phosphate is oxidized and phosphorylated simultaneously and converted into 1,3- diphosphoglyceric acid in the presence of NAD+, phosphoric acid and an enzyme phosphotriose dehydrogenase.

$$\text{D-Glyceraldehyde 3-phosphate} + NAD^{+} + \text{Pi} \xleftrightarrow{\text{Glyceraldehyde 3-dehydrogenase}} \text{1,3-diphosphoglyceric acid} + NADH + H^{+}$$

The coenzyme NAD in the oxidized form has a net positive charge and hence is written as NAD^{+}. When glyceraldehyde 3-phosphate is oxidized, NAD+ is reduced to NADH and one proton is released into the aqueous medium.

Step 7: Substrate level phosphorylation and ATP production - The high energy phosphate group in 1,3 – diphospho glycerate is transferred to ADP resulting in the formation of 3- phosphoglyceric acid and ATP. The reaction is catalyzed by phosphoglycerate kinase in the presence of Mg^{++}. Since 2-molecules of PGA are formed per molecule of glucose undergoing glycolysis, 2 molecules of ATP are generated.

The phosphorylation which occurs in this step is substrate level phosphorylation where a high energy phosphate bond is formed directly.

Phosphoglycerate kinase

1,3-diphosphoglyceric acid + ADP ⟷ 3-phosphoglyceric acid + ATP

Step 8: Isomerisation -The 3-phosphoglyceric acid arising from the above reaction undergoes internal rearrangement to form 2-phosphoglyceric acid in the presence of enzyme, phosphoglycerate mutase. The phosphate group is transferred from the third carbon atom to the second carbon atom.

Phosphoglycerate mutase

3-phosphoglyceric acid ⟷ 2-phosphoglyceric acid

Step 9: Dehydration -The 2-phosphoglyceric acid undergoes dehydration and redistribution of energy within the molecule, raising the phosphate on position 2 to the high energy state, thus forming phosphoenol pyruvic acid. The reaction is catalysed by enolase, whose activity can be inhibited by fluoride. Enolase requires Mg^{++} or $Mn^{++}$for the activity.

Enolase

2-phosphoglyceric acid ⟷ Phosphoenolpyruvic acid + H2O

Step 10: Substrate level phosphorylation and ATP generation - In the last step, there is a transfer of high energy phosphate from phosphoenolpyruvic acid to ADP by the enzyme pyruvate kinase. Thus, a molecule of ATP is directly synthesized and pyruvic acid is also formed. Pyruvic acid is accompanied by considerable loss of free energy as heat and must be regarded as physiologically irreversible.

Pyruvate kinase

Phosphoenolpyruvic acid + ADP ⟷ Pyruvic acid + ATP

At the end of glycolysis 2-molecules of pyruvic acid are produced per glucose molecule.

Under anaerobic conditions, the reoxidation of NADH by transfer of reducing equivalents through the respiratory chain to oxygen is prevented. Pyruvic acid is reduced by the NADH to lactic acid in the presence of enzyme, lactate dehydrogenase.

Lactate dehydrogenase

Pyruvic acid + NADH + H^+ ⟷ Lactic acid + NAD^+

2.4.3.1 Energy yield in glycolysis

The two molecules of ATP are generated in the conversation of glucose to pyruvic acid the reduced NAD ($NAD+H^+$) enters transport chain, where the reoxidation of NADH to NAD^+ occur with the liberation of 2 molecules of ATP.

2.4.3.2 Oxidation of cytosol NADH

It is mediated by substrate shuttles because NADH cannot penetrate the mitochondrial membrane. The shuttles used are:

1. ***Glycerophosphate shuttle:*** The mitochondrial enzyme is linked to the respiratory chain via FAD rather NAD, only 2 molecules of ATPase are produced.
2. ***Malate shuttle:*** 3 ATP molecules will be produced per 2H+ transferred into mitochondria. The hydrogen atoms are transferred to NADH in mitochondria.

2.4.3.3 Oxidation of pyruvic acid

The pyruvic acid is oxidatively decarboxylated to acetyl co-enzyme A (active/acetate) before entering the citric acid cycle. The conversion of pyruvic acid to acetyl-CoA is an irreversible reaction. It takes place in mitochondria. This occurs in the mitochondrial matrix and forms a link between glycolysis and the citric acid cycle.

The reaction is catalyzed by the multienzyme complex known as pyruvic acid dehydrogenase complex which is an aggregate of three kinds of enzymes, namely,

1. Pyruvic acid decarboxylase – Enzyme 1
2. Lipoate reductase transacetylase –Enzyme 2
3. Dihydrolipoyl dehydrogenase –Enzyme 3

The reaction is also assisted by TPP (Thymine Pyrophosphate), lipoic acid, FAD, CoA, and NAD^+ which act as coenzymes. There are four steps in the conversion of pyruvic acid to acetyl CoA.

Step 1: In the first step pyruvic acid reacts with enzyme bound thiamine pyrophosphate or thiamine diphosphate in the presence of magnesium ions (Mg^{++}) and pyruvate dehydrogenase to form "active pyruvate".

Step 2: The active pyruvate undergoes decarboxylation to produce hydroxyethyl thiamine diphosphate "active acetaldehyde" in the presence of pyruvate dehydrogenase.

Step 3: Hydroxyethyl thiamine diphosphate reacts with oxidized lipomide in the presence of enzyme, dihydrolipoyl transacetylase to produce acetyl lipoamide.

Step 4: Acetyl lipoamide reacts with coenzyme A to form acetyl-CoA and reduced lipoamide in the presence of dihydrolipoyl transacetylase. Acetyl-CoA contains one high energy bond.

Reduced lipoamide is then reoxidized by a flavoprotein FAD^+ in the presence of dihydrolipoyl dehydrogenase. As a result, FAD^+ gets converted to $FADH^+$ which in turn transfer the hydrogen to NAD.

The oxidation of pyruvic acid to acetyl-CoA can be summarized as follows:

$$\text{Pyruvic acid} + NAD^+ + CoA \longrightarrow \text{Acetyl-CoA} + NADH + H^+ + CO_2$$

Acetyl CoA is the end product of this reaction. It is an important intermediary metabolite which is formed not only from pyruvic acid but also from certain amino acids and fatty acids.

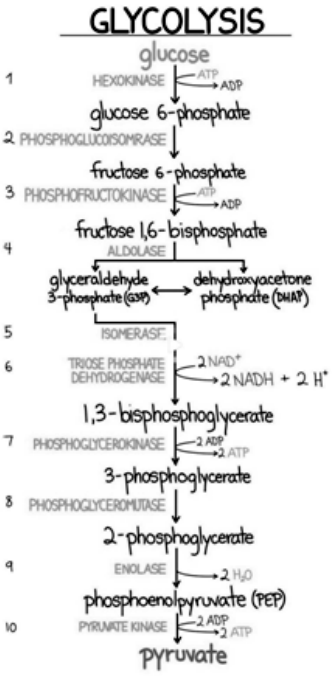


Figure 2.4 Glycolysis

2.4.4 Citric Acid Cycle

Citric acid cycle is a central pathway for the release of energy from acetyl CoA which is produced from the catabolism of carbohydrates, fatty acids and some amino acids.

The citric acid cycle is a series of reactions in mitochondria that bring about the catabolism of acetyl residues which are in the form of acetyl-CoA, an ester of coenzyme A.

The name citric acid cycle came from citric acid which is formed in the first step of this cycle. This cycle is also known as Krebs' cycle and tricarboxylic acid cycle or TCA cycle.

2.4.4.1 Reactions of the citric acid cycle

The citric acid cycle involves two important processes, namely electron transport and oxidative phosphorylation. The reduced enzymes of this cycle are oxidized and the available energy is used to synthesize ATP.

The various steps of citric acid cycle are as follows:

i. ***Condensation:*** The cycle starts with the joining of a 4 carbon unit, oxaloacetic acid and a two carbon unit, acetyl-CoA in the presence of a condensing enzyme, citrate synthetase to yield a six carbon unit, citric acid and coenzyme A(CoA).

$$\text{Acetyl-CoA + Oxaloacetate + } H_2O \longrightarrow \text{Citric acid + CoA}$$

ii. ***Dehydration:*** Under the action of enzyme aconitase, citric acid undergoes dehydration to form cis-aconitic acid.

$$\text{Citric acid} \xleftarrow{\text{Aconitase}} \text{Cis-aconitic acid + H2O}$$

iii. ***Hydration-I:*** Cis-aconitic acid undergoes hydration to form isocitric acid under the influence of aconitase.

$$\text{Cis – aconitic acid + } H_2O \xleftarrow{\text{Aconitase}} \text{Isocitric acid}$$

iv. ***Dehydrogenation I:*** Isocitric acid undergoes dehydrogenation to form oxalosuccinic acid. The pair of hydrogen atoms removed is accepted by NAD to form NADH+H^+ which enters the electron transport chain and 3 molecules of ATP are generated.

$$\text{Isocitric acid + NAD} \xleftarrow{\text{Isocitrate dehydrogenase}} \text{Oxalosuccinic acid + NADH + } H^+$$

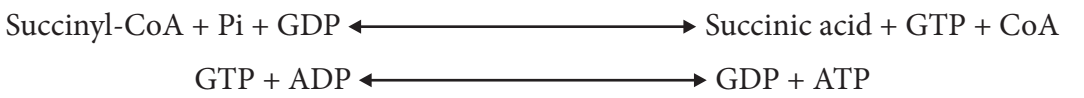
v. ***Decarboxylation I:*** The oxalosuccinic acid is oxidatively decarboxylated to α-ketoglutaric acid.One molecule of carbon dioxide is removed in the step and because of this loss of one carbon atom, the α- ketoglutaric acid molecule has five carbon atoms.

vi. ***Second Oxidative Decarboxylation (dehydrogenation II and decarboxylation II):*** α-Ketoglutaric acid undergoes oxidative decarboxylation and joins with coenzyme A to form succinyl CoA,a4 carbon atom derivate of coenzyme A.

This reaction is analogous to oxidative decarboxylation of pyruvic acid to acetyl CoA.This is an irreversible reaction and is catalyzed by α-ketoglutarate dehydrogenase complex which requires cofactors thiamine diphosphate, lipoate, NAD^+, FAD and CoA and results in the formation of succinyl-CoA, a thioester containing a high-energy bond.

vii. Formation of Succinic acid: Succinyl-CoA is converted to succinic acid by the enzyme succinate thiokinase (succinyl-CoA synthetase). The reaction requires GDP (Guanosine diphosphate) or IDP (inosine diphosphate) which undergoes phosphorylation in the presence of inorganic phosphate to produce GTP or ITP. GTP or ITP possesses a high energy phosphate bond, the energy is released.

In the citric acid cycle, this is the only example where energy is generated at the substrate level. By means of nucleoside diphosphate kinase, ATP may be formed from either GTP or ITP.



viii. ***Dehydrogenation III:*** Succinic acid undergoes dehydrogenation which is catalysed by succinate dehydrogenate, which is bound to the inner surface of the inner mitochondrial membrane. The hydrogen atoms are accepted directly by FAD.

ix. ***Hydration II:*** Fumaric acid is hydrated to malic acid and the reaction is catalyzed by the enzyme fumarase (fumarate hydrolase).

$$\text{Fumaric acid} + H_2O \longleftrightarrow \text{Malic acid}$$

x. ***Dehydration IV:*** In the final step malic acid is transformed into oxaloacetic acid by malate dehydrogenase. NAD+ accepts the hydrogen atoms to form NADH+ H^+ and by the passage of hydrogen atoms into the electron transport system 3 molecules of ATP are generated.

$$\text{Malic acid} + NAD^+ \longleftrightarrow \text{Oxaloacetic acid} + NADH + H^+$$

2.4.4.2 Energy yield in citric acid cycle

One molecule of glucose gives rise to two molecules of pyruvic acid by glycolysis, intermediates of citric acid cycle also result as two molecules.

2.4.4.3 Energetics of glucose metabolism

As a result of a glycolysis one molecule of glucose produces 2 molecules of pyruvic acid and in this process 6 molecules of ATP are synthesized.

In the conversion of the 2 molecules of pyruvic acid to 2 molecules of acetyl CoA, 6 ATP molecules are formed. The acetyl CoA enter the Krebs cycle and each acetyl CoA produces 122 molecules hence (2 × 12) 24 molecules of ATP are synthesized from 2 acetyl CoA molecules. Ultimately a total of 36 molecules of ATP are produced from the oxidation of a single molecule of glucose.

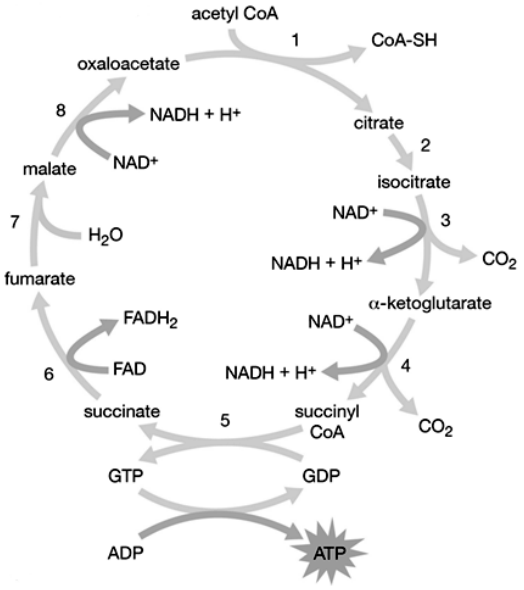


Figure 2.5 Citric Acid Cycle

2.4.5 Hexose Monophosphate (HMP) Shunt (Pentose Phosphate Pathway)

Hexose Monophosphate Shunt is an alternative pathway to glycolysis. It is an aerobic process. In this pathway glucose is used as the raw material.

HMP shunt is also known as alternative pathway or pentose phosphate pathway. This operates in liver, lactating mammary glands, adrenal cortex, adipose tissues, testis, thyroid, RBC and certain other tissues and is an alternative route for the oxidation of glucose.

The products formed in the HMP shunt are pentose sugar, CO, NADPH.The end products of HMP shunt can enter glycolysis and hence the name.

HMP shunt performs two major functions:

1. The generation of NADPH for reductive syntheses such as fatty acids and steroid biosynthesis
2. The provision of ribose for nucleotide and nucleic acid biosynthesis.

The HMP shunt reactions occur in the cytosol. The sequences of this shunt may be grouped into two phase,

1. The oxidative phase - conversion of hexose to pentose occurs
2. The non-oxidative phase - conversion of pentose to hexose occurs

1. **The oxidative phase:** During this phase NADPH are generated. The sequences of reactions are as follows

Step 1: Phosphorylation - The first step is the phosphorylation of glucose to glucose 6-phosphate in the presence of ATP and an enzyme hexokinase.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6-phosphate + ADP}$$

Step 2: Dehydrogenation - Glucose 6-phosphate is oxidized into 6-phosphogluconolactone in the presence of NADP and the enzyme glucose 6-phosphate dehydrogenase. NADP accepts the evolved hydrogen atoms and Mg^{2+} act as cofactors.

$$\text{Glucose6-phosphate} \xrightarrow[\text{Glucose 6-phosphate dehydrogenase}]{\text{NADP}^{+} \quad \text{Mg}^{+2} \quad \text{NADPH + H}^{+}} \text{6-phosphogluconolactone}$$

Step 3: Hydrolysis - 6-phosphogluconolactone is unstable and undergoes hydrolysis to produce 6-phosphogluconic acid. The enzyme that catalyzes the reaction is gluconolactone hydrolase in the presence of water and Mg^{+2} and Mn^{+2} ions.

$$\text{6-phosphogluconolactone} \xrightarrow[\text{Gluconolactone hydrolase}]{H_2O \quad Mg^{+2}, Mn^{+2}} \text{6-phosphogluconic acid}$$

Step4: Dehydrogenation - 6-phosphogluconic acid isoxidized by NADP into 3-keto-6-phosphogluconic acid in the presence of an enzyme 6-phosphogluconate dehydrogenase and cofactors Mg^{+2} and Mn^{+2} ions. NADP acts as hydrogen acceptor.

$$\text{6-phosphogluconic acid} \xrightarrow[\text{6-phosphogluconate dehydrogenase}]{NADP^+ \quad Mg^{+2}, Mn^{+2} \quad NADPH + H^+} \text{3-keto-6 phosphogluconic acid}$$

Step 5: Decarboxylation – Dehydrogenation is followed by decarboxylation to produce ketopentose, ribulose 5-phosphate. 3-keto-6-phosphogluconic acid acts as intermediate. Thus a hexose (glucose) is converted into a pentose (Ribulose 5-phosphate).

$$\text{3-keto-6-phosphogluconic acid} \longrightarrow \text{Ribulose 5-phosphate} + CO_2$$

Ribulose 5-phosphate is acted upon by two different enzymes. Ribulose 5-phosphate epimerase converts a portion of ribulose 5-phosphate to xylulose 5-phosphate while ribose 5-phosphate isomerase converts the rest of ribulose 5-phosphate into ribose 5 phosphate.

The above five steps constitute the first phase i.e. oxidative phase of the HMP shunt.

2. The non-oxidative phase: During this phase ribose precursors are generated. In the second phase of the HMP shunt the 5 carbon sugars, by a series of reversible reactions are converted into the glycolytic intermediates namely fructose 6-phosphate and glyceraldehyde 3-phosphate. The various sequences of reactions are as follows:

   Ribulose 5-phosphate generated during oxidative phase is acted upon by 2 different enzymes.

   Ribose 5-phosphateketoisomerase converts Ribulose 5-phosphate into an aldopentose, Ribose 5-phosphate and

   Ribulose 5-phosphate 3-epimerase converts ribulose 5-phosphate into xylulose 5-phosphate.

Ribose 5-phosphate keto Enediolform

Ribulose5-phosphate ⟷ isomerase ⟷

Ribose 5-phosphate

Ribulose 5-phosphate 3-epimerase

Ribulose 5-phosphate ⟷ xylulose 5-phophate

Step 6: Xylulose 5-phosphate and ribose 5-phosphate react to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate in the presence of the enzyme transketolase. Thus, the two pentose phosphate molecules react to form a heptose phosphate and a triose phosphate.

Ribose 5-phosphate + xylulose 5-phosphate ↔ Sedoheptulose7-phosphate + glyceraldehyde 3-phosphate

Transketolase thiamin – P_2 Mg^{+2}

Step 7: Sedoheptulose7-phosphate reacts with glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6 phosphate and this reaction is catalyzed by transaldolase.

Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate ↔ Fructose 6-phosphate + Erythrose 4-phosphate

Step 8: Xylulose 5-phophate may also react with erythrose 4-phosphate to produce fructose 6-phosphate and glyceraldehyde 3-phosphate in the presence of enzyme transketolase. The reaction also require thiamin-diphosphate and Mg+2 ions.

Xylulose 5-phosphate + Erythrose 4-phosphate ↔ Fructose 6-phosphate + Glyceraldehyde 3-phosphate

Transketolase thiamin – P_2 Mg^{+2}

Glyceraldehyde 3-phosphate and fructose 6-phosphate may enter the glycolytic cycle. These two products of the HMP shunt link up with the EMP pathway (Glycolysis). Fructose 6-phosphate is converted to glucose 6-phosphate in the presence of phosphoglucoisomerase and glyceraldehyde 3-phosphate is also converted to glucose 6-phosphate by the enzymes of the glycolytic pathway working in a reverse direction.

Figure 2.6 Hexose Monophosphate Shunt

2.4.6 Gluconeogenesis or neoglucogenesis

The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis. The mechanism involved in gluconeogenesis is reversal of citric acid cycle and glycolysis. Gluconeogenesis mainly occurs in liver and kidney. The most important substrates for gluconeogenesis are the glucogenic aminoacids, lactic acid, propionic acid and glycerol.

Gluconeogenesis is an important source for supplying glucose to various tissues when glucose is otherwise not available and is regulated by certain key enzymes. These enzymes allow reversal of glycolysis. However, Gluconeogenesis is not an exact reversal of glycolysis.

2.4.6.1 Reactions of Gluconeogenesis

1. Pyruvate is carboxylated to oxaloacetate at the expense of an ATP which is catalyzed by pyruvate carboxylase. Then oxaloacetate is decarboxylated and phosphorylated to yield phosphoenolpyruvate at the expense of one molecule of GTP which is catalyzed by phosphoenolpyruvate carboxykinase.
2. Fructose 6-phosphate is formed from fructose 1, 6-diphosphate by hydrolysis and the enzyme fructose 1, 6-diphosphatase catalyzes this hydrolysis.
3. Glucose is formed by hydrolysis of glucose 6-phosphate catalyzed by glucose 6- phosphatase.

Here six high energy bond, are used to synthesize glucose from pyruvic acid whereas only two ATP are generated in glycolysis in the conversion of glucose to pyruvic acid. In gluconeogenesis four high energy phosphate bonds per glucose are synthesized from pyruvic acid.

2.4.6.2 Gluconeogenesis of amino acids

Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination. The amino acids are thus metabolically routed through oxaloacetic acid and phosphoenol pyruvic acid resulting in the formation of glucose.

2.4.6.3 Gluconeogenesis of propionic acid

In ruminants, propionic acid - a saturated fatty acid which is the end product of carbohydrate fermentation in the rumen is converted to succinyl CoA where by citric acid cycle enters the process of gluconeogenesis.

2.4.6.4 Gluconeogenesis of lactic acid

The liver and skeletal muscles exhibit a special metabolic co-operation as far as carbohydrates are concerned by way of a cycle of conversions known as cori cycle or lactic acid cycle. This is the cycle where liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactic acid produced by the active skeletal muscles.

Hence glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus lactic acid gets accumulated in the muscles.The lactic acid diffuses out of the muscle and enters the liver through the blood. In the liver lactic acid is oxidized to pyruvic acid which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose.

The glucose is then converted into liver glycogen by a process of glycogenesis. The glycogen may be once again converted to glucose and may be recycled to the muscle through the blood. The process of glycogenesis completes the cycle by converting glucose once again to muscle glycogen. This cycle of conversion which repeats during muscle contraction is called the cori cycle.

2.4.6.5 Gluconeogenesis of glycerol

At the time of starvation glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolyzed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerolkinase necessary to phosphorylate it. Instead, the glycerol passes to the liver, where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerolkinase.

The glycerol 3 phosphate is then oxidized to dihydroxyacetone phosphate catalyzed by the enzyme glycerol phosphate dehydrogenase. This dihydroxy acetone phosphate enters the gluconeogenic pathway and gets converted to glucose.

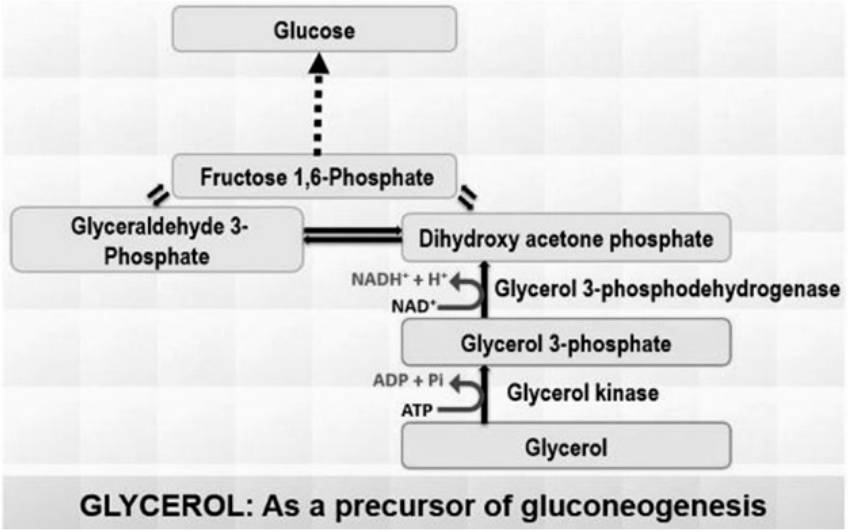


Figure 2.7 Gluconeogenesis

Overview:

Total net ATP produced under aerobic conditions:

a)	Glycolysis	8
b)	Pyruvic acid oxidation	6
c)	Citric acid cycle	24
	Total	**38**

Total net ATP produced under Anaerobic conditions = 2

If NADH produced during glycolysis is transported into mitochondria via glycerophosphate shuttle, only two ATP are produced hence total production of ATP would be 36 only instead of 38. If malate shuttle is used 38 ATP are produced.

CHAPTER

3 METABOLISM OF PROTEINS AND AMINO ACIDS

3.1 Protein Metabolism

The proteins present in the food are digested in the stomach and intestine and are hydrolysed into amino acids. The protein digestive enzymes are

1. Endopeptidases – pepsin, trypsin, chymotrypsin
2. Exopeptidases – carboxipeptidases, aminopeptidases, dipeptidases

The dietary proteins consist of 20 amino acids which exhibit L-configuration. The amino acids are classified into two types i.e. essential and non-essential amino acids. After digestion in the alimentary canal, the amino acids are absorbed through the intestinal epithelium into the blood. The sources of amino acids are dietary proteins, intra-cellular synthesis and tissue protein breakdown which constitute the "amino acid pool".

The amino acids undergo two main metabolic pathways:

1. Catabolic pathways
   i. Deamination
   ii. Transamination
   iii. Decarboxylation
2. Anabolic pathway
   i. Protein synthesis

3.2 Catabolic Pathways

3.2.1 Deamination

In the process of deamination an amino acid is converted into an α-keto acid and ammonia.

It occurs in the following ways:

i. **Amino acid oxidases –** The amino acid oxidases are flavoproteins and oxidize amino acids to the corresponding keto acids after generating a molecule of ammonia. Oxygen takes part in this oxidation process.

$$\text{Amino acid} + \tfrac{1}{2}\,O_2 \longrightarrow \alpha\text{-Keto acid} + NH_3$$

ii. **Amino acid dehydrogenases –** Amino acid dehydrogenases are NAD or NADP linked enzymes. This enzyme catalyses the removal of hydrogen atoms from an α-amino acid. The hydrogen atoms are accepted by NAD or NADP. The amino acid is hydrolysed to produce α-keto acid and ammonia.

$$\alpha\text{-Amino acid} \xrightarrow{NAD^+} \alpha\text{-Imino acid} + NADH + H+$$

$$\alpha\text{-Imino acid} + H_2O \xrightarrow{\text{Amino acid dehydrogenase}} \alpha\text{-Keto acid} + NH_3$$

3.2.2 Non-oxidative deaminatio

Serine and threonine dehydratases catalyse a non-oxidative deamination reaction resulting from a primary dehydration of the substrate and yield pyruvate or α-ketobutyrate.

$$\text{Serine} \longrightarrow \alpha\text{-imino acid} \xrightarrow{H_2O} \text{Pyruvate} + NH_3$$

$$\text{Threonine} \xrightarrow{H_2O} \alpha\text{-imino acid} \xrightarrow{H_2O} \alpha\text{-ketobutyrate} + NH_3$$

Non-oxidative deamination reactions also occur in microorganisms. Some of the reactions are as follows:

$$\text{Aspartate} \xrightarrow{\text{Aspartase}} \text{Fumarate} + NH3$$

$$\text{Histidine} \xrightarrow{\text{Histidinase}} \text{Uroeonic acid} + NH3$$

3.2.3 Transamination

In transamination reactions α-amino group is transferred to α-carbon atom of an α-keto acid. Transamination is catalysed by transaminases or aminotransferases. Pyridoxal phosphate acts as a cofactor.

$$\text{Amino acid 1 + Keto acid -1} \xleftrightarrow{\text{Transaminases}} \text{Keto acid 2 + Amino acid 2}$$

The transaminases are specific in their reactions. These are widely distributed in the tissues, however, their concentration in heart and liver is relatively high. Some specific examples of transaminases are as follows:

1. **GOT (Glutamic-oxaloacetate transaminase):** GOT activity is very high in heart tissue. It catalyses the reaction between glutamic acid and oxaloacetic acid to produce aspartic acid and α-ketoglutaric acid.

   $$\text{Glutamic acid + Oxaloacetic acid} \xleftrightarrow{\text{GOT}} \text{Aspartic acid + α-ketoglutaric acid}$$

   In a state of cardiac infarction, GOT is released in blood and its level in serum (SGOT) is abnormally raised.

2. GPT (Glutamic pyruvate transaminase): GPT concentration is very high in liver. Glutamic acid and pyruvic acid react in the presence of GPT to produce α-ketoglutaric acid and alanine.

   $$\text{Glutamic acid + Pyruvic acid} \xleftrightarrow{\text{GPT}} \text{α-ketoglutaric acid + Alanine}$$

   If liver cells are damaged, SGPT activity is abnormally raised.

3.2.4 Decarboxylation

Amino acids undergo decarboxylation to produce CO2 and an amine in the presence of amino acid decarboxylases. These enzymes require pyridoxal phosphate for their activity. The amines produced are called biogenic amines and have important pharmacological effects.

$$\text{Amino acids} \xrightarrow[\text{Pyridoxal phosphate}]{\text{Decarboxylase}} \text{Amine + } CO_2$$

Some metabolically significant amines formed as a result of decarboxylation are as follows:

1. **γ-Aminobutyric Acid (GABA):** GABA is formed in brain by the decarboxylation of glutamic acid. It stimulates neural activity and inhibits synaptic transmission in the central nervous system.

$$\text{Glutamic acid} \xrightarrow{\text{Glutamic acid decarboxylase}} \gamma\text{-Aminobutyric acid} + CO_2$$

2. **Tyramine:** Tyramine is obtained as a result of decarboxylation of tyrosine. It increases blood pressure.

$$\text{Tyrosine} \xrightarrow{\text{Tyrosine decarboxylase}} \text{Tyramine} + CO_2$$

3. **Histamine:** L-histidine is decarboxylated to produce histamine.

$$\text{Histidine} \xrightarrow{\text{Histine decarboxylase}} \text{Histamine} + CO_2$$

It's concentration is high in animal tissues such as liver, kidney, duodenum, large intestine and lungs. Histamine is a powerful vasodilator.

4. **Serotonin (5-hydroxytryptamine):** Serotonin is produced by the decarboxylation of 5-hydroxytryptophan.

$$\text{5-hydroxytryptophan} \xrightarrow{\text{5-hydroxytryptophan decarboxylase}} \text{5-hydroxytryptamine} + CO_2$$

Serotonin acts as a potent vasoconstrictor substance. It is mainly found in brain, blood platelets and intestinal tissues.

5. Dopamine (3,4-dihydroxyphenyl ethylamine): Dopamine is produced by the decarboxylation of 3,4-dihydroxyphenyl ethylamine (Dopa).

$$\text{Dopa} \xrightarrow{\text{Dopa decarboxylase}} \text{Dopamine} + CO2$$

Dopamine is present in kidney and adrenal tissues as well as sympathetic ganglia and nerves. It acts as an inhibitory neurotransmitter.

3.3 Anabolic Pathway

3.3.1 Protein Synthesis:

Protein synthesis is a process of synthesizing proteins in a chain of amino acids known as polypeptides. It takes place in the ribosomes found in the cytosol or those attached to the rough endoplasmic reticulum. It carries the information from DNA in the nucleus to a ribosome in the cytoplasm and then helps assemble the protein. It is called the central dogma of biology.

$$DNA \rightarrow RNA \rightarrow Protein$$

The protein synthesis involves two major steps:

- Transcription
- Translation

i. Transcription:

Transcription is the first part of the central dogma of molecular biology.

DNA → RNA

It is the transfer of genetic instructions in DNA to mRNA. During transcription, a strand of mRNA is made to complement a strand of DNA.

Steps of transcription

Transcriptiontakes place in three steps, called initiation, elongation, and termination.

1. Initiation is the beginning of transcription. It occurs when the enzymeRNA polymerase binds to a region of a genecalled the promoter. This signals the DNA to unwind so the enzymecan "read" the bases in one of the DNA strands. Theenzymeis ready to make a strand of mRNA with a complementary sequence of bases. The promoteris not part of the resulting mRNA.
2. Elongation is the addition of nucleotides to the mRNA strand.
3. Termination is the ending of transcription. As RNA polymerase transcribes the terminator, it detaches from DNA. The mRNA strand is complete after this step.

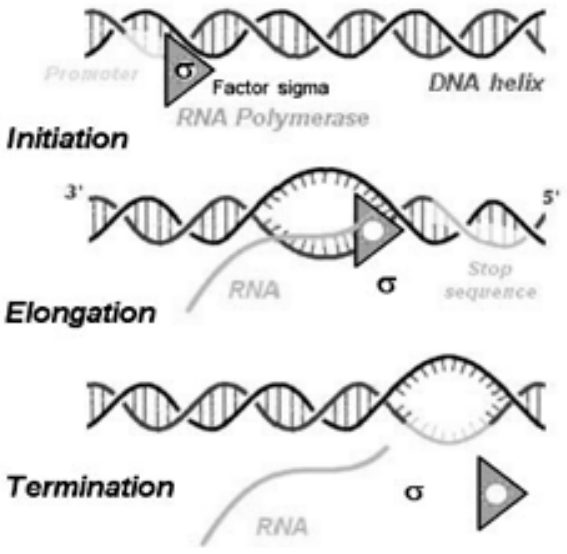


Figure 3.1 Protein Synthesis

Processing mRNA

In eukaryotes, the new mRNA is not yet ready for translation. At this stage, it is called pre-mRNA, and it must go through more processing before it leaves the nucleus as mature mRNA. The processing may include splicing, editing, and polyadenylation. These processes modify the mRNA in various ways. Such modifications allow a single gene to be used to make more than one protein.

- **Splicing** removes introns from mRNA. **Introns** are regions that do not code for the protein. The remaining mRNA consists only of regions called **exons** that do code for the protein. The ribonucleoproteins in the diagram are small proteins in the nucleus that contain RNA and are needed for the splicing process.
- **Editing** changes some of the nucleotides in mRNA. For example, a human protein called APOB, which helps transport lipids in the blood, has two different forms because of editing. One form is smaller than the other because editing adds an earlier stop signal in mRNA.
- **5′ Capping** adds a methylated cap to the "head" of the mRNA. This cap protects the mRNA from breaking down, and helps the ribosomes know where to bind to the mRNA
- **Polyadenylation** adds a "tail" to the mRNA. The tail consists of a string of As (adenine bases). It signals the end of mRNA. It is also involved in exporting mRNA from the nucleus, and it protects mRNA from enzymes that might break it down.

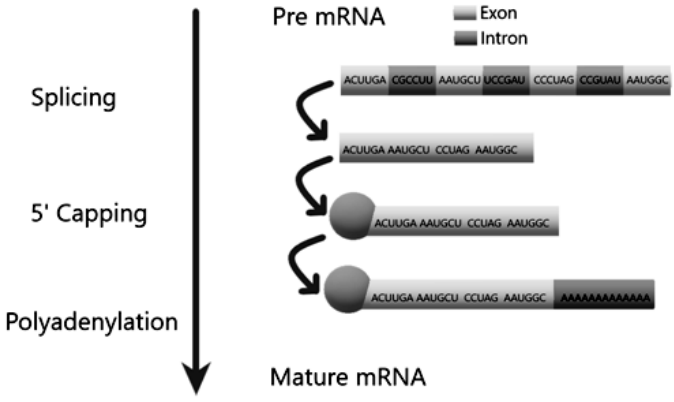


Figure 3.2 Pre mRNA processing

ii. Translation

Translation is the second part of the central dogma of molecular biology.

RNA → Protein

It is the process in which the genetic code in mRNA is read to make a protein. After mRNA leaves the nucleus, it moves to a ribosome, which consists of rRNA and proteins. The ribosome reads the sequence of codons in mRNA, and molecules of tRNA bring amino acids to the ribosome in the correct sequence.

Translation occurs in three stages: Initiation, Elongation and Termination.

a. Initiation

After transcription in the nucleus, the mRNA exits through a nuclear pore and enters the cytoplasm. At the region on the mRNA containing the methylated cap and the start codon, the small and large subunits of the ribosome bind to the mRNA. These are then joined by a tRNA which contains the anticodons matching the start codon on the mRNA. This group of molecues (mRNA, ribosome, tRNA) is called an initiation complex.

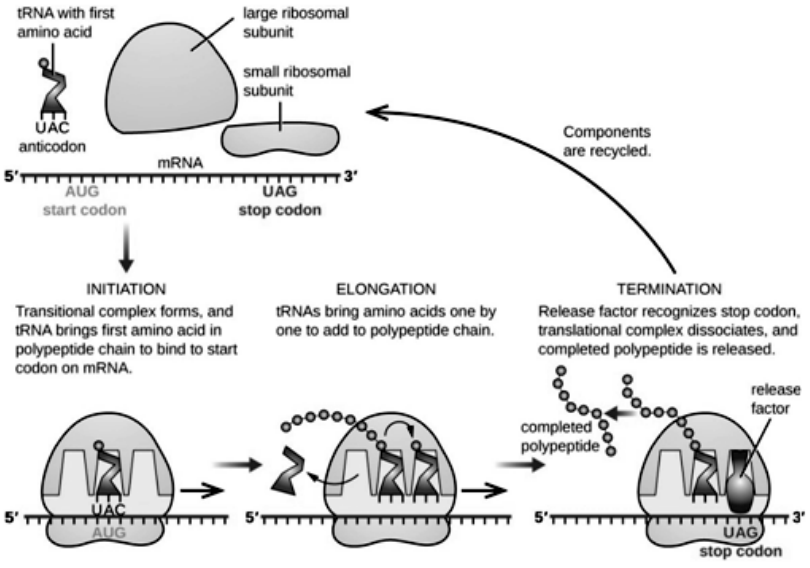


Figure 3.3 Translation takes place in three stages: Initiation, Elongation and Termination

b. Elongation

tRNA keep bringing amino acids to the growing polypeptide according to complementary base pairing between the codons on the mRNA and the anticodons

on the tRNA. As a tRNA moves into the ribosome, its amino acid is transferred to the growing polypeptide. Once this transfer is complete, the tRNA leaves the ribosome, the ribosome moves one codon length down the mRNA, and a new tRNA enters with its corresponding amino acid. This process repeats and the polypeptide grows.

c. Termination

At the end of the mRNA coding is a stop codon which will end the elongation stage. The stop codon doesn't call for a tRNA, but instead for a type of protein called a release factor, which will cause the entire complex (mRNA, ribosome, tRNA, and polypeptide) to break apart, releasing all of the components.

3.4 Amino acid Metabolism

The dietary proteins consist of 20 amino acids which exhibit L-configuration.

3.4.1 Essential amino acids (Indispensable amino acids)

Indispensable amino acids are those which cannot be synthesized in the body but must be supplied through food. These amino acids are:

- Arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine.

3.4.2 Non-essential amino acids (Dispensable amino acids)

Dispensable amino acids are those which can be synthesized at the desired rates in the body. These amino acids are:

- Alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine.

After digestion in the alimentary canal, the amino acids are absorbed through the intestinal epithelium into the blood.

3.5 Ornithine Cycle

Ammonia produced by deamination is highly toxic. It is converted into urea in liver. The formation of urea is known as urea cycle or ornithine cycle or Krebs-Henseleit cycle. The various steps of this cycle are as follows:

Step 1: Synthesis of Carbamyl phosphate - One molecule of NH3 reacts with CO2 to produce carbamyl phosphate. ATP donates phosphate group. Carbamyl phosphate synthetase catalyses the reaction in the presence of Mg^{+2} and N-acetyl glutamic acid.

$$CO2 + NH3 + 2ATP \xrightarrow[Mg+2]{\text{Carbamyl phosphate synthetase}} \text{Carbamyl phosphate} + 2ADP + Pi$$

Step 2: Synthesis of Citrulline – Carbamyl phosphate reacts with ornithine in the presence of ornithine carbamyl transferase to produce citrulline.

$$\text{Ornithine} + \text{Carbamyl phosphate} \xrightarrow{\text{Ornithine carbamyl transferase}} \text{Citrulline} + Pi$$

Step 3: Synthesis of Arginosuccinic acid – Citrulline reacts with aspartic acid (which injects one more NH3) to produce arginosuccinic acid. The reaction is catalysed by arginosuccinate synthetase in the presence of Mg^{+2}. ATP provides energy.

$$\text{Citrulline} + \text{Aspartic acid} + ATP \xrightarrow{\text{Arginosuccinate synthetase}} \text{Arginosuccinic acid} + AMP + Pi$$

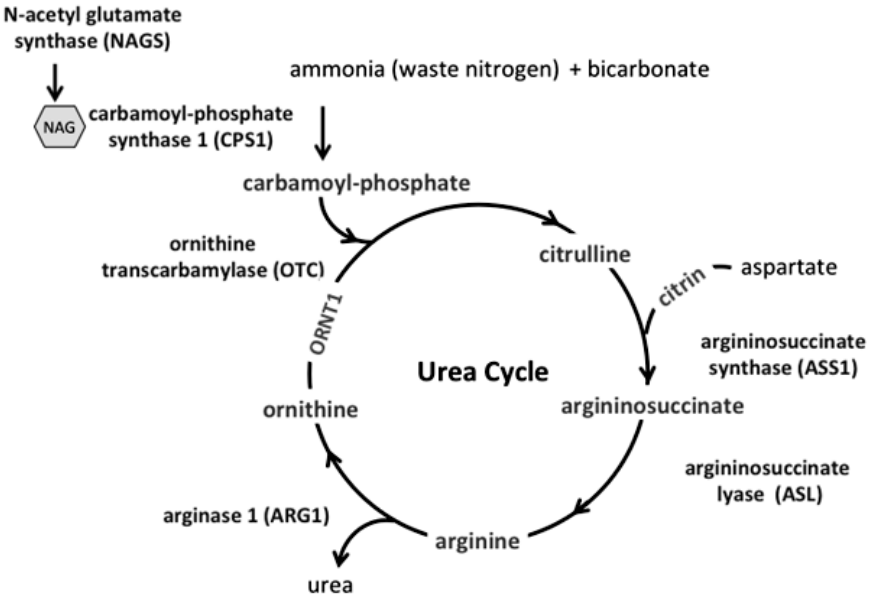


Figure 3.4 Ornithine Cycle

Step 4: Cleavage of Arginosuccinic acid: Arginosuccinase catalyses the cleavage of arginosuccinic acid into arginine and fumaric acid. The fumaric acid enters the Krebs cycle.

$$\text{Arginosuccinic acid} \xrightarrow{\text{Arginosuccinase}} \text{Arginine + Fumaric acid}$$

Step 5: Hydrolysis of Arginine: Arginine undergoes hydrolysis releasing urea and regenerating ornithine. This reaction is catalysed by the enzyme arginase.

$$\text{Arginine} + H_2O \xrightarrow{\text{Arginase}} \text{Urea + ornithine}$$

Urea is the main excretory products of ureotelic animals. It is less toxic than that of NH_3.

3.6 Catabolism of Phenylalanine and Tyrosine

Phenylalanine and tyrosine, the two aromatic amino acids have a common degradative pathway. The degradation is physiologically very important as it produces various substances of importance, namely thyroxine, adrenaline, melanin etc. The various steps of the catabolism are:

1. Phenylalanine undergoes hydroxylation to form tyrosine. This reaction is irreversible and is catalyzed by Phenylalanine hydroxylase which incorporates one oxygen atom from molecular oxygen into phenylalanine to yield tyrosine. The other oxygen atom is reduced to water. NADPH acts as a reducing agent which functions with another reductant namely tetrahydrobiopterin (a coenzyme which is a folic acid derivative).
2. Tyrosine formed from phenylalanine or taken from the diet enters into any one of the pathways mentioned below:

1. Acetoacetate and Fumarate Pathway:

This is the major pathway of degradation and most of the tyrosine and phenylalanine undergo degradation through this pathway. The various reactions in the pathway are:

i. Tyrosine is transaminated to parahydroxyphenyl pyruvic acid.

ii. Parahydroxyphenyl pyruvic acid is oxidized to homogentisic acid. Ascorbic acid is essential for this reaction which acts as a cofactor. The oxidation step is very complex which involves hydroxylation of the phenylring and decarboxylation, oxidation and migration of the side chain.

iii. The aromatic ring of homogentisic acid is then cleaved by oxidative reaction catalyzed by homogentisic acid oxidase resulting in 4-maleylacetoacetic acid. This reaction also needs ascorbic acid.

iv. 4-Maleylacetoacetic acid is isomerized to 4-fumaryl acetoacetic acid.

v. 4-Fumarylacetoacetic acid on hydrolysis by the enzyme fumarylacetoacetate hydrolase form fumaric acid (oxidized via citric acid cycle) and acetoacetate (a ketone body) hence phenylalanine and tyrosine are both glucogenic and ketogenic.

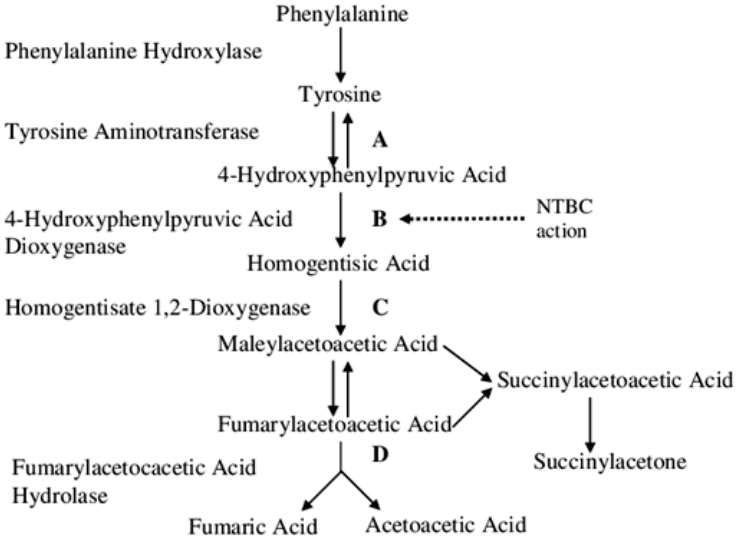


Figure 3.5 Acetoacetate and Fumarate Pathway

2. Epinephrine (adrenaline) Pathway:

i. Tyrosine is first oxidized to form 3,4-dihydroxy phenylalanine (DOPA) in the presence of tyrosine hydroxylase with tetrahydropteridine as cofactor.

ii. DOPA is then decarboxylated by a decarboxylating enzyme to dopamine (hydroxy tyramine). Pyridoxal phosphate acts as a cofactor in this reaction.

iii. Dopamine is further hydroxylated to nor-epinephrine and this reaction, catalyzed by dopamine hydroxylase needs ascorbic acid and molecular oxygen.

iv. Nor-epinephrine is converted to epinephrine by transmethylation and the active methionine (S-adenosyl methionine) is the source of methyl group.

3. Pathway to Melanin:

i. Tyrosine is oxidized to dihydroxy phenylalanine (DOPA) by tyrosinase in the presence of ascorbic acid which acts as a cofactor. This reaction occurs in specialized cells called melanocytes located in skin and eyes.

ii. DOPA is further oxidized to dopaquinone.

iii. Dopaquinone is further converted into 5,6-dihydroxy indole-2-carboxylic acid.

iv. 5,6-dihydroxy indole-2-carboxylic acid is oxidized to dihydroxy indole which then polymerises to melanin.

4. Pathway to Thyroxine:

This pathway leading to the synthesis of the hormone thyroxine occurs in the thyroid gland. The various steps of conversion are:

i. Tyrosine is iodinated at the 3rd position to form monoiodotyrosine.

ii. This is followed by the second iodination in the 5th position to form 3,5-di-iodotyrosine.

iii. Two molecules of di-iodotyrosine undergoes oxidative coupling to form one molecule of tetra-iodotyrosine, the hormone thyroxine. In this process, alanine is also eliminated.

iv. Coupling of one molecule of mono-iodotyrosine and one molecule of di-iodotyrosine results in the production of tri-iodothyronine.

Other Pathways:

i. A minor pathway in which tyrosine gets decarboxylated to tyramine which occurs in the kidney.

ii. Another minor pathway in which phenolic hydroxyl group of tyrosine conjugates with sulphate to form tyrosine-O-sulphate. This ester is a constituent of the peptide liberated in the transformation of fibrinogen to fibrin.

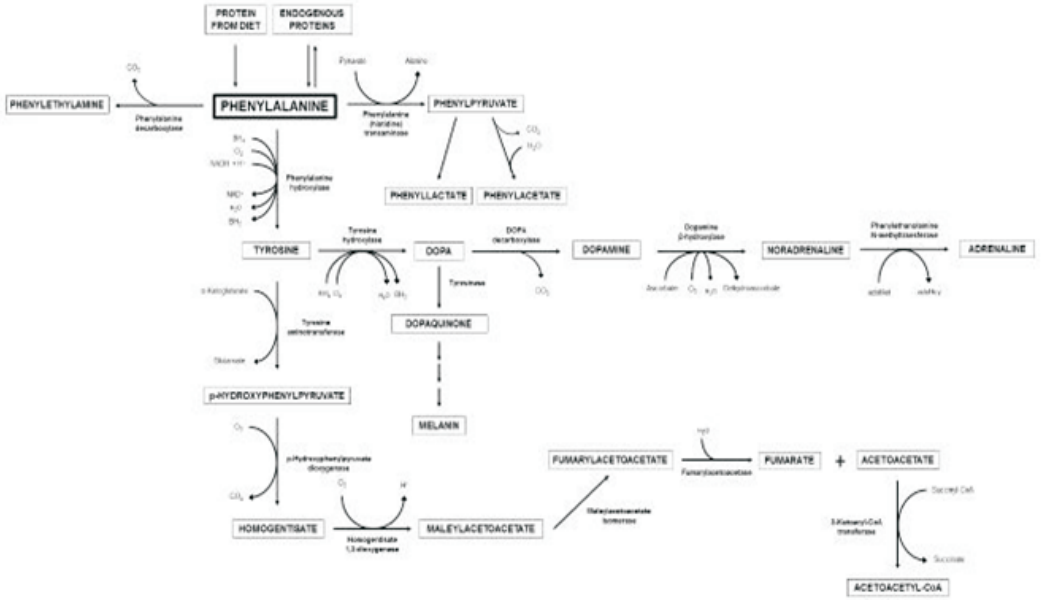


Figure 3.6 Epinephrine Pathway

3.7 Metabolism of Phenylalanine

3.7.1 Catabolism of Tryptophan:

The catabolism of tryptophan involves several pathways and it is both ketogenic and glucogenic. The various pathways are as follows:

1. Kynurenine-anthranilic acid pathway

This is the major pathway in the catabolism of tryptophan and the intermediates of this pathway are associated with some minor pathways which also result in the production of substances of physiological interest.

The various reactions of the pathway are:

i. The first step in the catabolism is catalyzed by the enzyme 2,3-dioxygenase also called tryptophan pyrrolase. This enzyme contains copper and heme groups which oxidizes tryptophan in the presence of molecular oxygen to N-formyl-L-kynurenine. In some human beings the enzyme is genetically defective, giving rise to mental retardation.

ii. Kynurenine formylase catalyzes the hydrolytic removal of the formyl group of N-formyl-kynurenine producing kynurenine.

iii. Kynurenine may take two different pathways.

   a. It may be hydroxylated to 3-hydroxy kynurenine by the enzyme kynurenine hydroxylase with molecular oxygen in the presence of NADPH.

   b. It may undergo spontaneous ring formation to form kynurenic acid. This is one of the side steps and not occurring in the main pathway.

iv. 3-hydroxy kynurenine of the major pathway may again undergo any one of the following steps.

   a. 3-hydroxy kynurenine is cleaved to alanine and 3-hydroxy anthranilic acid which is catalyzed by the enzyme kynureninase that needs Vitamin B6 (pyridoxal phosphate) as coenzyme. Alanine which is got in this step enters the citric acid cycle to be converted to glucose hence tryptophan is glucogenic.

   b. In deficiency of Vitamin B6 in mammals, large amount of kynurenine derivatives reach the extra hepatic tissues (for Eg.Kidney) where they are converted to xanthurenic acid which is found in the urine of mammals deficient in Vitamin B6.

   c. In some insects 3-hydroxy kynurenine is utilized as precursor of the pigment ommochrome.

v. 3-hydroxy anthranilic acid of the major pathway is then oxidized to 2-acroleyl-3-amino fumaric acid by the specific oxidase namely 3-hydroxy anthranilic acid oxidase.

vi. 2-acroleyl-3-amino fumaric acid is very unstable and it may take 2 different routes of catabolism.

   a. In the first route 2-acroleyl-3-amino fumaric acid is decarboxylated to form 2-amino muconic acid 6-semialdehyde.

   b. In the second route 2-acroleyl-3-amino fumaric acid is dehydrated to quinolinic acid which on decarboxylation produces nicotinic acid. This reaction is only a side step which further forms nicotinamides (NAD+ and NADP+).

vii. In the major pathway, 2-aminomuconic acid-6-semialdehyde on deamination and oxidation produces oxalocrotonic acid.

viii. Oxalocrotonic acid on reduction forms α-ketoadipic acid.

ix. α-ketoadipic acid is ultimately converted into acetoacetyl CoA and thus tryptophan is ketogenic.

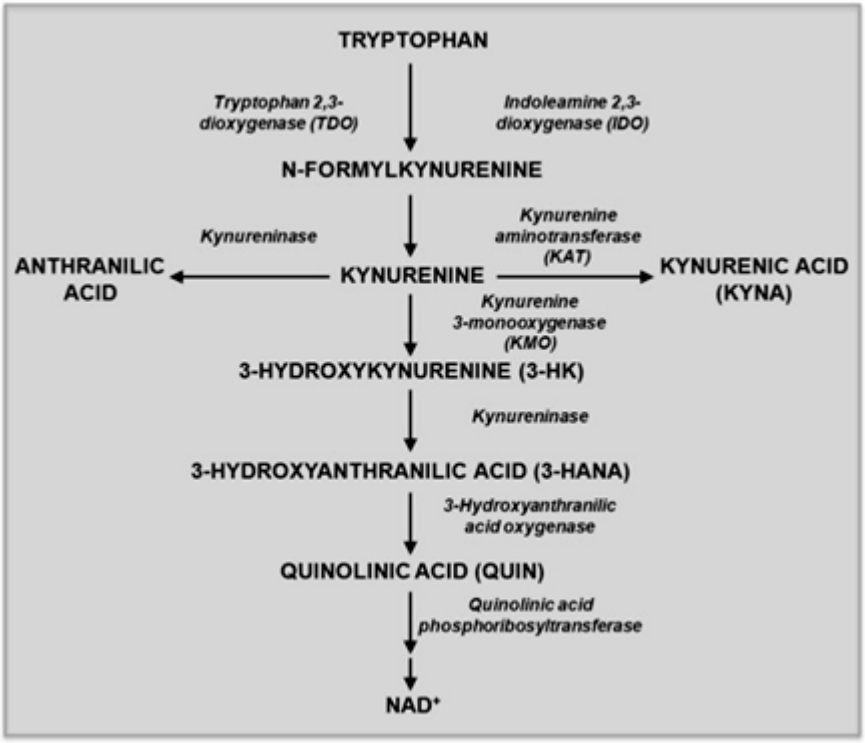


Figure 3.7 Kynurenine-anthranilic acid pathway

2. Serotonin Pathway

i. In the second pathway, namely serotonin pathway, tryptophan on dehydroxylation in the liver forms 5-hydroxy tryptophan by a hydroxylase enzyme.

ii. Next, 5-hydroxy tryptophan on decarboxylation produces 5-hydroxy tryptamine which is otherwise known as serotonin. Serotonin is a neurohormone and a vasoconstrictor in vertebrates. It also stimulates the contraction of smooth muscles. It is stored in blood platelets, gastro-intestinal tract and central nervous system. When platelets disintegrate during blood clotting, serotonin is liberated. Serotonin enters into two different minor pathways.

a. Serotonin is oxidatively deaminated to 5-hydroxy indole acetic acid (5-HIAA) by monoamine oxidase. About 5 to 10 mg of 5-HIAA is normally

excreted in the urine every 24 hours but when there is tumour of the argentaffin cells of the small intestine (malignant tumour) large amount of 5-HIAA are excreted in the urine and the amount of serotonin in the blood is also high.

b. Serotonin on acetylation with acetyl CoA and further methylation produces N-acetyl-5-methoxy tryptamine which is a hormone, otherwise known as melatonin. This is the hormone of the pineal gland and peripheral nerve of mammals. This hormone in frog has a lightening effect on the colour of the skin.

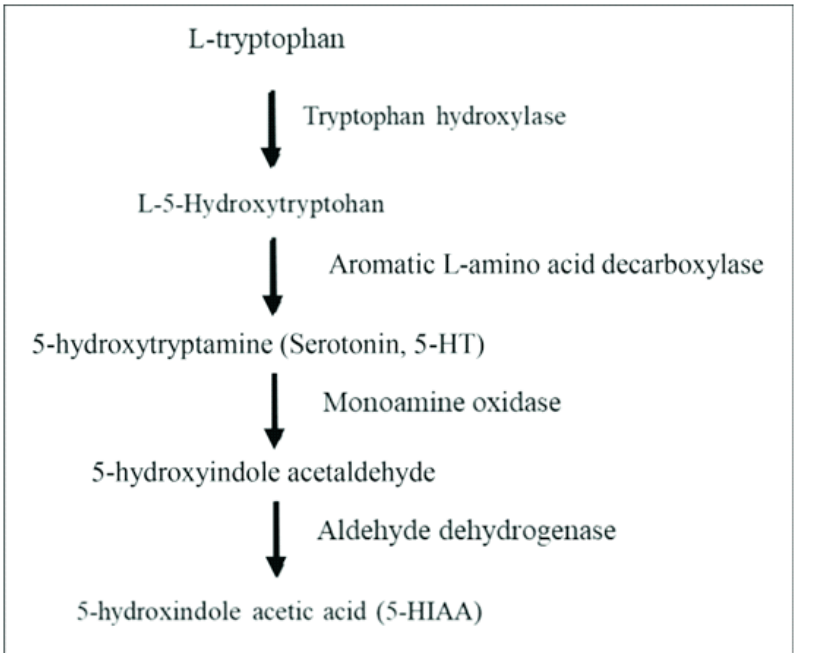


Figure 3.8 Serotonin Pathway

Hartnup Syndrome: It is an inborn error in the metabolism of tryptophan. It leads to increased excretion of tryptophan derivatives like indoleacetic acid and tryptophan itself, skin rash, mental deterioration.

Other pathways

1. Formation of Indole Acetic Acid: Under certain conditions, such as are found in the colon of human beings, tryptophan is converted to indole acetic acid and the various steps of conversion are:

a. Tryptophan is oxidatively deaminated to form indole acetic acid. In plants indole acetic acid functions as a growth hormone.

b. In humanbeings indole acetic acid is broken down by the intestinal bacteria to produce the evil smelling compounds skatole and indole which are responsible for the characteristic foul smell of the faeces and the related substance indoxyl which appears both in the faeces and urine.

2. Formation of Tryptamine: Some amount of tryptophan is converted by tryptophan carboxylase into tryptamine which is the precursor of the plant growth hormone indole acetic acid.

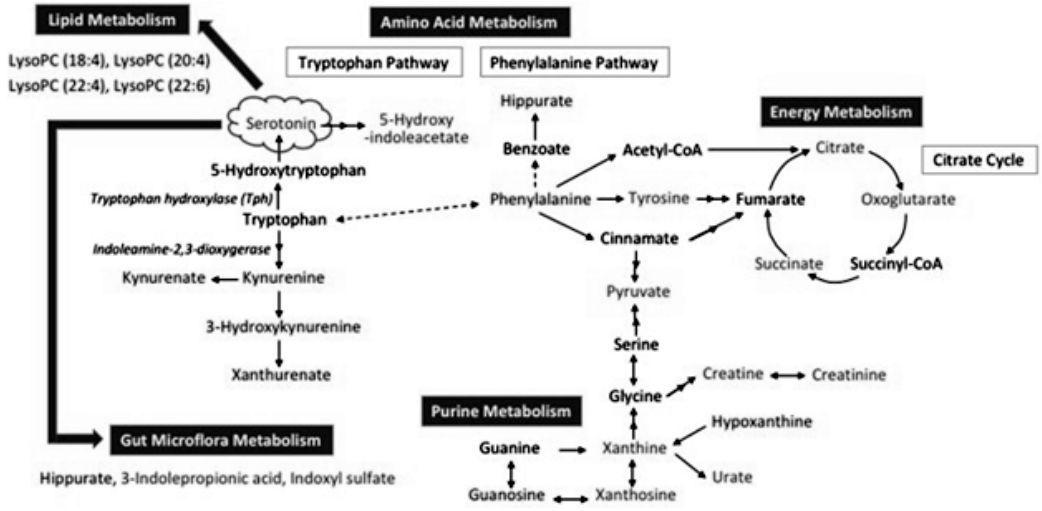


Figure 3.9 Amino Acid Metabolism

CHAPTER

4 LIPID METABOLISM

4.1 Introduction

Lipid metabolism refers to all chemical reactions concerned with lipids taking place in the cells. It includes anabolism and catabolism. Anabolism is the synthesis of lipids and catabolism is the breakdown of lipids. Lipids play a major role in the nutrition of man and other animals. The energy value of fat is extremely high when compared to carbohydrate or protein. One gram of fat releases 9.3 calories of energy; at the same time one gram of carbohydrate releases only 4.2 calories.

4.2 Catabolic Pathway

Fatty acid catabolism is the mechanism by which the body accesses energy stored as triglycerides. There are three steps in fatty acid catabolism. First the body must mobilize the lipid stores by breaking down triglycerides into free fatty acids and glycerols. Catabolism of fat (lipolysis) involves two separate pathways, glycerol pathway and fatty acids pathway.

4.2.1 Oxidation of glycerol

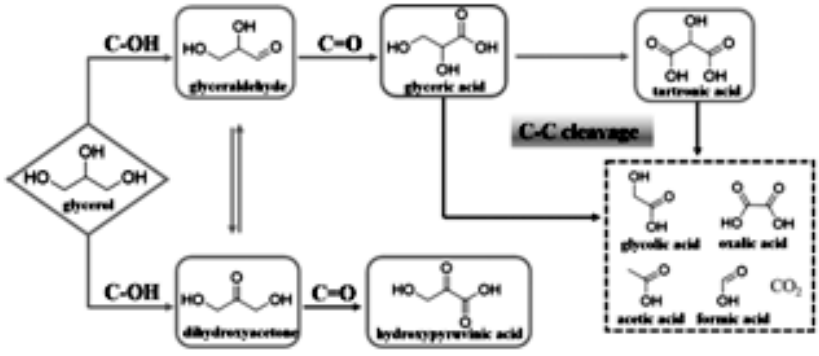


Figure 4.1 Oxidation of Glycerol

1. Glycerol with ATP converted into glycerol-3-phosphate by glycerol kinase and release one inorganic phosphate yields ADP.

2. Then, it is oxidized by NAD^+ into dihydroxyacetone-phosphate using glycerol-phosphate dehydrogenase. Theoxidized products will enter glycolysis pathway and produce energy.

4.2.2 Oxidation of fatty acids

Fatty acid catabolism through beta oxidation (the broken down process of Acyl-CoA molecules into Acetyl-CoA) occurred in mitochondria and/or in peroxisomes. Betaoxidation will produce:

1. Two-carbon acetic acid fragments, which are converted to acetyl-CoA and enter the Krebs cycle.
2. Reduced coenzymes, will enter the electron transportchain.
3. An acetyl-CoA used in the Krebs Cycle will make one ATP, 3 $NADH^+$, H^+ and 1 $FADH_2$. If a fatty acid has 18 carbon units, then 9 acetyl CoA units would be made.

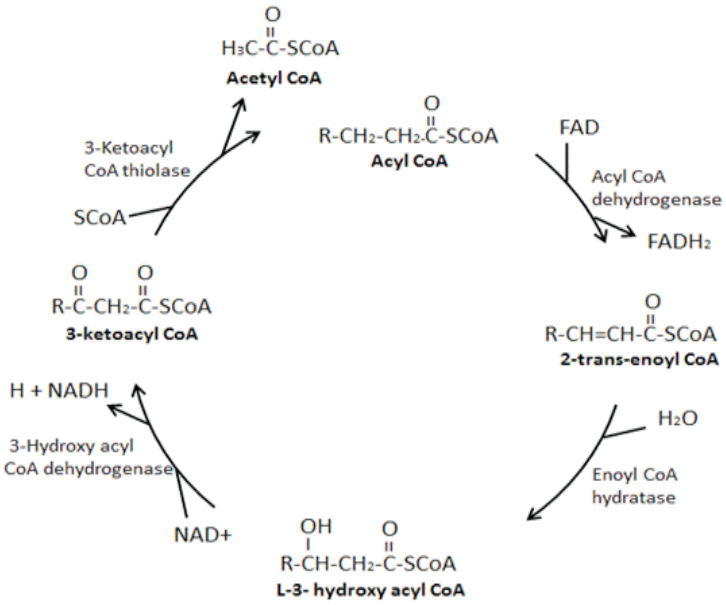


Figure 4.2 Oxidation of Fatty Acids

4.3 β - Oxidation

β – oxidation was first proposed by Knoop in 1905. In β – oxidation, the fatty acid is oxidised at the β carbon atom (second carbon atom from the carboxyl group) and is converted into fatty acid having two carbon atoms less and acetyl CoA.

This process is repeated where two carbon atoms are removed in each oxidation until a four carbon atom residue is left as butyric acid. The butyric acid is also oxidised in the β-position to form aceto-acetic acid. Both of these compounds undergo final oxidation into CO2 and water.

β – oxidation occurs in mitochondria. The acetyl CoA formed in β-oxidations of fatty acids is oxidised to CO2 and H2O with the release of energy through Krebs cycle. β-oxidation has the following steps:

1. Activation
2. Dehydrogenation I
3. Hydration
4. Dehydrogenation II
5. Thiolytic cleavage

1. Activation

The fatty acids, as such, are inert chemically. So in the first step, they are activated. The enzyme thiokinase (acyl-CoA synthetase) in the presence of ATP and CoA converts free fatty acid.

2. Dehydrogenation I

The acyl CoA is then oxidised by the removal of two hydrogen atoms, one form α-carbon and the other from the β-carbon. This reduction is brought about by the enzyme acyl CoA dehydrgenase and coenzyme flavin adenine dinucleotide (FAD). This reduction leads to the formation of an unsaturated double bond –CH=CH- and the substance is called α,β-unsaturated acyl-CoA.

3. Hydration

The α,β - unsaturated acyl-CoA undergoes a process of hydration with the addition of water under the influence of enol hydrase. The resulting compound is called beta-hydroxy acyl-CoA.

4. Dehydrogenation II

The beta-hydroxy acyl –CoA is again dehydrogenated by the removal of 2 hydrogen atoms from the beta carbon. This reaction leads to the formation of the beta-keto-acyl-CoA. It is catalyzed by beta-hydroxy acyl dehydrogenase and the coenzyme NAD functions as the hydrogen acceptor.

5. Thiolysis

The beta-keto-acyl-CoA is unstable and is cleaved into acetyl CoA and an activated fatty acid (acyl CoA), which is shorter by 2 carbon atoms than the original fatty acid. The cleavage is brought about by the addition of a new CoA to the beta carbon of the fatty acid. This reaction is catalyzed by beta-keto-acyl thiolase.

4.3.1 Energetics of Fatty acid Oxidation

The net production of energy from a single molecule of palmitic acid (a fatty acid) is 130 molecules of ATP.

4.3.2 Energetics of β Oxidation

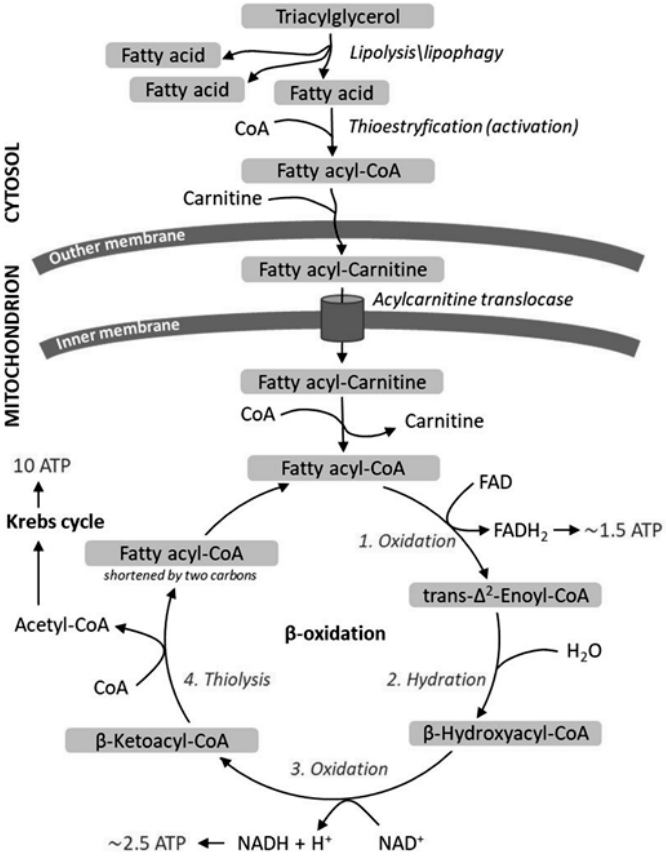


Figure 4.3 β Oxidation

The energetics of beta oxidation can be calculated by taking a typical fatty acid, palmitic acid – $C_{15}H_{31}COOH$.

Number of beta oxidation cycles - 7

Number of acetyl CoA produced - 8

Number of $FADH_2$ produced - 7

Number of $NADH_2$ produced - 7

Number of ATP produced by one acetyl CoA in Krebs cycle - 12

Number of ATP produced by 8 acetyl CoA – $8 \times 12 =$ 96

Number of ATP form each $FADH_2$ - 3

Number of ATP form 7 $FADH_2$ – $7 \times 3 =$ 21

Number of ATP form each $NADH_2$ – 2

Number of ATP form 7 $NADH_2$ – $7 \times 2 = 14$

131

ATP consumed for fatty acid activation 1

Net gain 130

So each palmitic acid can produce 130 ATP molecules.

4.4 Ketogenesis

- The process of formation of theketone bodies is known as ketogenesis.
- Acetoacetate, 3-hydroxybutryic acid and acetone are collectively referred to as ketone bodies (also called acetone bodies or ketones).
- Ketogenesis occurs only in the mitochondria of liver and the ketone bodies which are water soluble, lipid fuels are released continuously.
- Ketogenesis occurs when fatty acids undergo excessive oxidation in the liver, producing large amount of acetyl CoA. The entry of acetyl CoA into Kreb's cycle depends on the availability of oxaloacetate.
- When the amount of oxaloacetate is less, the acetyl CoA is diverted to form ketone bodies. In normal conditions, when carbohydrates are plenty and glucose is readily available to the tissues, the amount of ketone bodies in the blood is very low (1 mg/100 ml of blood) and the average excretion in the

urine in 24 hours is less than 125 mg. But, if break down of fat predominates, acetyl CoA is diverted to form ketone bodies.

- Certain chemical substances such as ammonia and phlorhizin are found to increase the formation of ketone bodies and such substances are known as ketogenic substances.
- On the other hand substances such as carbohydrates, oxaloacetic acid, thiamine pyrophosphate and α-keto acids such as pyruvic acid and α-keto glutaric acid are known as anti-ketogenic substances, as they are found to lower the formation of ketone bodies.

4.5 Ketosis

- The overall condition of increased concentration of ketone bodies in tissues and fluids is termed ketosis.
- Excretion of abnormally high amount of ketone bodies in urine is known as ketonuria and appearance of high levels of ketone bodies in the blood is known as ketonemia.
- Ketosis may occur due to many different physiological as well as pathological factors such as prolonged starvation, availability of less amount of carbohydrates or high amount of fat in the diet, severe exercise in the post absorptive state, increased metabolic demand (as in pregnancy and lactation) glycogen storage diseases, continued fever, mild pancreatic dysfunction, deficiency of insulin, diabetes mellitus and toxemia of pregnancy.
- In all the above cases there is a diminished utilization of carbohydrates and increased mobilization of fats.

4.5.1 Formation of Ketone Bodies

There are 3 steps in the formation of ketone bodies.

1. In the first step two molecules of acetyl CoA condense to form acetoacetyl CoA with the loss of one molecule of CoA and this reaction is catalyzed by 3-keto thiolase.
2. Next, acetoacetyl CoA reacts with one more molecule of acetyl CoA and H2O to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) in the presence of hydroxyl methylglutaryl synthase. In this process one molecule of CoA is also split off.

3. 3-hydroxy-3-methylglutaryl CoA is then cleaved to acetyl CoA and acetoacetate in the presence of hydroxyl methylglutaryl CoA lyase. Acetoacetate that is formed, spontaneously decarboxylates to form acetone. The odour of acetone may be detected in the breadth of a person who has a high level of acetoacetate in the blood. In the liver acetoacetate may also be reduced to 3-hydroxybutyric acid in the presence of NADH and this reaction is catalyzed by the enzyme 3-hydroxybutyric acid dehydrogenase.
4. Acetoacetate and 3-hydroxybutyric acid are normal fuels of respiration and quantitatively important as sources of energy. Tissues such as heart muscle and renal cortex use acetoacetate in preference to glucose whereas in well nourished individuals, the brain tissue use glucose as the major fuel. However, the brain adapts to the utilization of acetoacetate during starvation and diabetes.

4.6 Ketolysis

- The oxidation of ketone bodies to CO2 and water is known as ketolysis. Acetoacetate and 3-hydroxybutyric acid were carried to extrahepatic tissues such as kidney and muscle where they are converted to acetoacetyl CoA.
- The acetoacetyl CoA is then split by thiolase to acetyl CoA which is then oxidized by way of citric acid cycle to CO_2 and water with the liberation of energy.
- Acetoacetate and 3-hydroxybutyric acid are readily oxidized by extra-hepatic tissues. Whereas acetone is oxidized with difficulty and it is also utilized very slowly.
- Ketone bodies are not toxic if they are properly metabolized by the extra hepatic tissues.
- In this property of removing sodium ions along with them in the process of excretion results in acidosis which is accompanied by excretion of large amounts of water that is necessary to carry the ketone bodies.
- As a result the person becomes dehydrated and passes into a coma stage. In case of severe acidosis, death may result.

4.7 Anabolic Pathway

Lipid anabolism (lipogenesis) is synthesis oflipids on liver cells from amino acids which areconverted to acetyl-CoA and from glucose intoglyceraldehyde 3-phosphate. Both of acetyl-CoAand glyceraldehyde 3-phosphate converted intotriglycerides.

4.8 Biosynthesis of Fatty Acids

- Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the beta-oxidation process, but with a couple of key differences.
- The first of these occur in preparing substrates for the reactions that grow the fatty acid. Transport of acetyl-CoA from the mitochondria occurs when it begins to build up.
- Two molecules can play roles in moving it to the cytoplasm – citrate and acetylcarnitine. Joining of oxaloacetate with acetyl-CoA in the mitochondrion creates citrate which moves across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate.
- Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.
- Starting with two acetyl-CoA, one is converted to malonyl-CoA by carboxylation catalyzed by the enzyme acetyl-CoA carboxylase (ACC), the only regulatory enzyme of fatty acid synthesis.

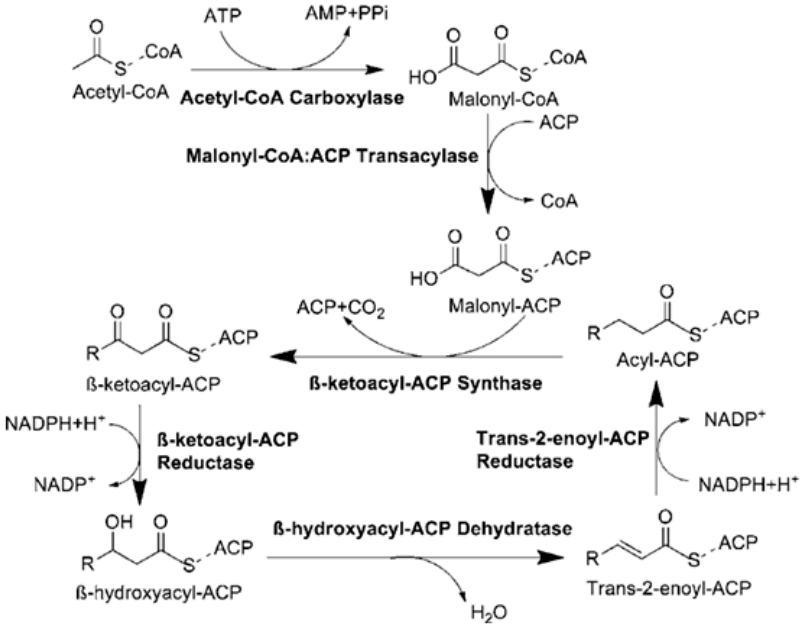


Figure 4.4 Biosynthesis of Fatty Acids

- Both molecules have their CoA portions replaced by a carrier protein known as ACP (acyl-carrier protein) to form acetyl-ACP and malonyl-ACP. Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with malonyl-ACP splits out the carboxyl that was added and creates the intermediate.
- From this point forward, the chemical reactions resemble those of beta oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH.
- In contrast to the hydroxylated intermediate of beta oxidation, the beta intermediate here is in the D-configuration. Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate to produce a trans doubled bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate. T
- This process cycles with the addition of another malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-CoA). At this point, the cytoplasmic synthesis ceases.

4.8.1 Enzymes of Fatty Acid Synthesis

- Acetyl-CoA carboxylase - catalyzes synthesis of malonyl-CoA, is the only regulated enzyme in fatty acid synthesis. Its regulation involves both allosteric control and covalent modification. The enzyme is known to be phosphorylated by both AMP Kinase and Protein Kinase A.
- Dephosphorylation is stimulated by phosphatases activated by insulin binding. Dephosphorylation activates the enzyme and favors its assembly into a long polymer, while phosphorylation reverses the process. Citrate acts as an allosteric activator and may also favor polymerization. Palmitoyl-CoA allosterically inactivates it.
- In animals, six different catalytic activities necessary for the remaining catalytic actions to fully make palmitoyl-CoA are contained in a single complex called Fatty Acid Synthase. These include transacylases for swapping CoA with ACP on acetyl-CoA and malonyl-CoA; a synthase to catalyze addition of the twocarbon unit from the three carbon malonyl-ACP in the first step of the elongation process; a reductase to reduce the ketone; a dehydrase to catalyze removal of water, and a reductase to reduce the trans double bond. In bacteria, these activities are found on separate enzymes and are not part of a complex.

4.8.2 Elongation of Fatty Acids

- Elongation to make fatty acids longer than 16 carbons occurs in the endoplasmic reticulum and is catalyzed by enzymes described as elongases.
- Mitochondria also can elongate fatty acids, but their starting materials are generally shorter than 16 carbons long. The mechanisms in both environments are similar to those in the cytoplasm (a malonyl group is used to add two carbons, for example), but CoA is attached to the intermediates, not ACP. Further, whereas cytoplasmic synthesis employs the fatty acid synthase complex, the enzymes in these organelles are separable and not part of a complex.

4.8.3 Desaturation of Fatty Acids

- Fatty acids are synthesized in the saturated form and desaturation occurs later. Enzymes called desaturases catalyze the formation of cis double bonds in mature fatty acids. These enzymes are found in the endoplasmic reticulum.
- Animals are limited in the desaturated fatty acids they can make, due to an inability to catalyze reactions beyond carbons 9 and 10. Thus, humans can make oleic acid, but cannot synthesis linoleic acid or linolenic acid. Consequently, these two must be provided in the diet and are referred to as essential fatty acids.

4.9 Synthesis of Triglycerides

- Glycerol accepts fatty acids from acyl-CoAs to synthesize glycerol lipids. Glycerol phosphate comes from glycolysis—specifically from the reduction of dihydroxyacetone phosphate using NADH as a cofactor.
- Then the glycerol phosphate accepts two fatty acids from fatty acyl-CoA. The fatty acyl-CoA is formed by the expenditure of two high-energy phosphate bonds from ATP.
- Fatty acyl-CoA is the donor of the fatty acyl group to the two non-phosphorylated positions of glycerol phosphate to make a phosphatidic acid.
- The third fatty acid can be added after the removal of the phosphate of the phosphatidic acid. This scheme results in a triacylglycerol, although other phosphatidic acids can be used as precursors to various membrane lipids.

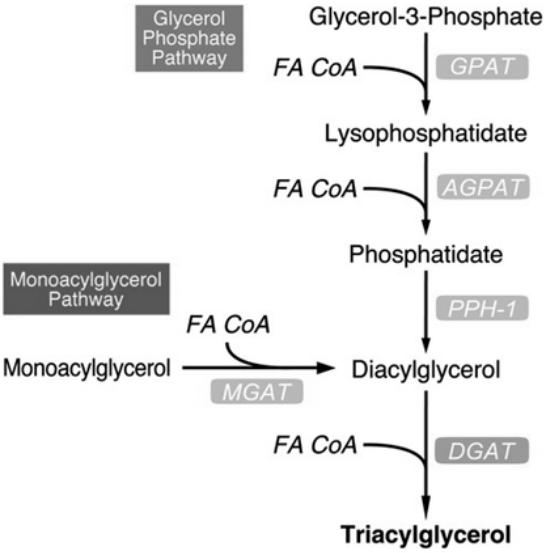


Figure 4.5 Synthesis of Triglycerides

4.10 Disorders of Fat Metabolism

4.10.1 Hypercholestrolemia

Hypercholesterolemia is an autosomal dominant disease is caused by the deficiency of the LDL receptor on the surface of cells in the liver and other organs. As a result, cholesterol is not moved into the cells. Under normal conditions, when enough cholesterol is present in the cell, feedback mechanisms signal enzymes to cease cholesterol synthesis. In familial hypercholesterolemia, these enzymes are relieved of feedback inhibition, thus inducing the production of still more cholesterol. The disease is characterized by early coronary vascular disease, strokes, and fatty deposits on the tendons. Blood cholesterol levels are very high from birth, and LDL cholesterol is also elevated. Treatment is by a low-cholesterol diet and drugs that inhibit cholesterol synthesis or increase its excretion in the gastrointestinal tract.

4.10.2 Hyperlipoproteinemia

Hyperlipoproteinemia is a common disorder. It results from an inability to break down lipids or fats in your body, specifically cholesterol and triglycerides. There are several types of hyperlipoproteinemia. The type depends on the concentration of lipids and which are affected. High levels of cholesterol or triglycerides are serious because they're associated with heart problems.Hyperlipoproteinemia can be a primary or secondary condition.

- Primary hyperlipoproteinemia is often genetic. It's a result of a defect or mutation in lipoproteins. These changes result in problems with accumulation of lipids in your body.
- Secondary hyperlipoproteinemia is the result of other health conditions that lead to high levels of lipids in your body.

These include:

- diabetes
- hypothyroidism
- pancreatitis
- use of certain drugs, such as contraceptives and steroids
- certain lifestyle choices

4.10.3 Atherosclerosis

Atherosclerosis is a hardening and narrowing of your arteries caused by cholesterol plaques lining the artery over time. It can put blood flow at risk as your arteries become blocked.It's the usual cause of heart attacks, strokes, and peripheral vascular disease together are known ascardiovascular disease.

Arteries are blood vessels that carry blood from your heart throughout your body. They're lined by a thin layer of cells called the endothelium. It keeps the inside of your arteries in shape and smooth, which keeps blood flowing.

Atherosclerosis begins with damage to the endothelium. Common causes include:

- High cholesterol
- High blood pressure
- Inflammation, like from arthritis or lupus
- Obesity or diabetes
- Smoking

CHAPTER

5 Metabolism of Nucleic Acids

5.1 Introduction

- Nucleotides consist of a nitrogenous base, a pentose and a phosphate.
- The pentose sugar is D-ribose in ribonucleotides of RNA while in deoxyribonucleotides of DNA, the sugar is 2- deoxy D-ribose.
- Nucleotides participate in almost all the biochemical processes, either directly or indirectly.
- They are the structural components of nuclei acid, coenzymes and are involved in the regulation of several metabolic reactions.

5.2 Biosynthesis of Purine Ribonucleotides

Many compounds contribute to the purine ring of the nucleotides.

1. N_1 of purine is derived from amino group of aspartate
2. C_2 and C_8 arise from formate of N10- formyl THF
3. N_3 and N_9 are obtained from amide group of glutamine
4. C_4, C_5 and N- are contributed by glycine.
5. C_6 directly comes from CO_2.

It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. The purines are built upon a pre-existing ribose 5- phosphate. Liver is the major site for purine nucleotide synthesis erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines.

The pathway for the synthesis of inosine monophosphate, the parent purine nucleotide. The reaction are briefly described in the next column.

1. Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate.

2. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides. This reaction is the committed step in purine nucleotide biosynthesis.

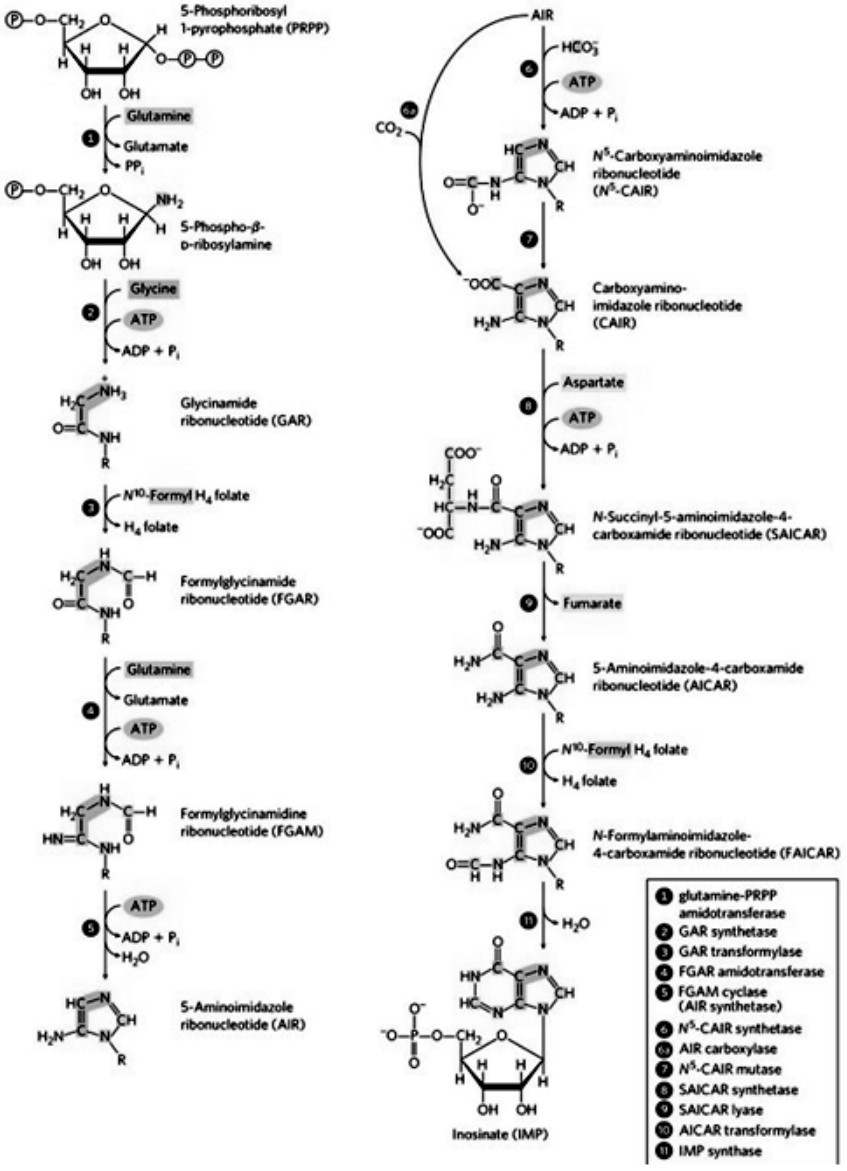


Figure 5.1 Biosynthesis of Purine Bionucleotides

3. Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide.
4. N^{10} formyl tetrahydrofolate donates the formyl group and the product formed is formyl glycinamide ribosyl 5-phosphate.
5. Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate.
6. The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5 amino imidazole ribosyl 5-phosphate.
7. Incorporation of co2 occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. This reaction does not require the vitamin biotin and or ATP which is the case with most of the carboxylation reactions.
8. Aspirate condenses with the product in reaction 7 to form aminoimidaze 4-succinyl carboxamide ribosyl 5-phosphate
9. Adenosuccinate lyase cleaves off fumarate and only the amino group of asparatae is retained to yield aminoimidazole 4- carboxamide ribosyl 5-phosphate,
10. N^{10} formyl tetrahydrofolate donates a one carbon moiety to produce formamino imidazole 4 carboxamide ribosyl 5-phosphate with this reaction all the carbon and nitrogen atoms of purine rings are contributed by the respective sources.

5.2.1 Inhibitors of Purine Synthesis

Folic acid (THF) is essential for the synthesis of purine nucleotide (reaction 4 and 10). Sulfonamides are the structural analogs of para-aminnobenzoic acid (PABA). These sulfa drugs can be used to inhibit the synthesis of folic acid by microorganisms. This indirectly reduces the synthesis of purines and therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on humans, since folic acid is not synthesized and is supplied through diet.

The structural analogs of folic acid (e.g. methotrexate) are widely used to control cancer. They inhibit the synthesis of purine nucleotides (reaction 4 and 10) and thus, nucleic acid. Both these reactions are concerned with the transfer of one carbon moiety (formyl group). These inhibitors also effect the proliferation of normally growing cells. This causes many side effects including anemia, baldness, scaly skin etc.

5.2.2 Synthesis of AMP and GMP from IMP

Inosine monophosphate is the immediate precursor for the formation of AMP and GMP. Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage forms AMP.

For the synthesis of GMP, IMP undergoes NAD dependent dehydrogenation to form xanthosine monophosphate (XMP). Glutamine then transfers amide nitrogen to XMP to produce GMP.

6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts in the enzymes adenylsuccinase (of AMP pathway) and IMP dehydrogenase (of GMP pathway).

5.2.3 Formation of Purine Nucleoside Diphosphates and Triphosphates

The nucleoside monophosphate (AMP and GMP) have to be converted to the corresponding di and triphosphates to participate in most of the metabolic reactions. This is achieved by the transfer of phosphate group from ATP, catalysed by nucleoside monophosphate (NMP) kinases and nucleoside diphosphate (NDP) kinases.

5.3 Salvage Pathway For Purines

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acid (particularly RNA) and also obtained from the dietary sources. The purines can be directly converted to the corresponding nucleotides and this process is known as salvage pathway.

Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine- guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine respectively to GMP and IMP. Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway.

The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where de novo (a new) synthesis of purine nucleotides is not operative.

A defect in enzyme HGPRT causes Lesch Nyhan syndrome.

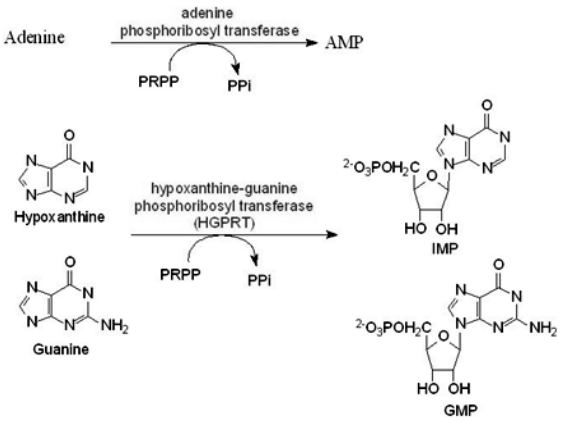


Figure 5.2 Purine Salvage Pathway

5.4 Regulation of Purine Nucleotide Biosynthesis

The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of PGPR regulates purine synthesis to a large extent. This, in turn is dependent on the availability of ribose 5-phosphate and the enzyme PROP synthetase.

PRPP glutamyl amidotransferase is controlled by a feedback mechanism by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements their synthesis is turned off at the amidotransferase reaction.

Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.

5.4.1 Conversion of Ribonucleotides to Deoxyribonucleotides

The synthesis of purine and pyrimidine deoxyribonucleotides occurs from ribo nucleotides by a reduction at the C2 of ribose moiety. This reaction is catalysed by a multisubunit enzyme ribonucleotides reductase.

5.4.2 Supply of reducing equivalents

The enzyme ribonucleotides reductase itself provides the hydrogen atoms needed for reduction from its sulfhydryl groups. The reducing equivalents, in turn are supplied by thioreedoxin a monomeric protein with two cysteine residues.

NADPH-dependent thioredoxin reductase converts the oxidized thioredoxin to reduced form which can be recycled again and again. Thioredoxin thus serves as a protein cofactor in an enzymatic reaction.

5.4.3 Regulation of deoxyribonucleotides synthesis

Deoxyribbonucleotides are mostly requires for the synthesis of DNA. The activity of the enzyme ribonucleotides reductase maintains the adequate supply of deoxyribonucleotides.

Ribonucleotides reductase is a complex enzyme with multiple sies (active sites and allosteric sites) that control the formation of deoxyribonucleotides.

5.5 Degradation of Purine Metabolism

The end product of purine metabolism in humans is uric acid. The sequence of reactions in purine nucleotide degradation is given in

1. The nucleotides monophosphates (AMP, IMPand GMP)are converted to their respective nucleoside forms (adenosine, inosine and guanosine) by the action of nucleotidase .
2. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosinerespectively.
3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by purine nucleoside phosphorylase. Adenosine is not degraded by this enzyme, hence it has to be converted to inosine.
4. Guanosine undergoes deamination by guanase to form xanthine.
5. Xanthine oxidase is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.

Uric acid is the final excretory product of purine metabolism in humans. Uric acid can serve as an important antioxidant by getting itself converted to allantoin it is believed that the antioxidant role of ascorbic acid in primates is replaced by uric acid, since these animals have lost the ability to synthesize ascorbic acid.

Most animals, however, oxidize uric acid by the enzyme uricase to allantoin, where the purine ring is cleaved. Allantoin is then converted to allantoic acid ad excreted in

some fishes. Further degradation of allantoic acid may occur to produce urea and, later to ammonia (in marine invertebrates).

5.6 Disorders of Purine Metabolism

5.6.1 Hyperuricemia and gout

- Uric acid is the end product of purine metabolism in humans. The normal concentration of uric acid in the serum of adults is in the range of 3-7 mg/dl. In women, it is slightly lower (by about 1 mg) than in men. The daily excretion of uric acid is about 500-700 mg.
- Hyperuricemia refers to an elevation in the serum uric acid concentration. This is sometimes associated with increased uric acid excretion (uricosuria).
- Gout is a metabolic disease associated with overproduction of uric acid. At the physiological pH, uric acid is found in a more soluble form as sodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as tophi. This causes inflammation in the joints resulting in a painful gouty arthritis. Sodium urate and/or uric acid may also precipitate in kidneys and ureters that results in renal damage and stone formation.
- Historically, gout was found to be often associated with high living, over-eating and alcohol consumption. In the previous centuries, alcohol was contaminated with lead during its manufacture and storage. Lead poisoning leads to kidney damage and decreased uric acid excretion causing gout.
- The prevalence of gout is about 3 per 1,000 persons, mostly affecting males. Post menopausal women, however, are as susceptible as men for this disease. Gout is of two types primary and secondary.

1. Primary gout

It is an inborn error of metabolism due to overproduction of uric acid. This is mostly related to increased synthesis of purine nucleotides. The following are the important metabolic defects associated with primary gout.

- PRPP synthetase: In normal circumstances, PRPP synthesis is under feedback control by purine nucleotides (ADP and GDP). However, variant forms of PRPP synthetase –which are not subjected to feedback regulation –have been detected. This leads to the increased production of purines.

- PRPP glutamylamidotransferase: The lack of feedback control of this enzyme by purine nucleotides also leads to their elevated synthesis.
- HGPRT deficiency: This is an enzyme of purine salvage pathway, and its defect causes Lesch-Nyhan syndrome. This disorder is associated with increased synthesis of purine nucleotides by a two-fold mechanism. Firstly, decreased utilization of purines by salvage pathway, resulting in the accumulation and diversion of PRPP for purine nucleotides. Secondly, the defect in salvage pathway leads to decreased levels of IMP and GMP causing impairment in the tightly controlled feedback regulation of their production.
- Glucose 6-phosphate deficiency: In type 1 glycogen storage disease, glucose 6-phosphate cannot be converted to glucose 6-phosphatae. This leads to the increased utilization of glucose 6-phosphate by hexose monophosphate shunt (HMP shunt), resulting in elevated levels of ribose 5-phosphate and PRPP and, ultimately, purine overproduction. Von Gierke's disease is also associated with increased activity of glycolysis. Due to this, lactic acid accumulates in the body which interferes with the uric acid excretion through renal tubules.
- Elevation of glutathiosine reductase: Increased glutathiosine reductase generates more NADP+ which is utilized by HMP shunt. This causes increased ribose 5- phosphate and PRPP synthesis.

  Among the five enzymes described, the first three are directly involved in purine synthesis. The remaining two directly regulate purine production. This is a good example to show how an abnormality in one metabolic pathway influences the other.

2. Secondary gout

Secondary hyperuricemia is due to various diseasecausing increased synthesis or decreased excretion of uric acid. Increased degradation of nucleic acid is observed in various cancers (leukemias, polycythemia, lymphomas, etc).

The disorders associated with impairment in renal function cause accumulation of uric acid which may lead to gout.

5.6.2 Uric acid pool in gout

By administration of uric acid isotope (N15), the miscible uric acid pool can be calculated. It is around 1,200 mg in normal subjects. Uric acid pool is tremendously increased to 3,000 mg. or even more, in patients suffering from gout.

Treatment of gout

The drug of choice for the treatment of primary gout is allopurinol. This is a structural analog of hypoxanthine that competitively inhibits the enzyme xanthine oxidase. Further, allopurinol is oxidized to alloxanthine by xanthine oxidase. Alloxanthine, in turn, is a more effective inhibitor of xanthine oxidase. This type of inhibition is referred to as suicide inhibition.

Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid , hence easily excreted.

Besides the drug therapy, restriction in dietary intake of purines and alcohol is advised. Consumption of plenty of water will also be useful.

The anti-inflammatory drug colchicine is used for the treatment of gouty arthritis. Other anti-inflammatory drugs such as phenylbutazone, indomethacin, oxyphenbutazone, corticosteroids-are also useful.

5.6.3 Pseudogout

The clinical manifestations of pseudogout are similar to gout. But this disorder is caused by the deposition of calcium pyrophosphate crystals in joints. Further, serum uric acid concentration is normal in pseudogout.

5.6.4 Lesch –Nyhan syndrome

This disorder is due to the deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme of purine salvage pathway .it was first described in 1964 by Michael Lesch and William L.Nyhan .

Lesh- Nyhan syndrome is a sex-linked metabolic disorder since the structural gene for HGPRT is located on the X-chromosome. It affects only the males and is characterized by excessive uric acid production , and neurological abnormalities such as mental retardation, aggressive behaviour, learning disability etc. the patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-multilation.

The overproduction of uric acid in Lesch-Nyhan syndrome is explained. HGPRT deficiency results in the accumulation of PRPP and decrease in GMP and IMP , ultimately leading to increased synthesis and degradation of purines (more details given under primary gout).

The biochemical basis for the neurological symptoms observed in Lesch – Nyhan syndrome is not clearly understood. This may be related to the dependence of brain on the salvage pathway for de novo synthesis of purine nucleotides. Uric acid is not toxic to the brain, since patients with severe hyperuricemia do not exhibit any neurological symptoms. Further, allopurinol treatment that helps to decrease uric acid production, has no affect on the neurological manifestations in these patients.

5.6.5 Immunodeficiency diseases associated with purine metabolism

Two different immunodeficiency disorders associated with the degradation of purine nucleosides are identified. The enzyme defects are adenosine deaminase and purine nucleoside phosphorylase, involved in uric acid synthesis.

The deficiency of adenosine deaminase (ADA)causes severe combined immunodeficiency (SCID)involving T-cell and usually B-cell dysfunction. It is explained that ADA deficiency results in the accumulation of dATP which is an inhibitor of ribonucleotide reductase and, therefore, DNA synthesis and cell replication.

The deficiency of purine nucleotide phosphorylase is associated with impairment of T-cell function but has no effect on B-cell function uric acid synthesis is decreased and the tissue levels of purine nucleosides and nucleotides are higher. It is believed that dGTP inhibits the development of normal T-cell.

5.6.6 Hypouricemia

Decreased uric acid levels in the serum (2mg/dl) represent hypouricemia. This is mostly associated with a rare genetic defect in the enzyme xanthine oxidase. It leads to the increased excretion of xanthine and hypoxanthine. Xanthinuria frequently causes the formation of xanthine stones in the urinary tract.

5.7 Biosynthesis of Pyrimidine Ribonucleotides

5.7.1 Introduction

The synthesis of pyrimidines is a much simpler process compared to that of purines. Aspirate, glutamine and CO_2 contribute to atoms in the formation of pyrimidines ring. Pyrimidines ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate. The pathway of pyrimidines synthesis are:

- Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase 2(CPS 2).

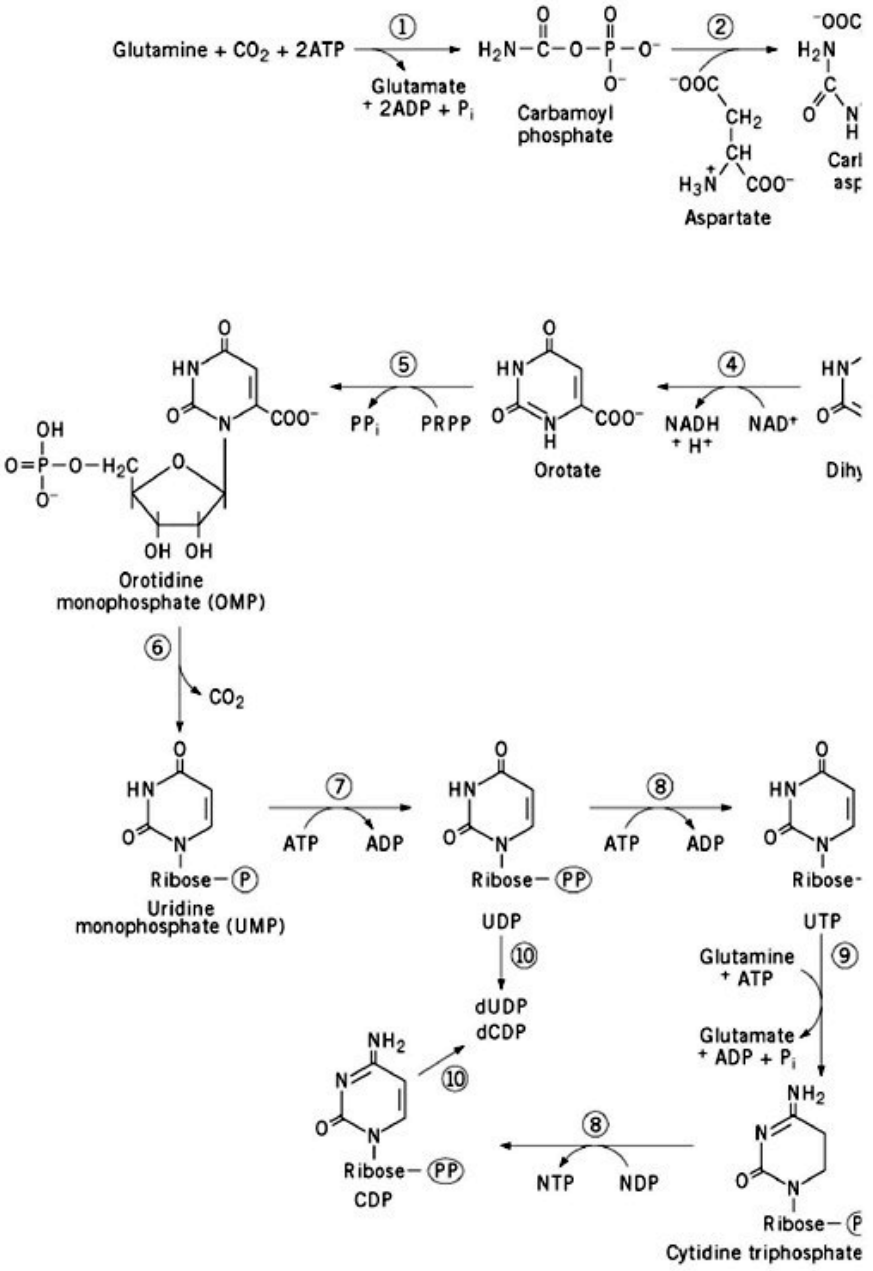


Figure 5.3 Biosynthesis of Pyrimidine Ribonucleotides

- CPS 2 is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase 1 (CPS 1) is a mitochondrial enzyme which synthesizes

carbamoyl phosphate from ammonia and CO_2 and, in turn urea. Prokaryotes have only one carbamoyl phosphate synthetase which is responsible for the biosynthesis of arginine and pyrimidines.

- Carbamoyl phosphate condenses with aspirate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidines ring with a loss of H_2O.
- The three enzymes –CPS 2, aspartate transcarbamoylase and dihydroorotase are the are the domins of the same protein. This is a good example of a multifunctional enzyme.
- The next step in pyrimidines synthesis is an NAD^+ dependent dehydrogenation, leading to the formation of orotate.
- Ribose 5-phosphate is now added to orotate to produce orotidine monophosphate(OMP). This reaction is catalysed by orotate phosphoribosyltransferase, an enzyme comparable with HGPRT in its function. OMP undergoes decarboxylation to uridine mono-phosphate (UMP).
- Orotate phosphoribosyltransferaseand OMP decarboxylase are domains of a single protein. A defect in this bifunctional enzyme causes orotic aciduria.
- By an ATP dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.
- Ribonucleotide reductase converts UDP to dUDP by a thioredoxin dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N5, N10 methylene tetrahydrofolate to produce deoxythymidines monophosphate (dTMP).
- UDP undergoes an ATP dependent kinase reaction to produce UDP. Cytidine triphosphate (CTP) is synthesized from UTP by amination CTP synthetase is the enzyme and glutamine provides the nitrogen.

5.7.2 Regulation of pyrimidine synthesis

In bacteria, aspartate transcarbamoylase (ATCase) catalyses a committed step in pyrimidine biosynthesis. ATCase is a good example of an enzyme controlled by feedback mechanism by the end product CTP. In certain bacteria, UTP also inhibits ATCase. ATP, however, stimulates ATCase activity.

Cabamoyl phosphate synthetase2 (CPS2) is the regulatory enzyme of pyrimidine synthesis in animals. It is activated by PRPP and ATP and inhibited by UDP and UTP. OMP decarboxylase, inhibited by UMP and CMP, also controls pyrimidine formation.

5.7.3 Degradation of Pyrimidine Nucleotides

The pyrimidine nucleotides undergo similar reactions (dephosphorylation, deamination and cleavage of gycosidic bond) like that of purine nucleotides to liberate the nitrogenous bases- cytosine, uracil and thymine. The bases are then degraded to highly soluble products β-alanine and β-aminoisobutyrate. These are the amino acids which undergo transamination and other reaction to finally produce acetyl CoA and succinyl CoA.

5.7.4 Salvage Pathway

The pyrimidines (like purines) ca also serve as precursors in the salvage pathway to be converted to the respective nucleotides. This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilize PRPP as the source of ribose 5-phosphate.

5.7.5 Disorder of Pyrimidine Metabolism

Orotic aciduria: This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes orotate phosphoribosyl transferase and OMP decarboxylase of pyrimidine synthesis. Both these enzyme activities are present on a single protein as domains (bifunctional enzyme).

Feeding diet rich in uridine and\or cytidine is an effective treatment for orotic aciduria. These compounds provide (through phosphorylation) pyrimidine nucleotides required for DNA and RNA synthesis. Besides this, UTP inhibits carbomyl phosphate synthetase II and blocks synthesis of orotic acid.

Reye's syndrome: It is considered as secondary orotic aciduria. It is believed that a defect in ornithine transcarbomoylase (of urea cycle) causes the accumulation of carbamoyl phosphate. This is then diverted for the increased synthesis and excretion of orotic acid.

CHAPTER

6

HORMONES

Hormones are the chemical messengers run through the blood stream to various organs in our body. They are very essential for various activities in our body. Exocrine glands have ducts to carry their secretions and endocrine glands have no ducts to carry their secretions. Endocrine glands are the ductless glands. Plants have no specialized glands for the secretion of plant hormones.

6.1 Hormones

The chemical substance or messenger is produced from one part of body by endocrine gland entered into the circulation. This was carried to distal target or cell to modify their structure and function is called hormone. Hormones may be steroids, proteins, peptides or amino acid derivatives. Steroid hormones (testosterones) are secreted by testes, adrenal cortex, placenta and ovaries. Proteinaceous hormones are secreted by pituatory gland and pancreas. Peptide hormones are secreted by pituatory gland, thyroid gland and parathyroid gland. Aminoacid derivatives are secreted by the thyroid gland and adrenal medulla. Some important activities of hormones in our body are

- Thyroid stimulating hormone controls the secretion of the hormones released by the thyroid.
- Adrenaline mobilizes glucose, increases heart rate and blood flow to skeletal muscles.
- Insulin controls blood glucose by lowering blood glucose levels.
- Glucagon increases blood glucose.
- Testosterone helps for the development of male sex organs and for change in voice.
- Oestrogen helps for the development of female sex organs and regulates the menstrual cycle.
- The main hormone secreting glands are intenstinal mucosa, pancreas, adrenals, thyroids, parathyroid, pituitary, ovaries and testes.

- Cranial endocrine glands are lying in the head. Example: Pineal and pituitary glands.
- Pharyngeal endocrine glands are lying on the neighborhood of the pharynx. Example: Thyroid and parathyroid glands.
- Abdominal glands are lying in the abdomen. Example: Pancreas, intenstinal glands and adrenals.

6.2 Properties

Hormones have low molecular weight and it can pass easily through capillaries.

- They are produced in trace amounts.
- They are soluble in water.
- They are carried by the blood to the target tissue.
- They act as catalysts.
- A single hormone may have multiple effects on a single target tissue or on several different target tissues.
- They can act in very low concentrations.
- They are destroyed or inactivated as soon as their functions are over.
- They are non-antigenic.
- They function as organic catalysts and act as coenzymes.
- The hormones show a high degree of target-specificity.
- Hormones act in very low concentration.
- The endocrine system is under the control of nerves.
- Hormonal activities are not heredity based.
- Hormones retard the reaction rate.
- Hormonal reactions are irreversible.

6.3 Biological Functions of Hormones

- Hormones control the growth of a body.
- Hormones maintain constant internal environment of organisms. Example:Insulin maintains a constant sugar level in the blood.

- Hormones promote Sexual maturation. Hormones are responsible for the sexual maturation in animals. Example: Lactogenic hormone brings about the secretion of milk, Testosterone in male produces male characters, Progesterone maintains the embryo in the uterus.
- Metabolic reactions are regulated by hormones.
- Emergency reactions are brought about by hormones. Example: Adrenaline is responsible for emergency reaction in the body.
- The development of an organism is controlled by hormones.

  Example: Thyroxin converts a tadpole into a frog. Ecdyson converts a pupa into a moth.

6.4 Plant Hormones

Plant Hormones are produced within the plants. They control the growth and development of plants. These hormones are involved in Cell enlargement, Cellular division, Phototropism, seed germination. Some hormones inhibit the growth. The organic compounds produced by higher plants regulate growth or other physiological functions. Plant hormones have no ducts. Plant hormones occur in very low concentration and they are transmitted through various parts of the plant.

Plant hormones available in plants are

1. Auxins
2. Gibberllins
3. Cytokinins
4. Ethylene
5. Traumatic acid
6. Abscisic acid
7. Marphactins.

6.4.1 Auxins

- Auxins are phytohormones that promote the plant growth. They organic compounds which promote growth along the longitudinal axis. Auxins occur in plants are Natural Auxin. It can also be synthesized artificially. Auxins are abundant in the growing tips of roots and leaves.
- Auxins induce cell differentiation and stimulate intake of water.
- Auxins control the premature fall of leaves.
- Auxins include elongation of plant cells, roots, buds and stem.

- Auxins promote cell division of parenchyma cells.
- In stem cuttings, rooting is favored by Auxins.
- Auxins modify flowering process.
- Auxins are used for the development of seedless fruits without pollination and fertilization.
- Auxins stimulate the process of respiration.
- Auxins act as herbicides.

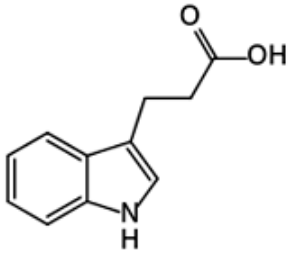


Figure 4.1 Structure of Auxin (Indole 3-acetic acid)

6.4.2 Gibberllins

- Gibberllins are plant hormones that promote stem elongation between nodes on the stem.
- The organs of flowering plants contain Giberllins.
- Gibberllins helps for the recovery from Genetic Dwarfism.
- Gibberllins induce bolting (shoot elongation) and flowering.
- Gibberllins helps for the reversal of light induced inhibition of stem growth.
- Gibberllins helps some plants like tobacco to germinate even in dark.
- Gibberllins regulate various developmental processes of plants.
- Gibberllins promote cell elongation that helps for the plant growth and they are very useful in agriculture.

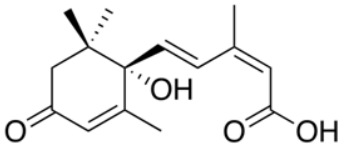


Figure 4.2 Structure of Gibberllin

- Gibberllins are used to increase the crop production. Gibberllins help for the germination of seeds.

6.4.3 Cytokinins

- Cytokinins are plant hormones promote cell division of plants.
- Cytokinins enhance the formation of chloroplast.
- Roots are the richest sources of vitamins.Fruits and endosperm contain large amount of cytokinins.
- Cytokinins promote Cell elongation.
- Cytokinins are effective in breaking seed dormancy in tobacco, lettuce.
- Cytokinins can be employed successfully to induce flowering in short day plants.
- Cytokinins helps for the shoot growth in plants and promote nutrient metabolism.

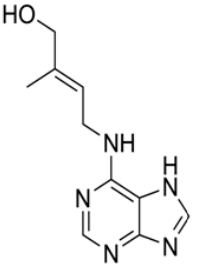


Figure 4.3 Structure of Cytokinin

6.4.4 Ethylene

- Ethylene plant hormone acts as both promoter and inhibitor. Ethylene is a gas produced by fungi and by the leaves and flowers. It is the most important hormone used much in agriculture
- Ethylene hormone promotes the elongation of petioles and internodes.
- It accelerates the colouring of harvested lemons.
- It fasten the fruit ripening.
- It induces root growth and root hair formation.
- It accelerates the ripening of fruits.

- It induces rooting.
- In pineapple, it induces flowering.

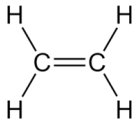


Figure 4.4 Structure of Ethylene

6.4.5 Jasmonates

- Jasmonates are isolated from Jasmine oil. In plants, it exist as jasmonic acid.

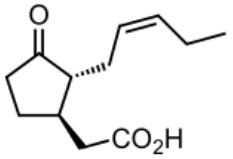


Figure 4.5 Structure of Jasmonic acid

6.4.6 Salicylic acid

- Salicylic acid hormone provide resistance to bio attacks

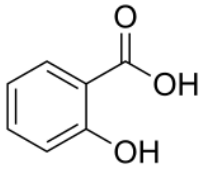


Figure 4.6 Structure of Salicylic acid

6.4.7 Traumatic acid

- Traumatic acid is a wound hormone. It promotes the healing of wounds and injured cells secret this hormone.This hormone is effective in inducing cell division.

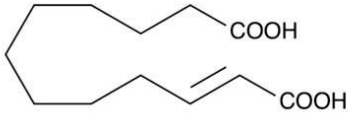


Figure 4.7 Structure of Traumatic acid

6.4.8 Abscisic Acid

- Abscisic Acid is a plant hormone.It is a weak organic acid exists in cis and trans forms.
- It helps for the developmental process including seed and bud dormancy.
- It inhibits growth and seed germination in lettuce.
- It helps the plants to tolerate heat and cold.

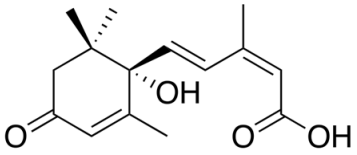
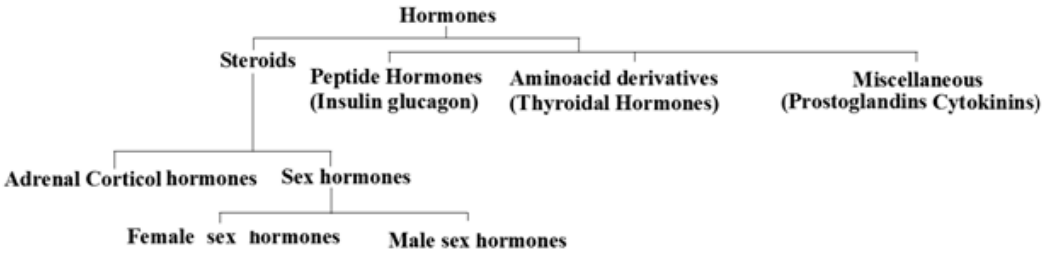


Figure 4.8 Structure of Abscisicc acid

6.4.9 Morphactins

- Morphactins are inhibiting hormones.They are the derivatives of fluorine compounds.
- It promotes the formation of branches.
- Morphactins are effective in inducing lateral bud development.

6.5 Animal Hormones



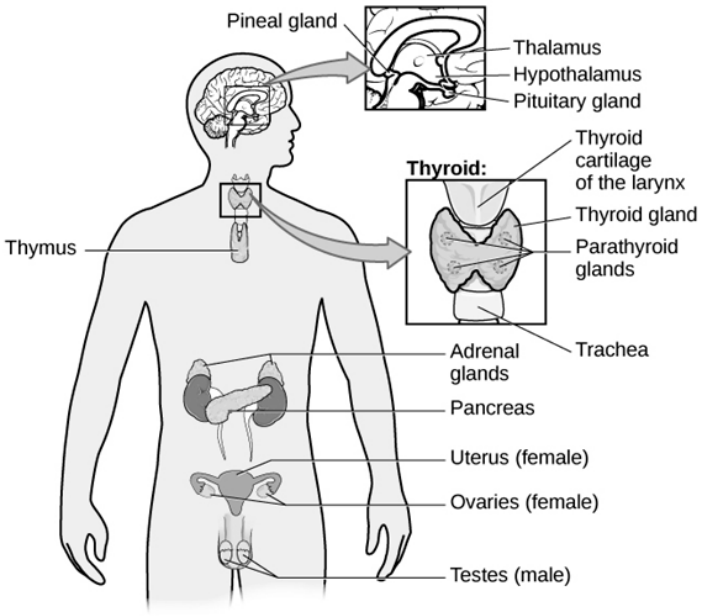


Figure 4.9 Human Physiology

6.5.1 Growth Hormone or Somatotrophic Hormone

- Growth hormone is also called as human growth hormone
- It is secreted by the adenohypophysis anterior lobe of the pituitary gland and stimulates the growth of tissues..
- It is protein in nature and is formed of a straight polypeptide chain having about 188 amino acids. It promotes the children growth.
- It stimulates the multiplication of cells.
- It increases the body growth and are essential for tissue growth.
- It increases the secretion of milk during lactation.
- STH has a remarkable effect on metabolism.It increases the release of fatty acids from the adipose tissue.
- STH decreases the utilization of carbohydrate for energy.
- GH increases intestinal absorption of calcium as well as its excretion. In addition to calcium, Na, K, Mg, PO4 and chloride are also retained.
- Children with growth hormone deficiency can be treated with the injections,

- Gigantism is a disorder caused by the over activity of pituitary glands in the child. This leads to over secretion of growth hormone. Acromegaly is caused by the hyperactivity of pituitary gland in the adult. It is due to the over secretion of growth hormone. Acromegaly is characterized by the over growth of the two jaws, the molar bones and the supraorbital ridges. Dwarfism is caused by the hypo activity of the pituitary in the child.

6.5.2 AdrenoCorticotrophic Hormone

Adreno Corticotropic hormone is secreted by the anterior lobe of the pituitary gland. It is a polypeptide hormone. It is made up to 39 amino acids. In morning, the hormone level is high and it will get decrease during the day time. It stimulates the activity of the adrenal cortex, inducing the secretion of glucocorticoids.

Oversecretion of this hormone leads to Cushing's disease Deficiency causes rheumatoid fever, Addison's disease, etc.

6.5.3 Thyrotrophin or Thyroid Stimulating Hormone

Thyroid Stimulating Hormone is secreted by the anterior lobe of pituitary gland. Hypothalamus in brain produces this hormone. It is a protein hormone. It stimulates thyroid gland there by increasing the thyroxine secretion.

6.5.4 Follicle Stimulating Hormone

FSH is secreted by the anterior lobe of the pituitary. It is a protein hormone. This hormone is important in sexual development. FSH in women changes in the menstrual cycle. In females it increases the number and size of Graffian follicles. FSH control the production of sperm. In males it stimulates the testis for spermatogenesis. Along with luteinizing hormone it control the sexual functions.

6.5.5 Luteinising Hormone

- LH is a gonadotrophic hormone secreted by the anterior lobe of pituitary.It is a glycoprotein with a molecular weight of about 3000. It makes the Graffian follicles grow and mature.It causes the Graffian follicles to secrete another sex hormone called oestrogen.
- In co-operation with FSH, it causes the rupture of the follicle and ovulation.LH causes the appearance, growth and persistence of corpus luteum in the ovary. In the male, LH stimulates the interstitial cells of testis and consequently the production of androgen.

6.5.6 Lactogenic Hormone or Prolactin or Luteotrophic Hormone (LTH)

- LTH is secreted by the anterior lobe of the pituitary gland.It is a protein with several disulphide bridges. It has a molecular weight of about 25000.It helps in initiating milk secretion in the breast.
- It stimulates the proliferation of the glandular elements of mammary glands during pregnancy and thus helps to complete the development of breasts.It helps the corpus luteum in the secretion of progesterone in co-operation with LH.

6.5.7 Thyroxin

Thyroxin is a thyroid hormone. It is a protein hormone. It is a derivative of tyrosine and it contains iodine. It increases basal metabolic rate (BMR). Hence it stimulates the production of more energy.

- It plays an important role in digestion, heart and muscle function, brain development.
- It improves growth.
- It stimulates protein synthesis.
- In increases the absorption of monosaccharaides.
- Deficiency of this hormone in children causes cretinism.
- In an adult deficiency causes **myxoedema**(myxa = mucus oedema = swelling). It is characterized by swelling of certain parts of skin, low BMR, low body temperature, under sensitivity of cold, anaemia, etc.

Over activity of thyroid gland or hyperthyroidism leads to a disease called exophthalmic goitre. It is characterized by considerable enlargement and protrusion of the gland below the chin; increased pulse rate and nervousness, bulging of the eyes, etc.

6.5.8 Insulin

Insulin is a peptide hormone. secreted by the islets of Langerhans. Chemically, is a polypeptide. Insulin helps to lower the blood sugar level. Deficiency of insulin causes a disease called diabetes mellitus. Insulin has two polypeptide chains namely A chain and B chain. A chain is acidic and it contains 21 amino acids. B chain is basic and it contains 30 amino acids. This two chains are joined by two disulphide (S-S) bonds.

- Insulin helps to break down fats or protein.
- Diabetes occurs because of this hormonal secretion will have the following symptoms. Hyper glycaemia is a pronounced increase in blood sugar level. Glycosuria shows the appearance of sugar in the urine. Polyuria is a condition in which Large volumes of urine about 10 litres per day will be released. Ketonuria: Ketonemia occurs due to increased appearance of ketone bodies in the blood.. Diabetes can be cured by the injection of protamine zinc solution.

CHAPTER

7

CELL RESPIRATION AND BIOLOGICAL OXIDATIONS

7.1 Introduction

Oxygen plays a major role in respiration. The exchange of gases between the air and organism is called respiration. We need energy to carry out all reactions and this energy is acquired through food.

7.2 Cellular Respiration

The process of respiration starts from cytoplasm and the process will complete in mitochondria. The exchange of gases between the body fluid and environment takes place by diffusion is called as breathing or external respiration in which oxygen is taken in and carbon dioxide is given out. Oxidative energy-yielding reactions of the cell is known as cell or internal respiration or biological oxidation. The main product of cellular respiration is ATP. By the process of cellular respiration, nutrients are converted into chemical energy

Biochemical reactions involve catabolic reactions, combustion and redox reactions. Biological oxidation refers to all enzyme catalyzed chemical reactions which utilize oxygen or lose hydrogen or electron (e). The complex organic food molecules are oxidized in the cells to release energy. During oxidation process, Carbohydrates and proteins are oxidized to form CO_2 and H_2O. Fats are oxidized to form acetate which was converted to CO_2 and H_2O by the process of oxidation and combustion.

7.3 Biological Oxidation

Biological oxidation is the process which takes place in living organisms in which the oxidation and reduction reaction takes place simultaneously.

- Biological oxidation is an important energy producing reaction in cell.
- Oxidation in living organism is a complex process.
- By the process of biological oxidation, living organism will get energy.
- Oxidation in living organism is a controlled process.

7.4 Theories of Biological Oxidation

There are two theories of Biological oxidation.

Oxygen Activation Theory

Oxidation reactions are catalyzed by oxidase enzymes which activate molecular oxygen.

$$SH_2 + \tfrac{1}{2}\, O_2 \xrightarrow{\text{oxidase}} S + H_2O$$

Hydrogen Activation Theory

Oxidation reactions are catalyzed by dehydrogenase enzymes which activate hydrogen atoms of the substrate. The hydrogen atoms are removed by a hydrogen acceptor 'A'.

$$SH_2 + A \xrightarrow{\text{Dehydrogenase}} S + AH_2$$

$$AH_2 + O_2 \longrightarrow A + H_2O_2$$

The above reactions will be catalysed by the enzymes known as oxidoreductases. Biological oxidations mostly take place by the removal of hydrogen in pairs. This process is known as dehydrogenation. The enzyme which catalyzes the dehydrogenation is known as dehydrogenase. The important hydrogen acceptors and carriers in biological oxidation are Nicotinamide nucleotides, Flavin Nucleotide and Cytochromes. NAD is the major electron acceptor in biological oxidation.

7.5 Cytochromes

Cytochromes are iron containing hemoproteins and they play a major role in electron transport. They transfer electrons from flavoproteins to molecular oxygen. They are mostly present in the mitochondria of aerobic cells. Cytochromes have a porphyrin ring with iron atom. It is a small water soluble protein.

7.6 Mitochondria

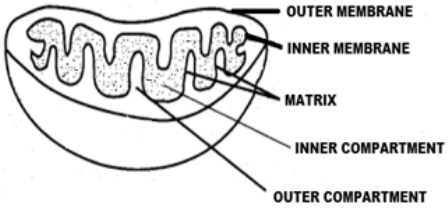


Figure 7.1 Mitochondria

- Mutochondria is found in the eukaryotic organism. They are the major sites of biological oxidations.
- Mitochondria float within the cytoplasm of the cell and they generate energy required for the chemical reactions. They are the "power house" of the cell because they are the sites of production of high energy compounds like ATP.
- All the enzymes and coenzymes required for biological oxidation are present in mitochondria.
- Mitochondria helps for the thermogenesis process. They support for the production of heat in living organism.
- The mitochondria have two membranes an outer membrane and an inner membrane.
- The inner mitochondrial membrane form folds called cristae. The inner compartment is filled with fluid matrix. The inner membrane has two layers a protein layer and a lipid layer. The protein layer is associated with electron transport chain of enzymes and the lipid layer with the enzymes of oxidative phosphorylation. The fluid matrix is associated with the enzymes of citric acid cycle and fatty acid oxidation.
- Mitochondria synthesis certain hormones and biochemicals.
- Mitochondria is very essential for the cholesterol and neurotransmitter metabolism.

7.7 Intermediatory Metabolism

Intermediary metabolism is the total intacellular processes in which the nutritive component is converted to cellular components. It is the total of all enzymatic reactions, occurring in the cell. A metabolic pathway is a series of chemical reactions that takes place within the cell. The metabolic pathways can be divided into 3 major types

1. ***Catabolic pathway:*** In this pathway, energy is released by breaking the molecules into simpler forms. It is responsible for degradation of energy rich nutrient molecules to release energy. Example: Krebs Cycle, Glycolysis.
2. ***Anabolic Pathway:*** It is responsible for synthesis of cellular components.

   Example: Synthesis of proteins from aminoacids.
3. ***Amphibolic Pathway:*** It includes both catabolic and anabolic pathways.

   Example: Glycogenosis, Lipogenosis.

Metabolites are the reactants, products and intermediates of enzymatic reactions. Biological oxidations occur in both catabolic and in amphibolic pathways.

7.8 Oxidative Decarboxylation

- Oxidative decarboxylation occurs in the mitochondrial matrix in which involves both oxidation and loss of carboxyl group as CO2 will takes place in the cell.
- In this process, the pyruvic acid is converted into acetyl coenzyme A and the removal of carbon dioxide will also takes place. The process takes place with the help of enzymes Pyruvic acid decarboxylase, Dihydroxylipoyl transacetylase, Dihydroxylipoyl dehydrogenase and the Co-factors such as Co-enzyme A, NAD, Lipoic acid and Thiamine pyrophosphate
- ***Step – 1:*** Thiamine pyrophosphate combines with pyruvic acid to form hydroxyethyl thiamine pyrophosphate . The carboxyl group is removed as CO2 with the help of enzyme Pyruvic dehydrogenase and forms acetaldehyde.

Pyruvic acid + Thiamine pyrophosphate $\xrightarrow[\text{dehydrogenase}]{}$ CO_2 + Pyruvic Acetaldehyde

- ***Step – 2:*** It is then oxidized to form acetyl lipoic acid.

Active acetaldehyde + lipoic acid $\longrightarrow$ 6-S-acetyl lipoic acid

- ***Step – 3:*** The acetyl group is then transferred to coenzyme A to form acetyl coenzyme A.

6-S-acetyl lipoic acid + CoA $\longrightarrow$ Acetyl CoA + Dihydrolipoic acid

Electron Transport System

- Energy is transferred from electron to ATP molecules by the process of cellular respiration. In the electron transport chain, 3 main steps will occur which involves the generation of proton across the mitochondrial membrane, reduction of molecular oxygen to water and ATP synthesis.
- Electron transport system is present in the cristae of mitochondria. Cristae contains coenzymes which acts as carrier and transfer molecules. The electron flow takes place through various components of the respiratory chain are arranged in the inner mitochondrial membrane in sequence an in the following order:

- Electrons from reduced NAD and reduced FAD are passed on to a common acceptor coenzyme Q. From CoQ electrons are passed on to cytochrome b, then to cytochrome c. From cytochrome c, the electrons are transferred to cytochrome a.
- The electrons are accepted by oxygen. Oxygen combines with hydrogen to form water.
- The electron transport chain is a enzymatic reactions which involves the electron acceptance and donors. Each electrons will be will passed to an acceptor of higher redox potential. The electrons are donated to another acceptor till the electrons are passed to oxygen. Energy is released in each step since the higher-energy donor and acceptor is converted into lower-energy products.
- The protein complexes which are involved for the electron transport from NADH to O_2 are NADH-Q reductase complex, Cytochrome C reductase complex, Cytochrome C, Cytochrome C oxidase complex.
- For the conversion of $FADH_2$ to O_2, the four protein complexes that catalyse the redox reaction are succinate dehydrogenase, Cytochrome C reductase complex, Cytochrome C, Cytochrome c oxidase complex.
- The electron transfer potential can be determined by placing an electrode in a solution containing 1M NADH and 1M NAD^+. The reference electrode is connected to 1M saturated H_2 gas. Then the electrodes are connected to voltmeter. Then the potential is measured.

Respiratory Chain	**Redox potential in Volt**
NADH	–0.32
↓	
FAD	–0.03
↓	
CoQ	–0.1
↓	
Cyt. b	0.04
↓	
Cyt.c	0.25

↓ Cyt. a ↓ O_2	0.29 0.8

Three greater jumps in the redox-potential values are observed in the conversion of NAD to FAD, Coenzyme Q to Cytochrome B, Cytochrome A to O_2.

Oxidative Phospholylation

- Oxidative phosphorylation is a final step of cellular respiration.
- The electrons are transported through redox reactions through the mitochondrial membrane. During biological oxidation, a large amount of energy is released.
- Energy is used to convert ADP into ATP.
- The conversion of oxidation-reduction energy into high energy phosphate bond is known as oxidative phosphorylation.
- Transfer of electrons takes place and the protons are pumped out of mitochondrial matrix.
- Hence a membrane potential is generated. When the protons are pumped in, ATP is synthesized. 4 ADP molecules are phosphorylated to 3 ATP molecules.
- The first molecule of ATP is formed in the reaction between NAD and FAD, the second between Cyt b and Cyt c and the third between Cyt a and O_2.

$$3\ NADH + H^+ + 3\ ADP + 3\ Pi + ½\ O_2 \longrightarrow NAD^+ + 4\ H_2O + 3\ ATP$$

The electron transfer takes place as follows

$$NADH + H^+ + ½\ O_2 \rightarrow NAD+ + H_2O;\ \Delta G = -51700\ cal/mole$$

In this reaction NADH is oxidized to NAD^+ and pair of electron is transferred to O_2 to form water. The process releases a free energy of 52700 cal/mole.

$$3\ ADP + 3\ Pi \rightarrow 3\ ATP + 3\ H_2O;\ \Delta G = +21900\ cal/mole$$

ADP is phosphorylated to ATP. The process requires a free energy of 21900 calories for the formation of one mole of ATP.

CHAPTER

8 BIOCHEMICAL TECHNIQUES

8.1 Introduction

Biochemical Techniques helps to identify the substance present in living organisms. Biochemical techniques are very important in the field of biology. Biochemical techniques helps to understand the biochemical process.

8.2 Microscopy

Microscope is a technique used to see objects that are not seen with the naked eye. Microscopy involves diffraction, reflection and refraction of electromagnetic radiation. Biochemical analysis is frequently accompanied by light and electron microscopic examination of tissue, cell or organelle preparations to evaluate the integrity of samples and to correlate structure with function.

8.2.1 Optical Microscope

Optical or light microscope involves passing light through a lens to magnify the image of an object. A condenser lens is used to produce a parallel beam of light. Thhe image is enlarged by objective and eyepiece lenses. The resolving power (resolution) of a microscope is given by Abbe's formula

$$R = 0.5 \lambda / n. \sin \alpha \ \mu m \text{ (or nm)}$$

Where λ = the wavelength of radiation in (or nm)

n = the refractive index (RI) of the medium between the specimen and the first lens

α = ½ angle of the aperture

As per Abbe's formula, small value for R gives a better resolution. Smaller values of R is obtained by making λ smaller (short wave) than longer wavelength. Also by using special oil immersion lenses, the value of n can be increased which lowers the value of R.

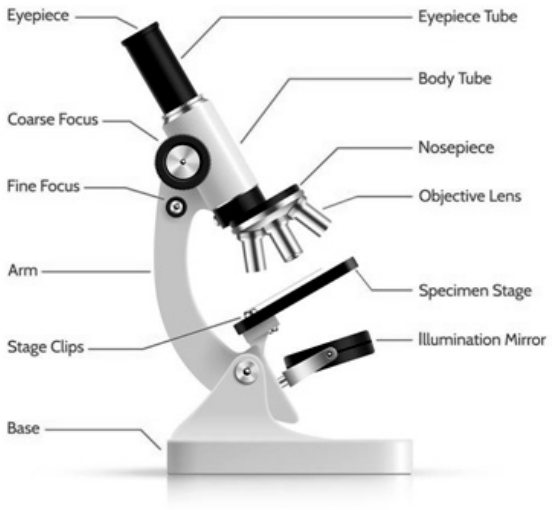


Figure 8.1 Optical Microscope

Making α as close to 90° as possible, R values can be smaller. The image clarity is reduced outside the focal plane. This technique can be used for strongly refracting objects.

The important features of optical microscopy are

- Sample preparation normally takes a few minutes / hours.
- Live or dead specimens is used for analysis.
- Running cost is very low.
- Morphology is detected in colour or black and white.
- Poor depth of focus
- Resolution 170 nm at best magnification x 2000 at best

8.2.2 Electron Microscope

For getting a high resolution, the electron beam is used in electron microscoy. This technique is carried out under vacuum since the electrons are easily scattered. Electrons are charged particles and they have wave-like properties, so they respond to magnetic fields. Powerful magnets are used to focus electrons. The wavelength of an electron is given by the formula:

$$\lambda = 1.23 / \sqrt{E} \text{ nm}$$

Where, E is the voltage through which an electron is accelerated.

Higher the voltage, the shorter the wavelength. Abbe's formula applies equally to both optical and electron microscopes, the best resolution are achieved at high voltages. The main types of electron microscopy are transmission electron microscopes (TEMs), scanning electron microscopes (SEMs) and Scanning Probe Microscopy (SPM).

Transmission Electron Microscopy:

In TEMs electrons are transmitted through the specimen. The image produced is due to the interaction of electrons with the sample. The image is then magnified using photographic film, fluorescent screen or a sensor. This technique is widely used in environmental research, medicinal and electronic applications.

Scanning Electron Microscopy:

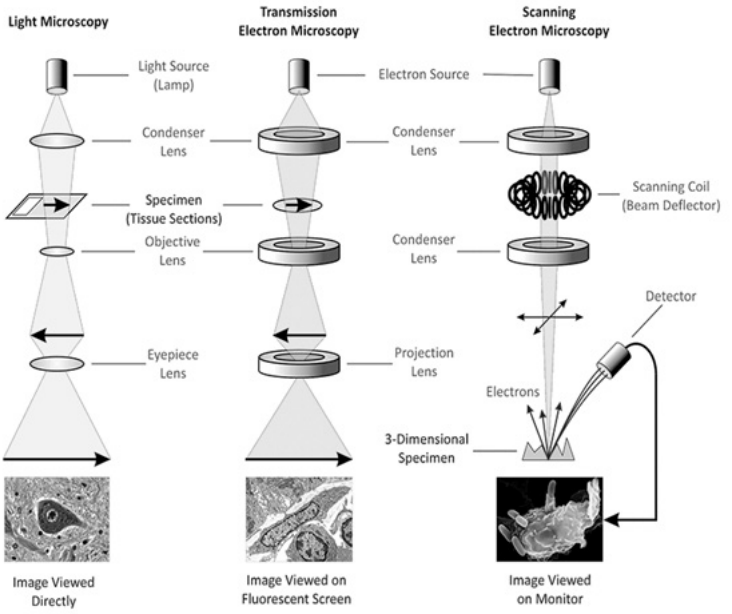


Figure 9.2 Electron Microscope

In SEMs the surface is scanned with a beam of electrons. Interaction between electron and the atoms take place. As a result, surface morphology of the sample can be determined. The important features of scanning electron microscope are

- Sample preparation often takes several days.
- Dead, dried specimens are used.
- Heavy running costs.
- Surface Morphology is detected in black and white only.
- Good depth of focus.
- Resolution 0.5 nm at best. Magnification x 5,00,000 at best

From electron gun, electron beam is emitted thermionically. Tungsten is used in thermionic electron guns. The beam passes through the scanning coils. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons. Images are created. Electronic amplifiers are used to amplify the signals.

8.3 Centrifuge

Centrifuge involves the process of centrifugation. The centrifugal force is involved to separate the particles in a solutioin. Centrifuge instrument is used to spin substances at a high speed. According to their size, shape, density, medium viscosity and rotor speed, the particles present in the liquid sample will be separated. On increasing the effective gravitational force of the solution, precipitate will settle down quickly to the bottom of the tube. The remaining liquid that lies above the precipitate is called a supernatant. Larger the size and the density of the particles, the faster they separate from the mixture. The rate of centrifugation is expresses by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as g. The conversion factor between RPM and g depends on the radius of the centrifuge rotor. The sedimentation rate of each particle is directly proportional to the applied centrifugal force.

Centrifugation techniques in research involves Microcentrifuge, Low speed centrifuge, High-speed centrifuge, Ultra centrifugation, Differential centrifugation, Density gradient and Isopycnic centrifugation.

Ultracentrifugation involves preparative centrifugation and analytical centrifugation.Preparative centrifugation technique requires large quantity of sample and it involves separation, isolation and purification of sub-cellular organelles, plasma membranes, ribosomes, chromatin, nucleic acids and viruses. This technique helps in the study of their morphology, composition and biological activity.

Analytical centrifugation technique requires only small amount of sample and is applied mainly to the study of purified macromolecules. By this technique, the purity,

chang in the molecular mass of complexes, relative molecular weight and shape of the material can be determined.

Depending on the operating speed, the centrifuge is classified as hand centrifuge, Desktop centrifuge, Continuous flow centrifuge, Gas centrifuge, Hematocrit centrifuge, low speed centrifuge, high speed centrifuge,Ultra centrifuge, Microcentrifuge, Refrigerated centrifuge and Vacuum centrifuge.

8.3.1 Hand Centrifuge

It is manually operated to separate the solid part of a solution.

Figure 9.3 Hand Centrifuge

8.3.2 Desktop Centrifuge or Small Bench Centrifuge

Figure 9.4 Desktop Centrifuge

Desktop centrifuge consists of an electric motor to rotate the tubes. They are used in clinical and research laboratories to separate red blood cells, yeast cells or bulky precipitate of chemical reactions. Their maximum speed is usually 3000 rpm They do not usually have any temperature regulatory system. Large volumes of crude samples can be used to separate. The centrifuge tubes must be placed opposite to each other after balancing their weights accurately.

8.3.3 Continuous Flow Centrifuge

Continuous Flow Centrifuge is a rapid centrifuge technique. It is used to separate large volumes of sample. A high centrifugal force is applied to remove the solid components of sample.

8.3.4 High Speed Centrifuge

High speed centrifuge operate at a very high speed. This centrifuge operates at a speed of 15,000 to 30,000 rpm. Due to large number of rotations, more heat energy will be generated. Hence it is connected with arefrigeration equipment to remove the heat generated due to friction between the air and the spinning rotor. The temperature can easily be maintained in the range 0°C to 4°C. A maximum volume of about 150 mL can be loaded. This centrifuge is used to separate sensitive biological samples.It can be used to isolate sub-cellular organelles such as the nuclei, mitochondria and lysosomes and to collect microorganisms, cell debris, precipitates of chemical reactions and immune precipitates.

Figure 8.5 High Speed Centrifuge

8.3.5 Gas Centrifuge

Gas centrifuge is used for the separation of gases based on their isotopes. Gas molecules are separated on the basis of their masses.

8.3.6 Hematocrit Centrifuge

Hematocrit Centrifuge is used to separate the blood samples. They are used for the volume fraction of RBCs in the blood sample. By using this technique, blood loss, anaemia, polycythemia, leukemia can be determined. This centrifuge quickly attains a speed of 11000rpm.

8.3.7 Ultracentrifuge

Ultracentrifuge is used for the separation of very smaller molecules. This centrifuge operates at a higher speed. The molecules like ribosomes, proteins and viruses can be separated. This centrifuge operates at a speed of 150, 000 rpm. Ultracentrifuge is used for both preparative and analytical methods. Due to large number of rotations, refrigeration system is equipped with this centrifuge. It is used for the determination of properties of macromolecules like size, shape and density.

8.3.8 Microcentrifuge

Microcentrifuge technique is used to separate very smaller volumes. It is operated at a speed of 12000 to 13000rpm. Also temperature control is attached to operate the temperature sensitive samples. It is used for the molecular separation of cell organelles like nuclei, DNA.

8.3.9 Refrigerated Centrifuge

Refrigerated centrifuges are used for the separation of various biological molecules like yeast cells, chloroplasts and erythrocytes. It is operated with temperature control ranging –20°c to 30°C.

8.3.10 Vacuum Centrifuge

Vacuum Centrifuge can centrifuge large number of samples at a time. This centrifuge is used on chemical and biological laboratories. By lowering the vapor pressure of the sample, the boiling point of the sample decreases which causes the solvent to get evaporated. The particles will get separated.

8.3.11 Advantages

The centrifugation technique is used to

- To separate the miscible substance.
- To purify the components.
- To separate crystalline drugs.
- To test the emulsion and suspensions for creaming and sedimentation at an accelerated speed.

8.3.12 Application Of Centrifugation

- Separation of particles in air.
- Removing fat from milk.
- Production of bulk drugs.
- Purification of cells.
- Production of biological products.
- Evaluation of suspensions and emulsion.
- Determination of molecular weight of colloids.
- Separating chalk powder from water.
- Purification of water.

8.4 pH meter

P.L.Sorenson introduced the concept of pH in 1909. pH meter is used to measure the acidity or alkalinity of a sample. It is used for various applications.

8.4.1 Principle

pH meter is a potentiometer which measures the voltage between two electrodes placed in a solution.pH meter has a glass and reference electrode or a combination electrode. An electric potential is generated when a thin glass membrane separates two solutions of different H^+ ion concentrations. The two electrodes used in pH meter are a calomel electrode and a glass electrode. Reference electrode used for this instrument is calomel electrode and the glass electrode is the standard test electrode whose electrical potential depends on the pH of the test solution.

8.4.2 Electrodes Used

The calomel electrode contains mercury, mercury chloride and a saturated solution of potassium chloride and it does not allow H^+ ions which indicates that its potential

is independent of pH. The calomel electrode is dipped in saturated solution of KCl. The glass electrode contains silver, silver chloride and 0.1M HCl solution which is permeable only to H^+ ions. This electrode is dipped in 0.1M HCl solution.

An electrical potential develops across the glass electrode and calomel electrode, which results in a flow of current between the electrodes. The magnitude of this current depends on the concentration of H^+ / OH- ions in the test solution.

Combination electrodes consist of a glass and a reference electrode in a single unit. The electrode is of high cost andsmaller volumes of solution are enough to measure.

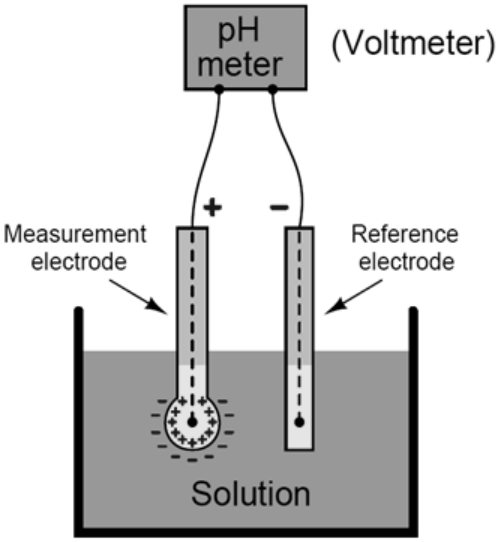


Figure 8.6 pH Meter

8.4.3 Application

- pH meter is used to measure pH of a given solution
- It is used in all industries.
- It is used in the clinical laboratory
- It is used in biochemical research.
- Used in agriculture for fertilizer testing
- Used for soil testing.
- Used to check water quality.

8.5 Electrophoresis

The movement of charged particles under the influence of an electric current to oppositely charged electrodes is called electrophoresis. The movement of the charged particles in an electric field depends upon time, electric currentand conductivity of the solvent and charge of the molecule to be separated. Electrophoresis of positively charged cation is called cataphoresis and the electrophoresis of negatively charged anion is called anaphoresis.

The electrophoretic movement is observed in clayparticles dispersed in water. Macromolecules can be separated. This technique is used in analysis of DNA, RNA and protein.

Electrophoretic mobility is defined as the distance travelled by the particles in one second under the potential gradient of one volt per centimeter. The different compounds in a mixture will have different electrophoretic mobility's and hence they can be separated. The two main types of electrophoretic methods are Moving boundary and Zone electrophoresis.

8.5.1 Zone Electrophoresis

In Zone electrophoresis, the migration of charged particles takes place with the supporting media. On the supporting medium, the components will get separate. For this technique, small volume of the smaple is enough. This technique is easy to maintain and it involves low cost. It is highly applicable to biochemical research.

This technique involves electrophoretic chamber, supportimg media, electrodes and diffusion barrier.On the basis of supporting media, zone electrophoresis is classified into

a) Paper Electrophoresis
b) Gel Electrophoresis
c) Thin Layer Electrophoresis
d) Cellulose acetate Electrophoresis

a) Paper Electrophoresis:

Under the influence of electric current, the charged particles migrate towards the positive and negative pole according to their charge. Paper Electrophoresis is a simple and low cost method. It is used for testing water samples, pharmaceutical industries and in clinical applications. The separation of particles depends upon size, charge, shape, electric field and pH.

Higher the charge of the sample, the mobility of the particles will be more. Larger particles have smaller electrophoretic mobility. Paper electrophoresis is very important for the study of normal and abnormal plasma proteins.The serum under investigation is mixed with bromophenol blue, a blue coloured stain and spotted at the centre of as trip of special filter paper, saturated with barbitone buffer of pH 8.6

When an electric current and voltage is passed through the paper, charged protein fractions bearing different charges migrate at different rates. The different fractions of plasma will migrate toward the anode at characteristically different rates. The paper is dried and stained with a solution containing bromophenol blue after a run of about 5 to 6 hours. Using paper electrophoresis, five different bands is observed in human serum in the order of decreasing mobility as albumin, alpha1-glubulin, alpha2-glubulin, beta-globulin and gamma-globulin. A band was identified in which Albumin, the fastest moving fraction of the proteins of plasma, forms the last band of the paper. Gamma globulin, which is the slowest moving protein, forms a band at the other end. The remaining fractions are seen in between these two bands.

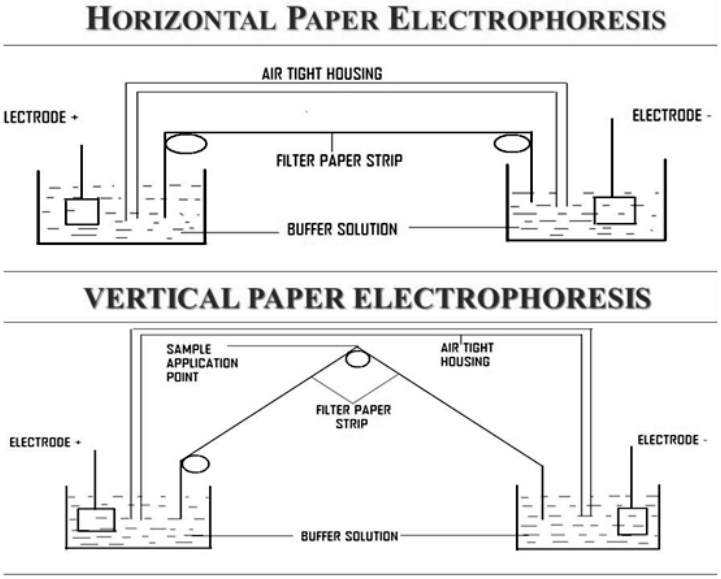


Figure 8.7 Paper Electrophoresis

b) Gel Electrophoresis

Based on the molecular size of the substances, separation is done by molecular sieving in gel electrophoresis. The supporting medium used in gel electrophoresis is electrically neutral. The gel sieves the macromolecules and allows only the smaller molecules to migrate.

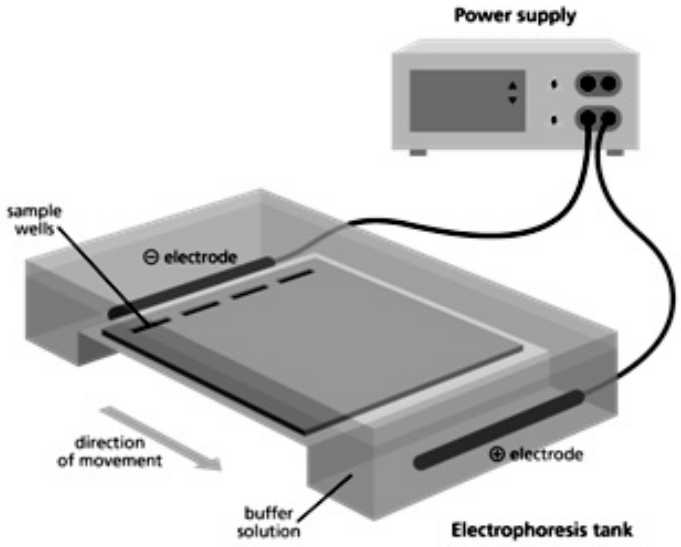


Figure 8.8 Paper Electrophoresis

Various types of gels are used as the supporting medium are starch gel, Agar gel, Polyacrylamide gel and Sephadex gel. The use of gels in electrophoresis is highly applicable for proteins and amino acids. Serum proteins can be separated into 15 bands.

Polacrylamide gel electrophoresis combined with sodium dodecyl sulphate is known as SDS-PAGE. It is the most widely used method for analyzing protein mixtures qualitatively. It is particularly useful for monitoring protein purification. It is also used to determine the relative molecular weight of proteins. PAGE is the most versatile electrophoretic system for the analysis and separation of proteins, small RNA molecules and very small fragments of DNA

c) Thin Layer Electrophoresis

In Thin layer Electrophoresis, thin layers of silica, alumina are used. Along with chromatography, this technique is useful in the study of proteins and nucleic acids. This technique requires less time and give good resolution. It is more advantageous than paper electrophoresis.

d) Cellulose Acetate Electrophoresis

This technique is more advantageous than paper electrophoresis.Biological acetate membrane is used for this type of electrophoresis. This membrane gives sharp bands. The cellulose acetate paper was dipped in the buffer solution. The sample is then applied

at one end. Then current was passed. Then the particles will migrate depending on their charge. This technique os used in the clinical and biological applications.

8.5.2 Moving Boundary Electrophoresis

Large volumes of sample is required for moving boundary electrophoresis. This technique is carried out in the absence of supporting medium.The apparatus consists of U- shaped cell containing buffer solution. On applying an electrical current, proteins migrate towards the anode. The migration of negatively charged proteins from the macromolecule solution to the pure buffer forms a boundary. A sharp change in the refractive index of the solution is identified. The changes in the refractive index are measured by Schlerin optics.

a) Capillary Electrophoresis

b) Isotachophoresis

c) Isoelectric Focusing

d) Immuno Electrophoresis

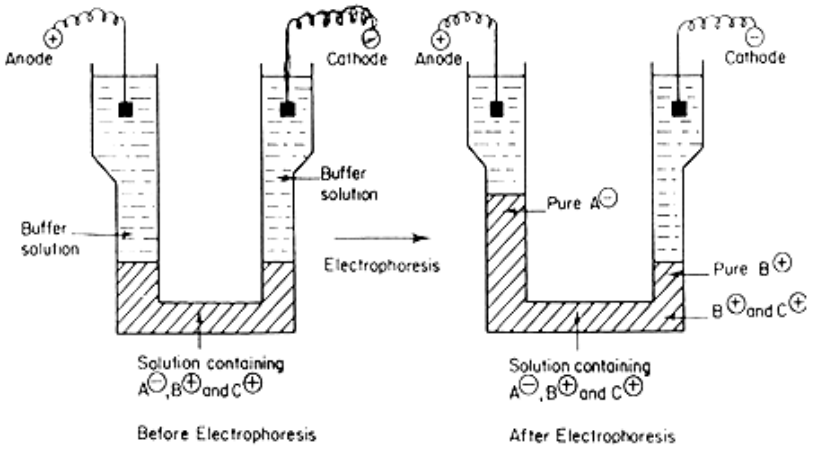


Figure 8.9 Moving Boundary Electrophoresis

Capillary electrophoresis is a separation method that takes place under the influence of electric field. Small highly charged solute will migrate faster. Thus the migration of solute depends on the charge and size of the particle.Proteins, Nucleic acids, organic and inorganic analysis can be done using this technique. UV, laser induced flourescence detectors can be used.

Capillary isoelectric focusing is used to separate peptides and proteins.

Capillary Isotachophoresis involves the migration of sample between electrolytes. Serum proteins can be separated into 40 bands. This technique is highly applicable for the purification of proteins.

Immuno Electrophoresis is used for the analysis of antigens and antibodies.

Spot test techniques are helpful to identify the separated components. Proteins are usually located by staining and enzymes by their specific activities. Amino acids can be detected by fluorescence under UV light. Radioactive substances can be located by autoradiography or by staining. Lipoproteins can be detected by staining with the fat-soluble dye such as sudan dye. Glycoprotein is detected by using modified Schiff's reagent.

In hepatic Cirrhosis, a decrease in albumin and an increase in globulin is identified. Albumin decrese is seen in protein malnutrition.In chronic infection (hepatitis) a relative decrease in albumin with a notable elevation in γ-globulin is observed.
The presence of an abnormal band (M protein) usually between ß and γ-globulin bands, closer to the γ band is identified in multiple myeloma.

In hypogammaglobulinemia a considerable drop in γ-globulin is readily observed. A slight increase in α2-globulin is also seen. In chronic liver diseases, a decrease in albumin band is observed. Occasionally an increase in γ and ß-globulin is also seen. α2-globulin level increase in nephritic syndrome.

8.5.3 Applications of Electrophoresis

- Used to separate insulin from plasma proteins.
- Helps to isolate a large number of proteins.
- Identify the purity of the isolated proteins.
- Molecular weight of proteins can be detected.
- Used for the separation of carbohydrates and vitamins.
- Helpful to determine the sequences of DNA.
- Used to find out the point of mutation in DNA or RNA.
- Helps to detect the precursor molecules of tRNA, rRNA and mRNA .
- Used to find out the number of subunits present in a protein.
- Used to determine the molecular weight of proteins and DNA.
- Haemoglobin separation can be done using this techniques.

8.6 Colorimeter

A colorimeter is a device which measures the transmittance and absorbance of a solution.

8.6.1 Principle

Colorimeter works on the principle of Beer-Lambert law which states that the concentration of a solute is directly proportional to the absorbance.

$$A = \epsilon cl$$

Where A is the absorbance, c is concentration, ϵ and l are constant.

Colorimeter involves a light source (Tungsten), Monochromator which selects the particular wavelength to pass, cuvette, filters and detector (Photocell).

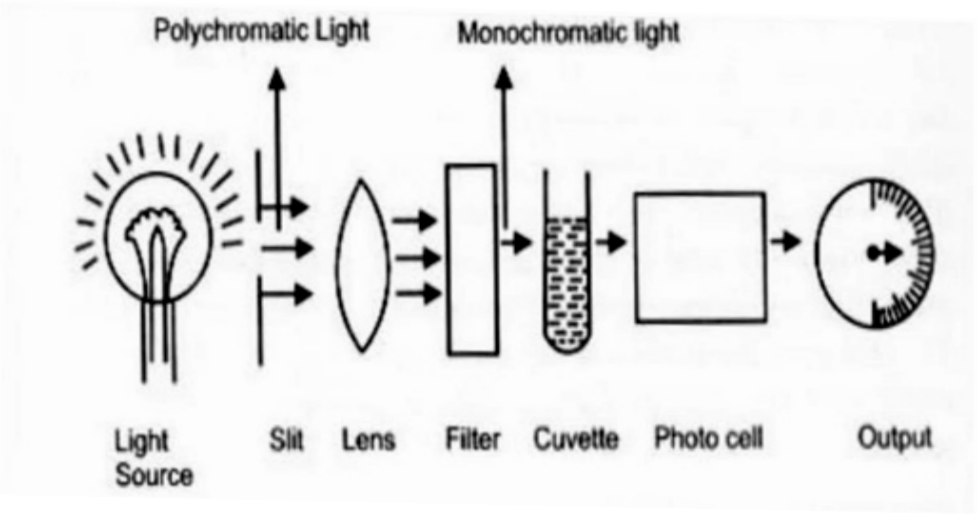


Figure 8.10 Colorimeter

A beam of light having particular wavelength is passed through a solution. A microprocessor then calculates the absorbance or percent transmittance. A sample of known concentration is first used to calibrate. Then the concentration of an unknown sample is determined.A graph is drawn between the concentration and absorbance, From the graph, the concentration of unknown sample is easily determined.

8.6.2 Applications

- Used to identify the food colours.
- Used in chemical Laboratories.

- Helps to monitor the growth of bacteria and yeast.
- Used to determine the concentration of plant nutrients.
- Used in paint industry.
- Used to determine the concentration of haemoglobin in blood.

CHAPTER

9 MINERALS

9.1 Introduction

Minerals play an important role and it is very essential for many activities in our body. For a healthy living, minerals play a major role. Minerals are responsible for growth. Also, they are required for hormones and enzymatic activities. Mineral balance must be maintained in our body or it will lead to many disease. Minerals does not decompose under the influence of heat or light. Cooking will not affect the composition of minerals. The two types of minerals required for our body are Macrominerals and microminerals.

9.2 Macrominerals

Macrominerals are the minerals which are required large amount in our body.

Example: Calcium, Sodium, Potassium, Phosphorous, Magnesium, Chloride and Sulphur.

9.3 Microminerals

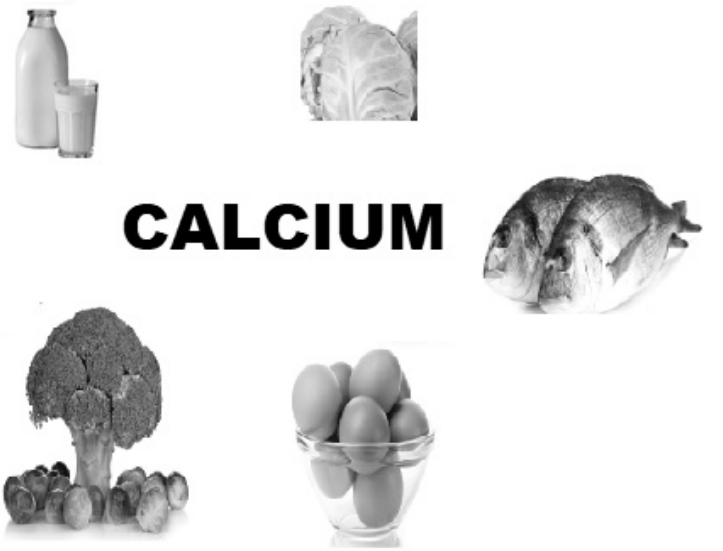
Microminerals are the minerals which are needed in trace amounts.

Example: Iron, Manganese, Copper, Iodine, Zinc, Cobalt, Fluoride and selenium.

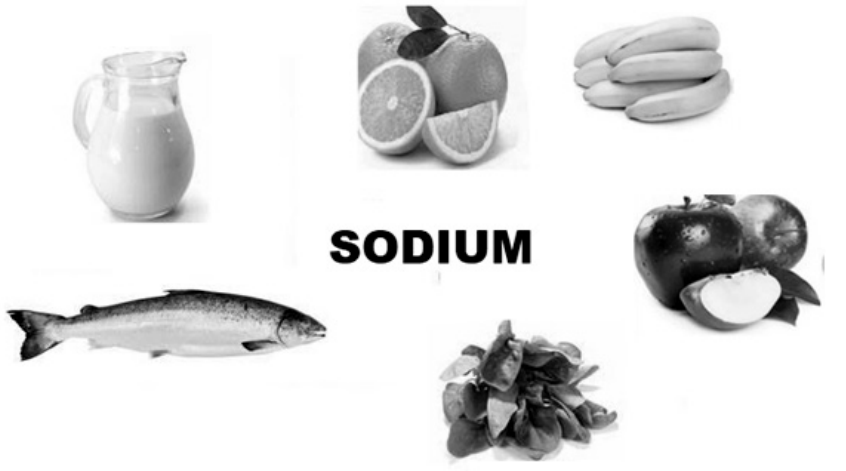
9.4 Role of Minerals In Human Body

9.4.1 Calcium

- The most abundant mineral in our body is calcium.
- Calcium along with vitamin D helps for good calcium absorption.
- Calcium is very essential to maintain healthy bones and teeth.
- Calcium is rich in milk, yoghurt, ghee, leafy green vegetables and cereals.
- Deficiency of calcium leads to rickets in children.
- Deficiency of calcium leads to osteoporosis for adults.
- Calcium helps for blood clotting.



9.4.2 Sodium

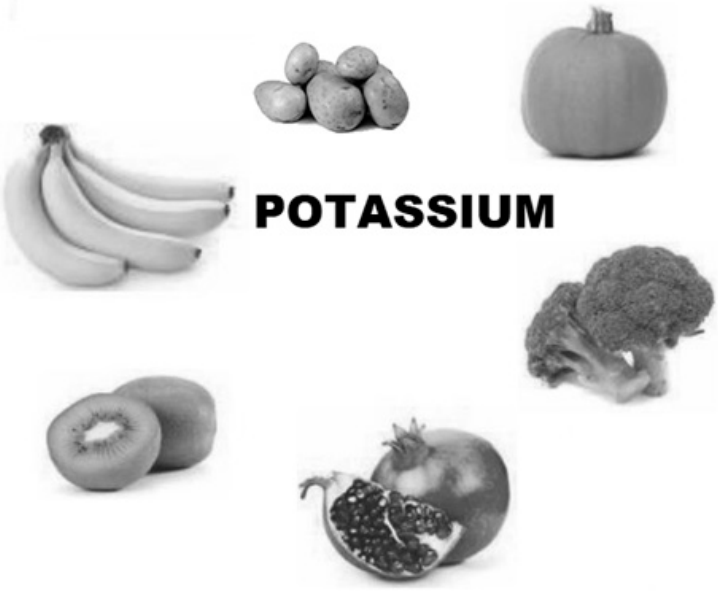


- Sodium is required in large amounts to keep our body healthy.
- Commonly we are taking sodium in the form of sodium chloride.
- Sodium helps to maintain water balance.
- Sodium also helps to control blood pressure of our body.
- Sodium transport nutrients and biomolecules in our body.

- Calcium imbalance will occur if we take excess of sodium.
- Deficiency of calcium leads to cardio vascular diseases.

9.4.3 Potassium

- Potassium mineral is required for organ function.
- Potassium is responsible for transmitting nerve impulses. It helps to keep the nervous system functioning properly.
- Potassium is third abundant mineral in our body.
- It helps in maintaining fluid balance of body.
- Potassium is high in banana, tomatoes, green vegetables and dairy products.
- Potassium is responsible for maintaining normal blood pressure and water balance of the body.
- Potassium helps to regulate digestion process.
- Deficiency of potassium leads to hypokalemia.



9.4.4 Phosphorous

- Phosphorous is rich in Mushrooms, Meat, Oats, Fish, Beans and Almonds.
- Phosphorous is essential for filtering waste secreted in the kidney.

- Phosphorous is required for cell repairing.
- ATP the energy bank of cell needs phosphorous.
- Phosphorous is essential for growth.

PHOSPHOROUS

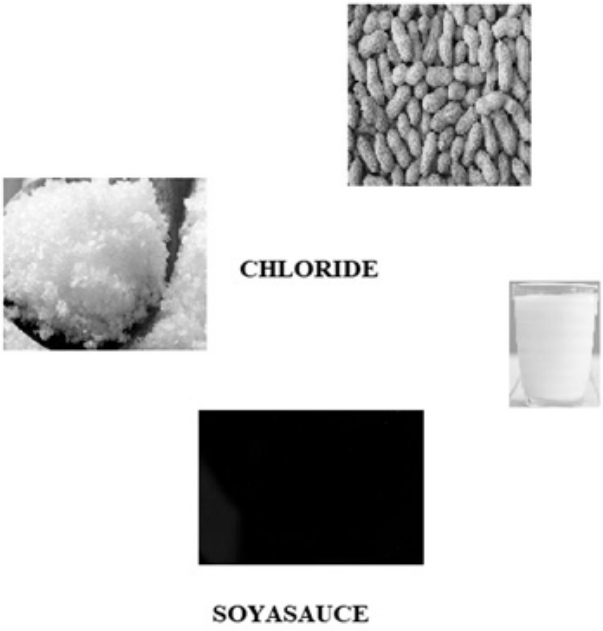
9.4.5 Magnesium

MAGNESIUM

- Magnesium is essential for healthy bones.
- Magnesium is essential for biochemical reactions in our body.

- Magnesium helps to regulate calcium and vitamin D in our body.
- Magnesium is essential for our heart to avoid cardio vascular problems. Magnesium intake lowers the risk of stroke.
- Magnesium plays a role in hypertension.
- Deficiency of Magnesium also leads to stress, anxiety and weakness.
- Magnesium deficiency causes hypomagnesemia.
- Excess of magnesium leads to gastrointestinal problems.
- Magnesium level fluctuates during menstrual cycle.

9.4.6 Chloride

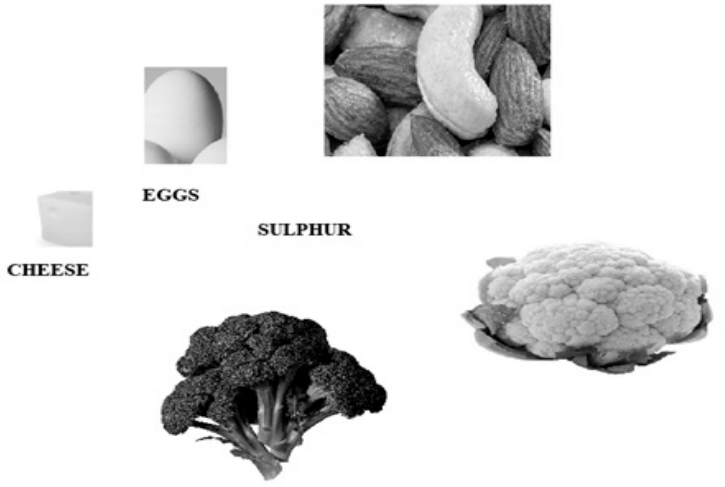


- Chloride helps to maintain pH and fluid level of our body.
- Chloride helps the red blood cells to exchange gases in our body.
- Cholride helps for the digestion of foods.
- Chloride helps to maintain blood pressure and blood volume.
- Chloride is rich in Table Salt, Soy Sauce, Milk and Peanuts.
- Excess of chloride leads to high blood pressure.

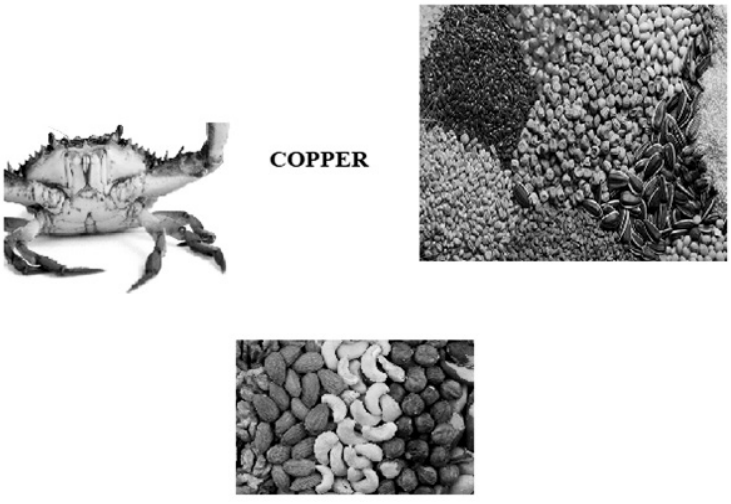
- Deficiency of chloride leads to vomiting, sweating and diarrhea.
- Immune system of our body needs chloride to perform it's functions.

9.4.7 Sulphur

- Suphur is an important mineral in protein synthesis.
- Sulphur is rich in Cheese, Eggs,Nuts, Onions, Cucumbers, Cauliflower and Broccoli.
- Sulphur has a special role to control cell damage.



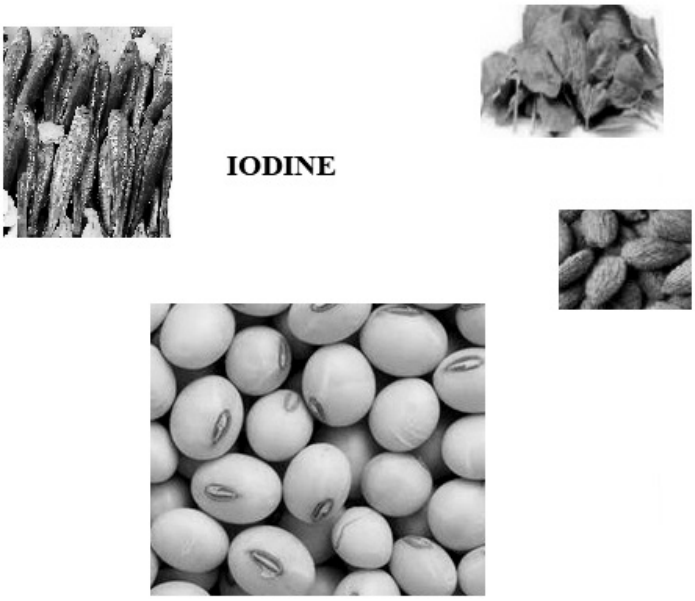
9.4.8 Copper



- Copper is rich in Oysters, Crab, Nuts, Wholegrains and Yeast extract.
- Copper is essential for the formation of red blood cells.

9.4.9 Iodine

- Iodine is an important mineral required for the normal functioning of the thyroid gland.
- Iodine is essential for normal growth and cell development.
- Iodine is necessary for thyroid hormones.
- Energy is generated from foods with the help of iodine.
- An iodine deficiency can lead to goiter.
- Swelling of thyroid gland occurs due to iodine deficiency.
- Radioactive iodine is used for treating thyroid cancer.
- Iodine promotes memory, concentration and thinking skills.
- Iodine is rich in salt, seaweeds and sea foods.



9.5 Microminerals

9.5.1 Iron

- Haemoglobin is an important constituent of blood and it contains iron.
- Iron is rich in meat and chicken, leafy green vegetables, dried fruits

- Iron helps for the transport of oxygen in blood.
- Deficiency of iron leads to anaemia.
- Excess of iron leads to liver and heart diseases and diabetes. In such cases, legumes, grains nuts can be taken to avoid iron absorption.
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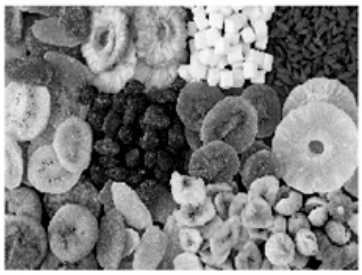
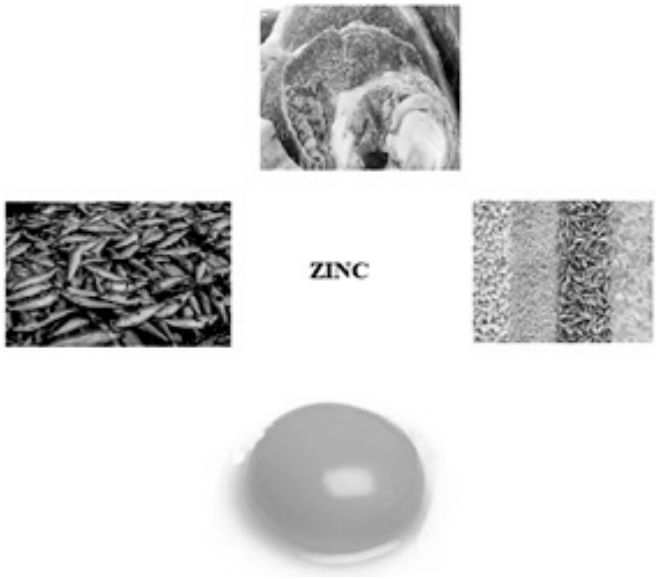


9.5.2 Boron

- Boron is important for strong bones.
- Boron is essential for muscle coordination.
- Boron helps to increase thinking skills.
- Boron is rich in coffee, apple, dried beans and milk.

9.5.3 Zinc

- Zinc is essential to maintain good health.
- Zinc is important for enzymatic reactions.
- Zinc helps to maintain growth and development.
- Zinc helps for supporting healthy skin and proper wound healing.
- Zinc supports sexual maturation and reproduction.
- Zinc is rich in egg yolk, fish, meat, seafood, seeds and grains.
- Zinc deficiency can result in loss of taste and smell.
- Zinc supports the immune system.
- Zinc controls the functioning of the sense organs in the nervous system.
- Zinc regulates division and reproduction.



9.5.4 Selenium

- Selenium helps to get healthy immune system.
- Selenium can be found in meat and grains.
- It helps to keep the blood sugar level.
- It helps to regulate cholesterol levels.

SELENIUM

9.5.5 Chromium

- Chromium is found in whole grains, cereals, mushrooms and meat.
- Chromium helps to break down fats and carbohydrates.
- Chromium is essential for metabolic processes.
- Deficiency of chromium leads to weight loss.
- It enhance protein, carbohydrate and lipid metabolism.

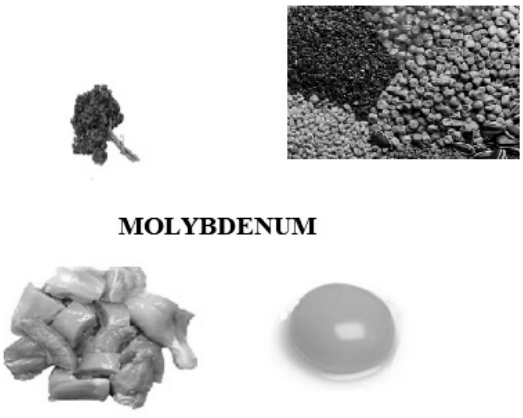
CHROMIUM

9.5.6 Manganese

- Manganese is essential for normal brain and nerve function.

- Manganese supports connective tissue, bones, blood clotting factors and sex hormones.
- It helps for fat and carbohydrate metabolism.
- Manganese is required for calcium absorption and blood sugar regulation.
- Manganese is rich in spinach, pineapple, nuts.
- Manganese deficiency leads to poor bone growth.

9.5.8 Molybdenum



- Molybdenum is rich in beans, liver, cereal grains, peas, legumes and dark green leafy vegetables.
- Molybdenum helps to break down proteins.
- Molybdenum is necessary to process proteins and genetic material.
- Molybdenum is vital for basic functions in our body.
- Molybdenum deficiency leads to poor growth.

CHAPTER

10 GENERAL BIOCHEMICAL PROCEDURES

10.1 Basics of Analysis

10.1.1 Qualitative Analysis

Qualitative analysis is the determination of chemical composition of a sample. By this method, the elements present in a sample can be identified.

10.1.2 Quantitative Analysis

Quantitative analysis is the determination of amount of substance present in a sample.

10.1.3 Solution

A homogeneous mixture of two or more substances which may be solids, liquids, gases, or a combination of solid, liquid and a gas.

10.1.4 Solvent

A substance in which another substance is dissolved, forming a solution.

10.1.5 Solute

The substance dissolved in a solvent in forming a solution.

10.1.6 Strength

The amount of solute in gram present in one litre of the solution.

10.1.7 Normality

Normality is the number of gram equivalents of the substance dissolved per litre of the solution. It is denoted by N.

For example, equivalent weight of NaOH is 40g.

For preparing 1N NaOH, 40g of NaOH is dissolved in 1L of water and for 0.1N NaOH, 4g of NaOH is dissolved in 1L of water.

For 1000mL of water, amount of NaOH required for 0.1N = 4g

For 100mL of water, amount of NaOH required for 0.1N = 0.4g

10.1.8 Molarity

Molarity is the number of moles of solute per litre of the solution. It is denoted by M.

10.1.9 Molality

Molality is the number of moles of the substance dissolved in 1000gms of the solvent.

10.1.10 Standard solution:

A solution whose concentration is known.

10.1.11 Percent Solution

A percentage solution is an amount or volume of chemical or compound per 100 mL of a solution. It is a relative expression of solute to solvent: X amount/100 ml = X%

For preparing 2% NaOH solution, we have to dissolve 2g of NaOH in 100 mL of water.

10.1.12 Buffer

A buffer is a solution that can resist pH change upon the addition of an acid or base. Using buffer solution, pH of a sample can be maintained. Blood acts as buffer in many biological reactions.

10.2 Qualitative Analysis of Biomolecules

10.2.1 Test for Carbohydrate

Molisch's Test

To about few ml of the substance , two drops of Molisch's reagent is added and shaken well. 2ml of conc.H_2SO_4 is added carefully along the sides of the test tube. A violet ring appears which indicates the presence of carbohydrate.

Fehling's Test

To few ml of the solution, 2ml of Fehling's solution(1ml of Fehling's A and 1ml of Fehling's B) is added. It is then boiled in a water bath for 5 mins. Reddish brown precipitate indicates the presence of monosaccharide.

Benedict's test

To 1ml of the solution, 2 drops of Benedict's solution is added. It is then boiled in a water bath for 5 mins. The Colour of solution change from blue to green , yellow, orange or red which indicates the presence of monosaccharide.

Tollen's test

To 1ml of the solution, 2ml of Tollen's reagent (1ml of Tollen's A and 1ml of Tollen's B) is added to the test tube It is then boiled in a water bath for 5 mins.

Iodine test

To about 1ml of the solution , 2 drops of 0.1N HCl and 2 drops of iodine solution are added.

Blue colouration indicates the presence of starch.

10.2.2 Test for monosaccharide

1. Barfoed's Test

To 1ml of the solution, 2ml of Barfoed's solution is added. It is then boiled in a water bath for 5mins. Indication of brick red precipitate shows the presence of monosaccharide.

2. Anthrone test

To 1ml of the solution, 2 drops of anthrone reagent is added along the sides of the test tube and shaken well. (If there is no colour change keep it in a water bath). Green coloration of solution shows the presence of monosaccharide.

3. Seliwanoff's test

To 1ml of the solution, 3 drops of Seliwanoff's reagent is added and boiled in a water bath for 5 mins.Cherry red colour indicates the presence of fructose.

4. Fougler's test

To one drop of a solution in a test tube, 3 drops of Foulger's reagent is added and boiled for 2minutes.Appearance of deep blue colour indicates the presence of fructose.

5. Bial's test

To 2ml of the solution in a test tube, 5 drops of Bial's reagent is added. It is then boiled in a water bath for 5 minutes. Appearance of green colour within 10 minutes shows the presence of pentose.

10.2.3 Test for Proteins

To 1ml of the solution, 5 drops of biuret reagent is added and mixed well. A violet or purple colour indicates the presence of proteins.

10.2.4 Test for Aminoacids

To 1ml of the solution , 1ml of 2% ninhydrin is added. It is then heated in a water bath for 5 mins. A purple colour will appear which shows the presence of aminoacids.

1. Xanthoproteic test

To about 1ml of the solution in a test tube, 0.5ml of conc.HNO3 is added, boiled, cooled. To this excess of 40% NaOH is added. Yellow coloured solution will formed which indicates the presence of aromatic amino acids Tyrosine and Tryptophan.

2. Pauly's test

To 1ml of the solution in a test tube, 1 drop of sulphanilic acid is added and cooled in ice. To this, add 1 drop of sodium nitrite solution, heat, cool and add 2 drops of 1% Na2CO3 solution. Solution turns to red colour which shows the presence of tyrosine and tryptophan.

3. Millon's test(Modified Millon's Test)

To 1ml of the solution in a test tube, 0.5ml of Millon's reagent is added and boiled in a water bath for 10 mins. Cool the mixture and add 5 drops of 1% sodium nitrite solution. Solution turns red which indicates the presence of tyrosine and tryptophan.

4. Ehrlich's Test

To 1ml of the solution, add 1 drop of Ehrlich's reagent is added. The appearance of deep red colour indicates the presence of tryptophan.

5. Hopkins-Cole Test

To 1ml of the solution added 2ml of glacial acetic acid, mixed well and then carefully added 2 drops of conc.H_2SO_4 along the sides of the test tube. A violet ring appears at the junction of liquids indicates the tryptophan.

6. Sodium Nitroprusside Test

To 1ml of the solution 5 drops of freshly prepared 2% solution of sodium nitroprusside and 5 drops of 10% NaOH is added. The solution turns red in colour which shows the presence of cysteine and cystine.

7. Sulphur Test

To 1ml of the solution in a test tube, add 2 drops of 40% NaOH and 1 drop lead acetate solution. The test tube is boiled for a minute and cooled. Brown colour of the solution will turn into black which shows the presence of cystine.

8. Sakaguchi Reaction

To 3ml of the solution in a test tube, add 1drop of 10% NaOH and 2 drops of 1% α-napthol in alcohol. After a few minutes add 1 drop of sodium hypobromite solution (Br_2 in NaOH). The appearance of intense red colour shows the presence of arginine.

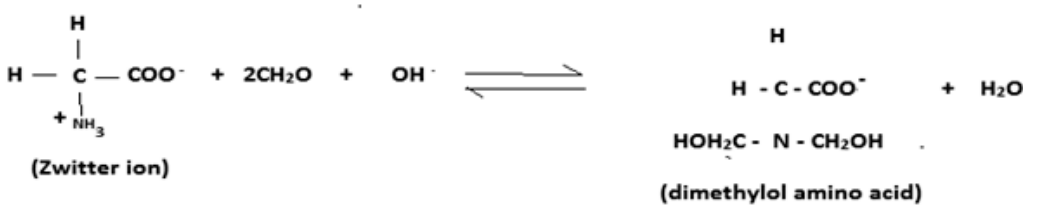
10.3 Estimation of Aminoacids (Glycine) by Formal Titration

10.3.1 Aim

To estimate the amount of glycine present in the whole of the given solution by formal titration. You are provided with exactly 0.1N oxalic acid solution and approximately decinormal solution of sodium hydroxide solution.

10.3.2 Principle

Amino acids contain amino group and carboxyl group. The carboxyl group of α-amino acids react with the basic amino groups to form zwitter ions. Zwitter ions are held together by electrostatic attraction. The zwitterions are not completely decomposed at the end point of alkaline indicators such as phenolphthalein. When amino acid solutions are treated with large excess of neutralysed formaldehyde the amino group combines with formaldehyde to form dimethylol amino acid. This reacts with alkali in the presence of phenolphthalein indicator to give a sharp end point.



$$\text{Equivalent weight of glycine} = \frac{\text{Molecular weight}}{1} = \frac{75}{1} = 75$$

10.3.3 Procedure

Titration I: Standardization of NaOH:

Pipette out 20mL solution hydroxide solution into a clean conical flask. Add a drop of phenolphthalein indicator. Titrate this solution against the standard oxalic acid taken in the burette. The end point is the disappearance of pink colour. Repeat the titration for concordant values. From the titre value the strength of sodium hydroxide solution can be determined.

Titration III

Estimation of Glycine

Titration II: Formaldehyde Versus Sodium hydroxide:

Pipette out 10mL of formalin and 20mL of water into a conical flask. Keep the mixture as such with occasional shaking. After 10 minutes, add a drop of phenolphthalein indicator. Titrate this mixture against the sodium hydroxide taken in the burette. The end point is the appearanceof pale permanent pink colour. Repeat the titration for concordant values.

Titration III: Estimation of Glycine:

Make up the given solution of glycine in a 100mL standard flask. Pipette out 20mL of this made up glycine into a conical flask. Add 10mL of formalin shake the contents well and allow the reaction to take place for 10 minutes. Now add a drop of phenolphthalein indicator. Titrate the contents against the sodium hydroxide taken in the burette. The end point is the appearance of pale, permanent pink colour. Repeat the titration for concordant values. From the titre value the strength and hence the weight of glycine in the whole of the given solution can be calculated.

10.3.4 Result

Weight of glycine present in the whole of the given solution = _____ g.

Titration I: Standardisation of NaOH Std Oxalic acid Vs NaOH

S.No	Volume of NaOH (mL)	Burette Reading (mL)		Volume of Oxalic acid (mL)	Indicator
		Initial	Final		
2.	20				

Volume of Oxalic acid (V_1) = _____ mL

Strength of Oxalic acid (N_1) = _____ 0.1N

Volume of Sodium hydroxide (V_2) = _____ mL

Strength of Sodium hydroxide (N_2) = _____ N

Strength of glycine (N_2) = $\frac{V_1 N_1}{V_2}$ = _____ N

Titration II: Formaldehyde Vs NaOH

S.No	Volume of formaldehyde (mL)	Burette Reading (mL)		Volume of NaOH (mL)	Indicator
		Initial	Final		
1.	20				
2.	20				

Titration III: NaOH Vs Given glycine

S.No	Volume of glycine (mL) + 10mL of formalin (mL)	Burette Reading (mL)		Volume of NaOH (V_y) (mL)	Indicator
		Initial	Final		
1.	20				
2.	20				

Volume of Sodium hydroxide (V_1) = (V_y – V_x) mL

Strength of Sodium hydroxide (N_1) = _____ N

Volume of glycine (V_2) = 20 mL

Strength of glycine (N_2) = $\frac{V_1 N_1}{V_2}$ =_____ N

Weight glycine present in the whole of the given solution = $\frac{N \times 75}{10} g$

= _____ g

From this, the amount of glycine present in the whole of the given solution is calculated.

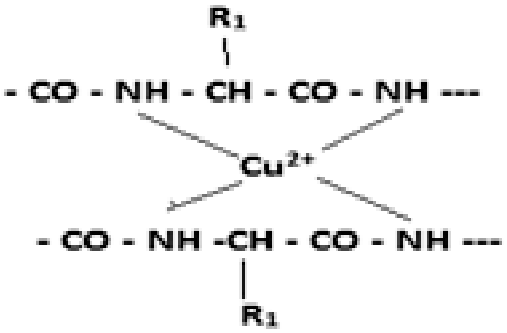
10.4 Estimation of Protein by Biuret Method

10.4.1 Aim

To estimate the amount of protein present in the whole of the given sample of serum by biuret method.

10.4.2 Principle

Cu^{2+} in alkaline solution complexes with nitrogen atoms of the peptide bonds in proteins. This complex gives a purple colour. The colour is measured at 520 n.m (green filter).



10.4.3 Procedure

Prepare a standard solution of protein (stock solution) by dissolving 15 g of Bovine serum albumin in 250 mL of distilled water. Prepare a working standard by diluting 10 mL of stock solution into 100 mL in a standard flask using distilled water. This working standard contains 6 mg of protein / mL. Pipette out into a series of tubes (S1 to S10) 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL of the protein solution and make up the total volume to 5.0 mL with addition of distilled water. A blank tube (B) will contain only 5.0 mL of water. Add 6.0 mL of biuret reagent to each tube and mix well. Keep the test tubes at room temperature for 10 minutes. Measure the optical density of each tube at 520 n.m (green filter) using the reagent blank. Draw a standard graph using concentration along x-axis and optical density along y-axis. Make up the given serum to 100 mL in a standard flask. Pipette out 2 mL and 4 mL from this made up solution into different test tubes (T_1 and T_2) and make up the volume to 5 mL with water and repeat the above same procedure with these test solutions also. Cut the standard graph using the Optical density obtained for the test solutions. This gives the concentration of protein in the test solutions. From this calculate amount of protein present in the sample of serum given.

Estimation of Protein by Biuret method

Volume of Protein (mL)	Volume of Water (mL)	Volume of Biuret reagent (mL)	Optical Density (%T)	Concentration (mg)
1	4	6		
1.5	305	6		
2	3	6		
2.5	2.5	6		

3	2	6		
3.5	1.5	6		
4	1	6		
4.5	0.5	6		
5	0	6		
Unknown I		6		
Unknown II		6		

From the graph, the concentration of unknown protein is determined.

10.5 Estimation of Carbohydrate by Anthrone Method

10.5.1 Aim

To estimate the amount of carbohydrate present in the given sample.

10.5.2 Principle

Carbohydrates react with concentrated sulphuric acid to form furfural or 5- hydroxyl methyl furfurol. Then it condenses with anthrone to form a green coloured complex. Then the absorbance of the sample was measured colorimetrically at 620-640nm.

10.5.3 Procedure

100mg of glucose is dissolved in 100mL of water (stock solution). Pipette out 10mL from stock solution into an 100mL SMF (100µg/mL). Dissolve 0.2g anthrone in 5mL ethanol. Make up the solution to 100mL using 75% sulphuric acid.

0.1 to 1mL of the working standard solution is taken in a series of test tube. Make up the volume of all test tube to 1mL with distilled water. Keep the test tubes in an ice bath and slowly add 5mL of cold anthrone reagent. Mix well. Close the test tubes with aluminium foil and place it in a boiling water bath for 10 minutes.

Cool the test tubes . Measure OD at 620nm. Plot the graph between concentration and absorbance. Then the concentration of carbohydrate in the sample can be determined from the graph.

Estimation of Carbohydrate by anthrone method

Volume of working standard (mL)	Volume of Water (mL)	Concentration of working sample (µg/mL)	Volume of anthrone (mL)	OD at 620nm
0.1	0.9	10	5	

0.2	0.8	20	5	
0.3	0.7	30	5	
0.4	0.6	40	5	
0.5	0.5	50	5	
0.6	0.4	60	5	
0.7	0.3	70	5	
0.8	0.2	80	5	
0.9	0.1	90	5	
1	0	100	5	

From the graph, the concentration of unknown carbohydrate is calculated.

10.6 Techniques for Sample Preparation

Sample preparation is the series of steps required to prepare a sample in a suitable form for analysis. The faster these steps can be done, the more quickly the analysis will be completed. Sample preparation has been considered not as a part of the analytical process, rather the "procedure" that had do be done to develop and perform analytical methods.

Sample preparation may involve dissolution, extraction, reaction with some chemical species, pulverizing, treatment with a chelating agent (e.g. EDTA), masking, filtering, dilution, sub-sampling or many other techniques. Treatment is done to prepare the sample into a form ready for analysis by specified analytical equipment. Sample preparation could involve: crushing and dissolution, chemical digestion with acid or alkali, sample extraction, sample clean up and sample pre-concentration.

However, the significance of the sample preparation for the total analytical performance is nowadays widely recognized.

10.7 Ultrafiltration

Ultrafiltration (UF) is a membrane filtration process similar to Reverse Osmosis, using hydrostatic pressure to force water through a semi-permeable membrane. The pore size of the ultrafiltration membrane is usually 103 - 106 Daltons. Ultrafiltration (UF) is a pressure-driven barrier to suspended solids, bacteria, viruses, endotoxins and other pathogens to produce water with very high purity and low silt density.

Ultrafiltration (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semi permeable membrane. Suspended solids and

solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. Ultrafiltration is not fundamentally different from reverse osmosis, microfiltration or nanofiltration, except in terms of the size of the molecules it retains.

A membrane or, more properly, a semi permeable membrane, is a thin layer of material capable of separating substances when a driving force is applied across the membrane. Once considered a viable technology only for desalination, membrane processes are increasingly employed for removal of bacteria and other microorganisms, particulate material, and natural organic material, which can impart color, tastes, and odors to the water and react with disinfectants to form disinfection by products (DBP).

10.8 Lyophilization

10.8.1 Introduction

Lyophilization is a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Lyophilization works by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate.

10.8.2 Phases of Lyophilization

Lyophilization occurs in three phases, with the first and most critical being the freezing phase. Proper lyophilization can reduce drying times by 30%.

i) Freezing Phase

There are various methods to freeze the product. Freezing can be done in a freezer, a chilled bath (shell freezer) or on a shelf in the freeze dryer. Cooling the material below its triple point ensures that sublimation, rather than melting, will occur. This preserves its physical form.

Lyophilization is easiest to accomplish using large ice crystals, which can be produced by slow freezing or annealing. However, with biological materials, when crystals are too large they may break the cell walls, and that leads to less-than-ideal freeze drying results. To prevent this, the freezing is done rapidly. For materials that tend to precipitate, annealing can be used. This process involves fast freezing, then raising the product temperature to allow the crystals to grow.

ii) Primary Drying (Sublimation) Phase

Lyophilization's second phase is primary drying (sublimation), in which the pressure is lowered and heat is added to the material in order for the water to sublimate. The

vacuum speeds sublimation. The cold condenser provides a surface for the water vapor to adhere and solidify. The condenser also protects the vacuum pump from the water vapor. About 95% of the water in the material is removed in this phase. Primary drying can be a slow process. Too much heat can alter the structure of the material.

iii) Secondary Drying (Adsorption) Phase

Lyophilization's final phase is secondary drying (adsorption), during which the ionically-bound water molecules are removed. By raising the temperature higher than in the primary drying phase, the bonds are broken between the material and the water molecules. Freeze dried materials retain a porous structure. After the lyophilization process is complete, the vacuum can be broken with an inert gas before the material is sealed. Most materials can be dried to 1-5% residual moisture.

10.8.3 Problems in Lyophilization

- Heating the product too high in temperature can cause melt-back or product collapse
- Condenser overload caused by too much vapor hitting the condenser.
  - Too much vapor creation
  - Too much surface area
  - Too small a condenser area
  - Insufficient refrigeration
- Vapor choking – the vapor is produced at a rate faster than it can get through the vapor port, the port between the product chamber and the condenser, creating an increase in chamber pressure.

10.8.4 Critical Temperature

During lyophilization, the maximum temperature of the product before its quality degrades by melt-back or collapse.

Lyophilization is a commonly used technique for formulation development of small molecules which are unstable in aqueous medium and are thermolabile in nature. Lyophilization of drug alone, however, presents certain formulation development challenges, which may be overcome by incorporation of excipients (e.g. bulking agents, buffering agents, tonicifying agent, wetting agent and cosolvents, preservatives and collapse temperature modifiers) in the formulation.

10.9 Quantitative Estimation of Lipid

10.9.1 Determination of Iodine Number

- The iodine number of a fat is the amount in gm. of iodine taken up by 100 gm. of fat. Not only iodine but also equivalent amounts of other halogens will add at double bonds; so bromine is often used instead of iodine because it is more reactive.
- The halogenating reagent used in this method is pyridine sulphate di-bromide. This reagent can be prepared by adding carefully 8.1 ml pyridine in 20 ml glacial acetic acid and making the volume up to 1 litre with glacial acetic acid.
- Weigh the bottle containing sample of oil plus a medicine dropper and then transfer about 0.1 to 0.3 gm. of oil to a flask. Reweigh the bottle containing oil and dropper to find out the exact quantity of the sample transferred. Add 10 ml of chloroform and then 25 ml of the pyridine sulphate di-bromide reagent.
- Shake thoroughly; allow standing for 5 minutes and then determine the residual bromine. To do this, add 10 ml of 10% KI and titrate the equivalent amount of iodine liberated by the residual bromine with the help of 0.1 (N) $Na_2S_2O_3$ (sodium thiosulphate). The titration can be done by adding sodium thiosulphate solution through a burette to the flask.
- When the colour of the solution in flask becomes light yellow add 1 ml of starch solution. It will become blue. Slowly add the thiosulphate solution again till it becomes colourless. Note the total volume of thiosulphate used.
- The total amount of bromine originally added is found by titrating 25 ml of the pyridine sulphate di-bromide reagent with thiosulphate after adding KI as in the previous case. The amount of bromine taken up by the fat sample can be determined by the difference between the two titers and then the iodine number can be calculated.

10.9.2 Quantitative Estimation of Cholesterol

- Shake the tubes well and keep them at room temperature for 30 minutes. Blue colour will develop in all the tubes except blank tube. Measure the absorbancies at 625 m|a. against the blank tube and plot these against the amount of cholesterol.
- Acetic anhydride-sulphuric acid reagent has to be freshly prepared before use. Acetic anhydride (20 ml) is taken in a glass stoppered flask which is then chilled in ice water. When cold, add 1 ml of conc. H_2SO_4 to it drop by drop.

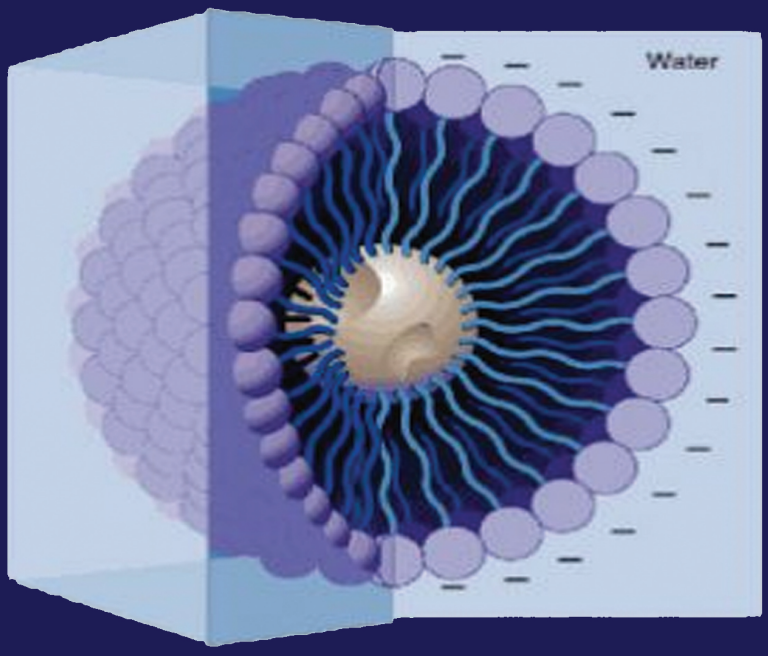
- The contents are mixed and cooled during the addition. After completion of the addition the flask is stoppered and shaken vigorously for a few minutes. The solution has to be kept cold in ice and should be used within an hour.

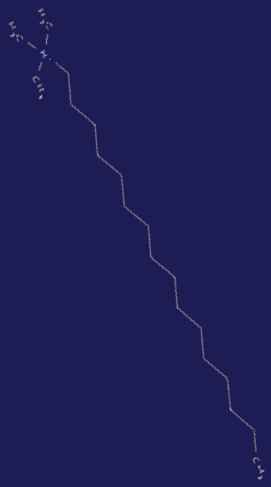
10.10 Quantitative Estimation of Fatty Acid

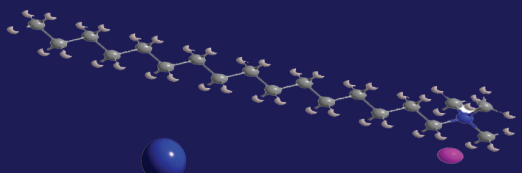
Free fatty acids (FFA) in plant oils and fats (e.g. edible oils and fats) are a quality feature for these fats. Fats with high levels of FFA are more susceptible to oxidative aging, they become rancid more quickly. The FFA should be removed during a refining process.

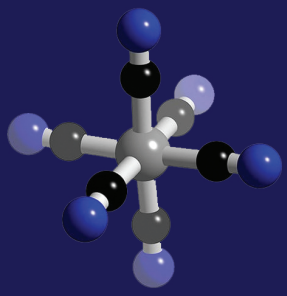
Determination of the FFA in Oils and fats is done by potentiometric titration in Ethanol / Diethyl ether as solvent with KOH in Isopropyl alcohol.

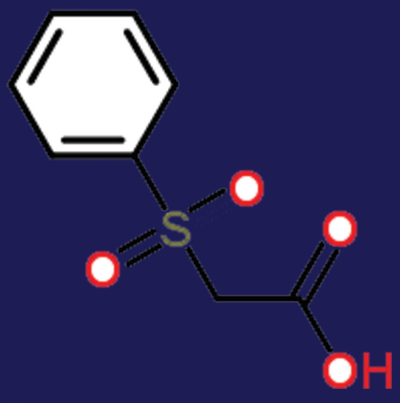
Kinetic and Mechanistic Approach to Oxidation Reaction

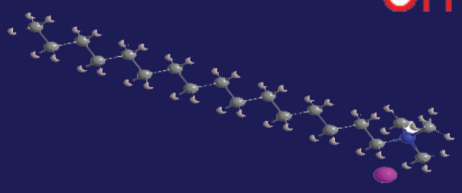


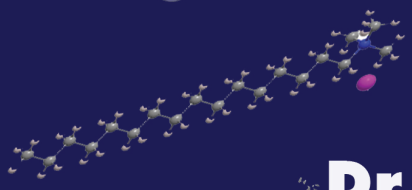












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Kinetic And Mechanistic Approach To Oxidation Reaction

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ABBREVIATION

PSAA - Phenylsulphonylacetic acid

CTAB - Cetyltrimethylammonium bromide

CMC - Critical Micellar Concentration

I - Ionic Strength

s - Standard deviation

r - Correlation coefficient

k_1 - Observed rate constant

k_2 - Overall rate constant

CONTENT

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CHAPTER I

INTRODUCTION

1. Introduction to Chemical Kinetics

Chemical kinetics is the study of the speed with which a chemical reaction occurs and the factors that affect this speed. This information is especially useful for determining how a reaction occurs.

Chemical kinetics tells us the speed at which chemical species transform into new substances by breaking and reforming their molecular bonds. In other words, it is the study of how fast chemical reactions proceed from reactants to products.

Chemical kinetics also called reaction kinetics helps us understand the rates of reactions and how it is influenced by certain conditions. It further helps to gather and analyze the information about the mechanism of the reaction and define the characteristics of a chemical reaction.

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1.1 Importance and Aim of Chemical Kinetics

This is an important topic because while thermodynamics tells us about the direction of spontaneous change, it is silent as to how fast processes will occur, i.e., in itself tells nothing about its rate.

A major goal in chemical kinetics is to determine the sequence of elementary reactions or the reaction mechanism that comprise complex reactions. It is useful to derive rate laws for complex reaction mechanisms, including reversible, parallel and consecutive reactions.

1.2 Terminology

1.2.1 Reactants

Substances which undergo chemical reactions are called reactants. In a chemical reaction, these reactants are converted into new substances.

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1.2.3 Products

The substances which are the end products of a chemical reaction are called products. In other words, new substances that are formed due to the chemical reactions are all called products.

1.2.4 Rate of a reaction

The reaction rate or rate of reaction is the speed at which a chemical reaction takes place, defined as proportional to the increase in the concentration of a product per unit time and to the decrease in the concentration of a reactant per unit time. Reaction rates can vary dramatically.

A rate law describes the relationship between reactant rates and reactant concentrations. Reaction rates are usually expressed as the concentration of reactant consumed or the concentration of product formed per unit time. The unit of rate of

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reaction is given by concentration/time that is (mol/L)/sec.

1.2.4.1 Rate of Formations and Disappearances

In any chemical reaction, as the reaction proceeds, the amount of reactants decreases, whereas the amount of products increases. One has to understand that the rate of the overall reaction depends on the rate at which reactants are consumed or the rate at which the products are formed.

If a graph is plotted between the concentration of reactants and products and time, rate of formation of products and rate of disappearance of reactants can be easily calculated from the slope of curves for products and reactants. The overall rate of the reaction may or may not be equal to the rate of formations and disappearances.

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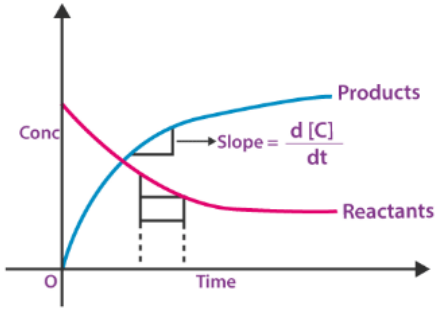
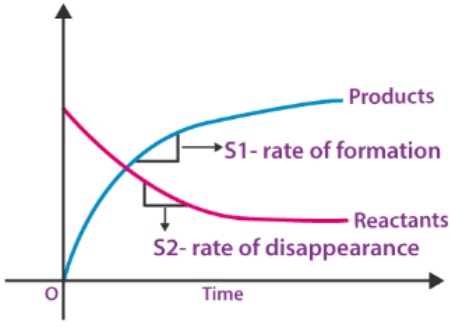


Figure 1.1 *(a) Product concentration is zero at time t = 0*



(b) at time t = 0, both reactants and products are present

From the graph, it is understood that the slope of the reactants curve is negative and that for product curve is positive, indicating the concentration of reactants and products, decreases and increases respectively. We will take a simple reaction as an example to

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illustrate how the rate of overall reactions, rate of disappearances of reactants and rate of formation of products are related.

1.2.4.2 Factors Affecting the Reaction Rate

The various factions that can affect the rate of a chemical reaction are listed in this subsection.

i) Nature of the reaction

- The rate of reaction highly depends on the type and nature of the reaction. As mentioned earlier, few reactions are naturally faster than others while some reactions are very slow.

- The physical state of reactants, number of reactants, complexity of reaction and other factors highly influence the reaction rate as well.

- The rate of reaction is generally slower in liquids when compared to gases and slower in solids when compared to liquids. Size of the

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reactant also matters a lot. The smaller the size of reactant, the faster the reaction.

ii) Concentration

- According to the collision theory, the rate of reaction increases with the increase in the concentration of the reactants.
- As per the law of mass action, the chemical reaction rate is directly proportional to the concentration of reactants.
- This implies that the chemical reaction rate increases with the increase in concentration and decreases with the decrease in the concentration of reactants.
- Time plays a major role in changing the concentration of reactants and products. Therefore, even time is a vital factor affecting the reaction rate.

iii) Pressure factor

- Pressure increases the concentration of gases which in turn results in the increase of

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the rate of reaction. The reaction rate increases in the direction of less gaseous molecules and decreases in the reverse direction.

- Thus, it can be understood that pressure and concentration are interlinked and that they both affect the rate of reaction.

iv) Temperature

- According to collision theory, a chemical reaction that takes place at a higher temperature generates more energy than a reaction at a lower temperature.

- This is because colliding particles will have the required activation energy at high temperature and more successful collisions will take place.

- There are some reactions that are independent of temperature. Reactions without an activation barrier are examples of chemical reactions that are independent of temperature.

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v) Solvent

The rate of reaction also depends on the type of solvent. Properties of solvent and ionic strength highly affect the reaction rate.

vi) Order of the reaction

The order of reaction manages how the reactant pressure or concentration affects the rate of reaction.

vii) Electromagnetic Radiation

Electromagnetic radiation is a form of energy and its presence at the chemical reaction may increase the rate of reaction as it gives the particles of reactants more energy.

Viii) Intensity of Light

Even the intensity of light affects the rate of reaction. Particles absorb more energy with the increase in the intensity of light thereby increasing the rate of reaction.

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ix) Presence of Catalyst

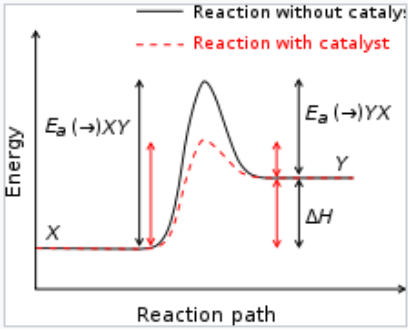

Figure 1.2 *Effect of catalyst*

- A catalyst can be defined as a substance that increases the rate of the reaction without actually participating in the reaction. The definition itself describes its effect on chemical reactions.
- The presence of a catalyst increases the speed of reaction in both forward and reverse reaction by providing an alternate pathway which has lower activation energy.

x) Surface Area of the Reactants

The surface area of reactants affects the rate of reaction. If the size of a particle is small,

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the surface area will be more and this increases the speed of heterogeneous chemical reactions.

Meanwhile, chemical kinetics has gained a critically significant role in the world today. The reaction rate (both average and instantaneous) is enabling engineers and scientists around the globe to optimize the process parameters in order to get the most desired results from a chemical reaction in the most economical and safe way.

Chemical kinetics along with its critical role in the manufacturing industry has also served as a base for further advances in the fields of reaction engineering and biochemical engineering.

Any chemical reaction contains the following two constituents,

- Reactants
- Products

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The role these constituents play in chemical reactions is briefly described below. Important concepts in chemical reactions such as activation energy are also described.

1.2.5 Order of the Reaction

From experimental observations, scientists have established that reaction rates almost always have a power-law dependence on the concentrations of one or more of the reactants. In the following sections, we will discuss different power laws that are commonly observed in chemical reactions.

1.2.6 Molecularity of the Reaction

If the reactions are elementary reactions, (i.e. they cannot be expressed as a series of simpler reactions), then we can directly define the rate law based on the chemical equation.

1.3 Effect of Temperature

When molecules collide, the kinetic energy of the molecules can be used to stretch,

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bend, and ultimately break bonds, leading to chemical reactions. If molecules move too slowly with little kinetic energy, or collide with improper orientation, they do not react and simply bounce off each other. However, if the molecules are moving fast enough with a proper collision orientation, such that the kinetic energy upon collision is greater than the minimum energy barrier, then a reaction occurs.

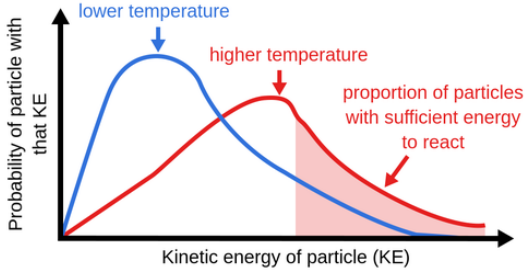

Figure 1.3 *Effect of temperature*

1.4 Potential Energy Surfaces

A potential energy surface (PES) describes the potential energy of a system, especially a collection of atoms, in terms of certain parameters, normally the positions of

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the atoms. The surface might define the energy as a function of one or more coordinates; if there is only one coordinate, the surface is called a potential energy curve or energy profile.

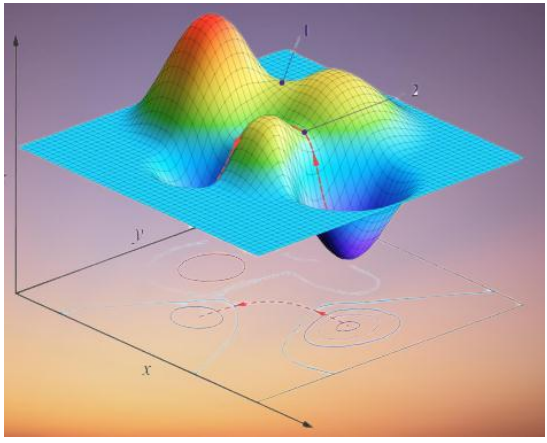

Figure 1.4 *Potential Energy Surface*

1.6 Theories of Reaction Rates

The macroscopic discussion of kinetics discussed in previous sections can be now expanded into a more microscopic picture in terms of molecular level properties (e.g, mass and velocities) involving two important theories:

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i) Collision theory

ii) Transition-state theory

1.7 Isotope Effects in Chemical Reactions

The kinetic isotope effect (KIE) is a phenomenon associated with isotopically substituted molecules exhibiting different reaction rates. Isotope effects such as KIEs are invaluable tools in both physical and biological sciences and are used to aid in the understanding of reaction kinetics, mechanisms and solvent effects.

1.8 Reactions in Solution

Most of the complications of kinetics and rate processes in liquid solutions arise from the much higher density of the liquid phase. In a typical liquid solution, the solvent molecules massively outnumber the reactant solute molecules, which tend to find themselves momentarily (~10 – 11 sec) confined to a "hole" within the liquid.

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1.9 Activation Energy

Activation energy can be defined as the minimum amount of energy that is required to activate molecules or atoms so that they can undergo chemical transformation. This minimum energy is to overcome the energy barrier is called activation energy.

Similarly, chemical kinetics is a part of physical chemistry that is related to the study of reaction rates. It has many applications that include enzymology, chemical engineering, and environmental engineering.

In a chemical reaction, products are formed due to the collision between the reactant molecules.

The conditions for the collisions to form products are:

- Collisions should be effective.
- The right orientation of reactant molecules towards each other.

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- All molecules should possess a minimum amount of energy to form product molecules.

As the chemical reaction advances, the concentration of reactants will decrease and the concentration of products will increase.

1.10 Kinetics in Redox reactions

Redox reactions have long been a central focus in mechanistic organic chemistry. This interest stems partly with the arrival of new oxidising agents which will bring about certain oxidations selectively. The development of new strategies for the selective oxidation of organic substrates continues to be an important goal in chemistry. Inorganic redox reactions are also of primary importance in biological systems.

There are two major mechanistic pathways for the oxidation of organic substrates by oxidising agents. The first one involves S_N2 type mechanism, where

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nucleophilic attack of a donor atom of the substrate on the oxidising agent takes place. The second mechanism involves a single electron transfer from organic substrate to oxidant with the formation of cation radical.

Though bimolecular nucleophilic substitution of organic substrate on the oxidant has been postulated in most of the oxidation reactions, an electron transfer to a metal ion or metal complex in the rate determining step is suggested only in a few cases. The redox reaction of organic substances with metal complexes is not as simple as in the case of inorganic reactants. Only recently, electron transfer (ET) concept in organic chemistry is being given much attention.[1-3] The electron detachment from most electron-rich organic donor (D) generates transient cation radical and the analogous electron attachment to electron-poor organic acceptors (A) generally affords transient anion radical.[4,5] This leads to a mechanistic situation

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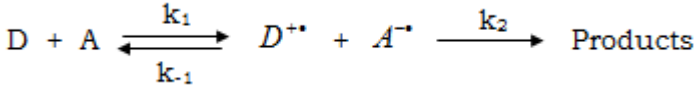
in which the stepwise formation of products via electron transfer is kinetically difficult to distinguish from a concerted single step process, especially when back electron transfer and the follow up steps are facile.



Iron is truly ubiquitous in living systems. It is at the active centre of molecules responsible for oxygen transport and electron transport and it is found in, or with, such diverse metalloenzymes as various oxidases, hydrogenases, reductases dehydrogenases, deoxygenases and dehydrases.[6-10] Not only iron is involved in an enormous range of functions, it is also found in the whole gamut of life forms from bacteria to man.

In biological systems, there are three well-characterised iron systems. Proteins that contain one or more iron-porphyrin[7] units such as haemoglobin, myoglobin and

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cytochrome P-450, a diverse group of proteins that contain non-heam iron, in particular the iron-sulphur clusters[11] like nitrogenase[12], rubredoxin[13] and ferridoxins[14] and the non-heam diiron oxo-bridged species, most of which have carboxylates such as haemerythrin[15], methane mono oxygenase[16] and ribonucleotide reductase.[17]

Organic sulphur compounds play an important role as structural elements in many materials and biological macromolecules.[18,19] Because of the high susceptibility to oxidation, any attempt to maintain the integrity of the sulphur function requires a detailed understanding of the underlying mechanism of oxidation as well as the physico-chemical parameters which control the oxidation reaction. The nature of the sulphur functional group, the nature and the strength of the coupling of the neighbouring group affect the ultimate course of the oxidation in organic sulphur compounds. The detailed studies on

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the mechanism of these oxidation reactions have provided important information on the reaction intermediates as well as steric and electronic effect of substituents on the reactivity of such intermediates

Some of the oxidation reactions of organic sulphur compounds, viz., organic sulphides, sulphoxides, thioacetic acids and sulphur containing aminoacids with different oxidising agents are briefly discussed below.

1.10.1 Oxidation with iron compounds

Rajagopal and co-workers[20] have used iron(III)-polypyridyl complexes $[Fe(NN)_3]^{3+}$ (where, NN = 2,2′-bipyridine and 1,10-phenonthroline) for the oxidation of organic sulphides in aqueous methanol medium. The redox reactions are of total second-order, first-order each in oxidant and the substrate. The author proposed an electron transfer from the sulphide to the oxidant with the formation of the sulphide radical cation. The study of effect of anionic and cationic micelles on this

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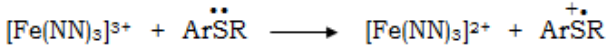
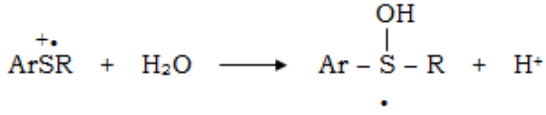
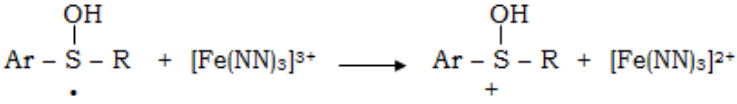
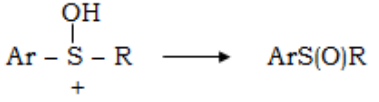
reaction showed that the reaction was catalysed by both micelles.[21]









A mechanism similar to the sulphide oxidation has been proposed for the kinetics of electron transfer from sulphoxides and arylthioacetic acid to $[Fe(NN)_3]^{3+}$ by John Adaikalasamy et al.[22]

The oxidation of sulphides and sulphoxides to (sulphoxides and sulphones respectively) by microsomal cytochrome P-450, horse radish peroxidase and iron porphyrins have been extensively studied.[23-30] This

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reaction involves an oxygen atom transfer to sulphur from oxo-iron compound or electron transfer reaction leading to the formation of cation radical.

A detailed study on the kinetics of oxygenation of organic sulphides[31] and sulphoxides[32] with six oxo(salen) iron complexes in CH_3CN has been carried out by Sivasubramanian and co-workers. The reaction is found to be first order in the oxidant and fractional-order in the substrate. The redox reaction proceeds through Michaelis–Menten kinetics.

Naruta et al.[33-34] have studied the asymmetric oxidation of sulphides with iodosylbenzene catalysed by ferric porphyrins bearing chiral binaphthalene moieties.

1.10.2 Oxidation with chromium compounds

Srinivasan et al.[35-39] and others have investigated the oxidation of aryl methyl sulphides, aryl methyl sulphoxides,

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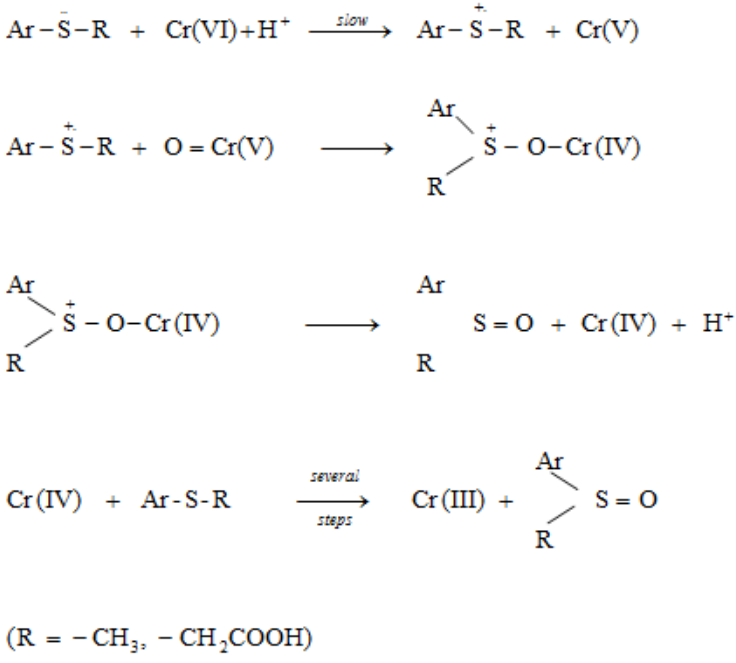
arylthioacetic acids by Cr(VI) in 50% (V/V) aqueous acetic acid. The reaction follows on overall second-order kinetics, first-order in each reactant. It has been observed that $[H^+]$ accelerated the oxidation rate. Studies with para-substituted phenyl methyl sulphides, sulpoxides and thioacetic acids reveal that electron-donating groups facilitate the rate of oxidation while electron-withdrawing groups retard it.



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On the basis of the observed kinetic results, a mechanism involving one-electron transfer from the sulphur atom of the substrate to Cr(VI) in the rate determining step has been postulated.

A similar electron transfer mechanism has been postulated for the oxidation of organic suphoxides. They have also studied the effect of picolinic acid on the Cr(VI) oxidation of above substrates. The picolinic acid catalysed Cr(VI) oxidation follows third-order kinetics, first-order each in the oxidant, sulphide and catalyst at constant $[H^+]$.

The oxidation of organic sulphide by pyridinium fluorochromate has been studied by Banerji[40] and a mechanism involving a rate-determining electrophilic oxygen atom transfer from oxidant to the sulphide has been proposed. The kinetics of organic sulphides and several para-substituted phenylmehtyl sulphides by pyridinium chlorochromate has been studied in binary solvent mixtures of 60%

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(V/V) aqueous acetic acid and 50% (V/V) chlorobenzene – nitrobenzene by Panigrahi et al.[41] and Rajasekaran et al.[42] A Michaelis-Menten kinetics has been proposed for the reaction.

The oxidation of 34 organic sulphides in 19 different solvents by 2,2′-bipyridinium chlorochromate by Vyas et al[43] and pyridinium bromochromate by Loonker et al.[44] have been carried out at various temperatures. The authors have brought out the important role played by the solvent. A mechanism involving electophilic oxygen atom transfer from oxidant to the sulphide in the rate-determining step has been proposed.

Ganesan et al.[45,46] have studied the oxygenation reaction of organic sulphides with carboxylate bound oxochromium(V) complexes in aqueous acetonitrile. A mechanism involving outer sphere electron transfer from sulphide to Cr(V) in the rate determining step has been proposed. Picolinic acid, 2,2′-bipyridine and

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1,10-Phenanthroline and anionic and cationic micelles catalyse the above reactions.[47,48]

Oxosalen complexes of Cr(V) have also been used for the oxidation of sulphides and sulphoxides by Sevvel et al.[49] and Venkataramanan.[50]

1.10.3 Oxidation with manganese compounds

Chellamani et al.[51,52] have studied the kinetics of oxidation of several aryl methyl sulphides with several in situ generated cationic oxo(salen)manganese(V) complexes in acetonitrile. Electron-donating groups in the phenyl ring of ArSMe accelerate the rate, while electron-withdrawing groups have the retarding effect. On the other hand, electron withdrawing substituents at 5-position of the salen ligand enhance the reactivity, while the electron-donating substituents retard it. The authors have also studied the oxidation of organic sulphoxides[53] oxo(salen) manganese(V) complexes.

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The reaction between MnO_4^- and organic sulphides involving a rate determining electrophilic oxygen transfer from permanganate ion to the sulphide has been suggested by Banerji.[54] However, Lee and co-workers[55,56] have shown that the oxidation of arylthioacetic acids by permanganate ion is more consistent with a mechanism initiated by a reaction between an unshared pair of sulphur electron with an empty d-orbital of manganese. However, a mechanism involving nucleophilic attach of MnO_4^- on the sulphur atom of the sulphoxide has been proposed for the permanganate ion oxidation of diaryl sulphoxides.[57]

The kinetics and mechanism of the oxidation of organic sulphides[58] by [bis(2,2′-bipyridyl)copper(II)] permanganate follows Michaelis – Menten type kinetics.

1.11 Catalysis by surfactant aggregates

The enormous recent interest in supramolecular chemistry, the chemistry of

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molecular organised assemblies, reflects the current emphasis on non-covalent inter molecular interactions and molecular recognition. In this field, chemical research has entered into new dimensions leading to the design and understanding of hierachies of physically associated molecules.

Surfactant self-organisation constitutes a major area of interest. Particularly micelles and vesicles have received great attention. The special properties of surfactants are important in a wide variety of applications in chemistry, biology, engineering, materials science and other areas. The relation between surfactant structure and morphology of the aggregate as well as the analysis of the different molecular interaction determine the properties of the aggregate.

Catalysis by micelles involves at least three main steps.

i) Binding of the substrate(s) to the micelle

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ii) The actual chemical transformation in the micellar surface)

iii) Release of product(s)

The micellar solution can be viewed as a micro heterogeneous system, the micelle-catalysed reaction is always influenced by a local medium effect, characteristic of the "surface micropolarity" of the micelle.

1.12 Micelles

Micelles are aggregates of surfactant molecules suspended in water. Surfactant dissolves completely in water at very low concentration, but above a certain level, the Critical Micellar Concentration (CMC), the molecules form globular aggregates, called micelles.

Surfactant molecules self-aggregate into super-molecular structures when dissolved in water. The simplest aggregate of these surfactant molecules is called a micelle, and the dispersion of the aggregates in water is referred to as a micellar solution. A typical

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micelle has size of ~50 A^0 and is made of about 100 surfactant molecules. In general, these pseudo particles could be spherical, cylindrical, ellipsoidal or disc-like in shape.

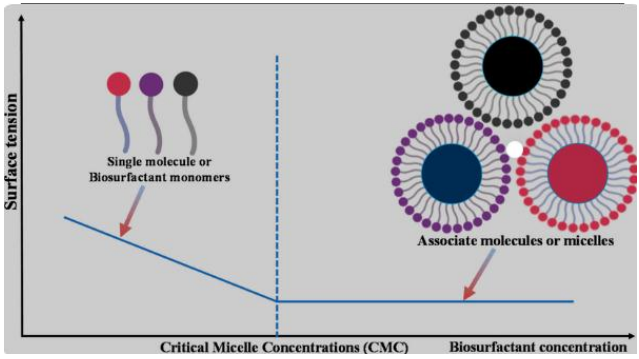

Figure 1.5 *Critical Micellar Concentration (CMC)*

The intermolecular forces between the surfactant molecules in presence of water are weak and can be easily modified by manipulating them by addition of salts (that is by reducing or increasing electrostatic effects). Hence, micellar solutions exhibit interesting properties on addition of salt or with a change in temperature.

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For example, the micellar solutions of CTAB become extremely viscous on addition of small quantities of sodium salicylate. Triton X-100 micellar solutions separate into two phases - one rich and the other dilute in micellar concentration. The structure of a micelle depends both on the architecture of the constituent surfactant molecule and the solution conditions such as temperature presence of impurities etc. The inter-particle interaction between micelles also depends on several different parameters. Individual surfactant molecules that are in the system but are not part of a micelle are called “monomers”.

A typical micelle in aqueous solution forms an aggregate with the hydrophilic “head” regions in contact with surrounding solvent. The polar region, called, the head group may be either neutral, cationic, anionic or zwitter ionic. Depending on the charge of the head region, micelles can be cationic, anionic or

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nonionic. However, the lipophilic 'tails' of surfactant molecules have less content with water when they are part of a micelle - this being the basis for the energetic drive for micelle formation. In a micelle, the hydrophobic tails of several surfactant molecules assemble into an oil-like core, the stable form of which has no contact with water.

Micelles composed of ionic surfactants have an electrostatic attraction to the ions that surround them in solution, the latter known as counter ions. Although the closest counter ions partially mask a charged micelle, the miceller charge affects the structure of the surrounding solvent at appreciable distance from the micelle. Ionic micelles influence many properties of the mixture, including its electrical conductivity. Adding salts to a colloid containing micelles can decrease the strength of electrostatic interactions and lead to the formation of larger ionic micelles. This is more

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accurately seen from an effective change in hydration of the system.

1.12.1 Cetyltrimethylammonium bromide

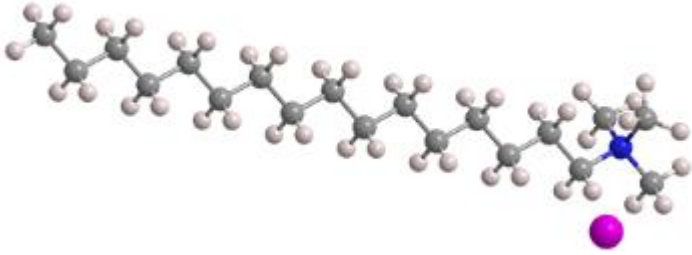

Figure 1.6 *Structure of Cetyltrimethylammonium bromide*

N-Cetyl-N,N,N-trimethylammonium bromide (CTAB) is a cationic detergent, soluble in water and readily soluble in alcohol. At 303 K it forms micelles with aggregation number 72-120. Cardiovascular effects of CTAB has been extensively used as a core for controlled drug release[59] and enhancement of radiotherapy.[60] The cardiovascular action of CTAB monolayer – protected nanogold has been described by Brust et al.[61] The control studies with nanogold and CTAB did not affect the blood pressure. The cetyltrimethylammonium cation

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has a wide spectrum of anti-infective against bacteria and fungi. CTAB is one of the components of the topical antiseptic cetrimide. It also provides a buffer solution for the extraction of DNA.

The kinetics of binding of cationic surfactant, CTAB, with sodium salt of carboxy methyl cellulose was studied[62] by the electrometric method. The beneficial effect of the addition of CTAB during electro deposition of lead dioxide was also investigated.[63]

The inhibitive action of CTAB towards the corrosion of mild steel in sulphuric acid has been investigated by weight loss and polarization techniques. The percentage inhibition varies with the nature and concentration of the inhibitors, temperature and pH of the medium. The corrosion inhibition is explained by considering adsorption or complex formation by the inhibitors on the corroding mild steel surface.

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1.13 General survey of phenylsulphonyl acetic acid

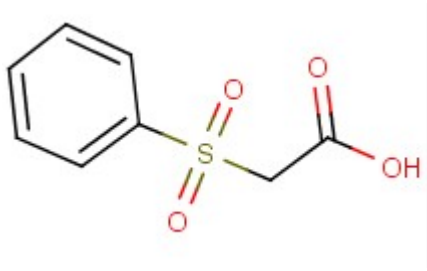

Figure 1.7 *Structure of phenylsulphonylacetic acid*

The existence of so many valence states for sulphur has generated selective and novel ways to effect oxidation, dehydration and carbon-carbon bond formation. The sulphonyl group has an ability to stabilize negative charge on adjacent carbon atom thus providing a method to form carbon-carbon bonds. These α-sulphonyl carbanions continue to evolve new reactions and sequences which facilitate the design of the total synthesis of complex organic molecules. One such example is the synthesis of α,β-unsaturated sulphones by phase transfer condensation[64] of α-sulphonyl carbanions with aldehydes using aqueous

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sodium hydroxide-dichloromethane in presence of a small amount of triethylbenzyl-ammonium chloride. α,β-unsaturated sulphones have also been synthesized by Baliah[65] through the condensation of arylsulphonylacetic acid with aldehydes in the presence of benzylamine catalyst in acetic acid.

Eventhough carbonyl and sulphonyl groups are analogous in nature, the sulphonyl group cannot participate in the kind of conjugation observed for the carbonyl group. This fact has been considered by Baliah and Shanmuganathan[66] as an evidence to show that the sulphur-oxygen bonds in sulphones are semi-polar.

Phenylsulphonylacetic acid and several substituted phenylsulphonylacetic acids are used as a starting material for the preparation of a number of diiodomethyl sulphones of the type $ArSO_2CHI_2$, which are useful as pesticides and seed disinfectants. They are also useful for

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the preparation of latex coatings and vinyl films.

Only the ionisation constants[67,68] of arylsulphonylacetic acids have been measured. No other detailed studies have been carried out with arysulphonylacetic acids. Pasto et al.[68] have determined Hammett ρ values for a series of phenylmercapto, phenylsulfinyl and phenylsulphonylacetic acids in water and 50% (v/v) dioxane-water medium. Comparison of these ρ values indicates that the sulfinyl group is the least effective group for the transmission of inductive effects, with the sulphonyl group of intermediate effectiveness. This phenomenon can be explained by hydrogen bonding. In phenylsulphinylacetic acid no intra-molecular hydrogen bonding is possible. The transmission of inductive effects by the sulphinyl group may be partially shunted into the highly polar sulphur – oxygen bond. In phenylsulphinylacetic acid intra-molecular hydrogen bonding is possible and a portion of

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the substituent effect may be transmitted directly the sulphur oxygen bond, as well as through the methylene group.

Pasto and Kent[67] have measured the ionisation constants of phenylmercapto-, phenylsulfinyl- and phenylsulphonyl acetic acids in water ethanol and water-dioxane solvent systems. An inversion of the relative acidities of phenylsulphinyl and phenylsulphonylacetic acids is observed, the phenylsulphonyl acetic acid being the stronger acid of the two in pure water but the weaker acid of the two in highly non-aqueous solvent systems. One might be led to expect that the greater inductive effect of the sulphonyl group would make the sulphonylacetic acid a stronger acid than the sulphinylacetic acid regardless of the solvent system. This anomalous behavior can be rationalised on the basis of a preferred conformation for phenylsulphinylacetic acid in which intra-molecular hydrogen bonding is not possible (I).

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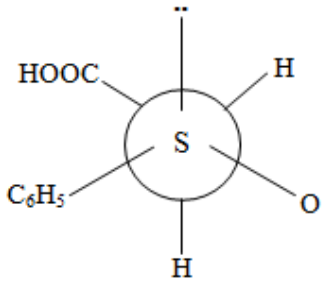

(I)

In contrast, phenylsulphonylacetic acid is not capable of existing in a conformation in which intra-molecular hydrogen loading is not possible. Conformers (II) and (III) may be used to represent phenylsulphonylacetic acid. The possible existence of intra-molecular hydrogen bonding in all conformations should lead to a greater decrease in acidity in poorer solvating solvents leading to the anomolous behaviour.

Ganesan[69] has recorded and discussed the ^{1}H and ^{13}C NMR spectra of several para-substituted phenylsulphonylacetic acids in order to understand the influence of substituents on the chemical shifts. He has

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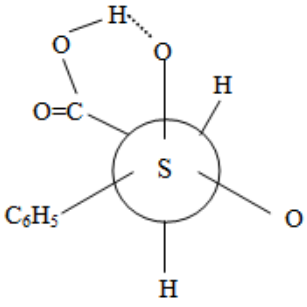
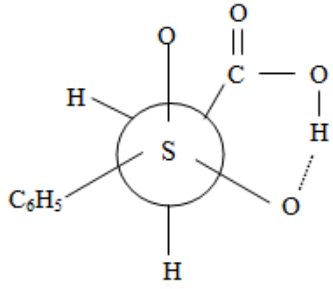
also studied the substituent effects on the mass spectra of arylsulphonyl acetic acids.



(II)



(III)

An examination of ^1H NMR data reveals that the chemical shifts of methylene protons and 2,6-protons of substituted phenyl sulphonylacetic acids are fairly sensitive to substituent effects. The substituent effect on 2,6-protons is more pronounced compared to the methylene protons in phenylsulphonyl acetic acids. The observed Hammett ρ value of phenylsulphonylacetic acid shows that the methylene protons are more deshielded due to carboxyl group. The magnitude of ρ_I and ρ_R indicate that the chemical shifts of methylene

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proton and 2,6-protons are influenced more by the resonance effect then the inductive effect.

The ^{13}C NMR chemical shift of carbon atoms of phenylsulphonylacetic acid shows that only C-1 and methylene carbons are related to the polar effects of the substituents. One notable difference between the correlation results of chemical shifts of C-1 and methylene carbons lie in the signs of the susceptibility parameters. The sign of the susceptibility constants of methylene carbon is negative indicating electron donating substituents cause downfield shifts while electron withdrawing groups cause upfield shifts.

In electron impact studies of arylsulphonylacetic acids the skeletal rearrangement via aryl-oxygen bond formation is predominant with a weak competing alkyl migration.

Arylsulphonylacetic acids are extremely used in the synthesis of variety of organic macromolecules. The heterocycles like

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thiadiazoles, triazoles and oxadiazoles were prepared from sulphonyloacetic acids in good yields.[70] The synthesis of α-methylsulphonyl alkyl phenyl sulphones was described by Wladislaw et al.[71] from α-pheynylsulphonyl carloxylic acids wtih NaH in DMSO and dimehtyl sulphide. A variety of tin salphonyl compounds were synthesised by Bao et al.[72] and their structures were characterized by elementary analysis, IR, 'H NMR, MS and XPS. The data of IR and XPS indicate that these compounds are four co-ordinated organotin compounds for the tricyclohexyltin aryl-sulphonyl acetates and five co-ordinated, carboxylate-bridged polymers for the triphenyl tin arylsulphonyl acetates.

Coupling of diazonium salts with $ArSO_2CH_2COOH$ afforded good yield of 1,6-sym -metrical disubstituted-3-sulphonylformazans. The sulphonyl macrocyclic crown formazans were prepared by coupling diazonium salts with arylsulphonyl acetic acid.[73]

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A new route for the preparation of methyl arylsulphones[74] in good yield in the attempted Knoevenagol condensation of $ArSO_2CH_2COOH$ with 2-methylcyclopentanone in acetic acid containing $PhCH_2NH_2$. Benzyl and arylsulphonylacetic acids have condensed with benzenedicarboxaldehydes to give a new class of unsaturated sulphones, 1,2-, 1,3-, and 1,4-bis(arylsulphonylethenyl) benzenes.[75] Their configurations were also assigned on the basis of IR and proton and ^{13}C NMR spectral data.

α,α-dianions derived from arenesulphonylacetate esters of 2,3-epoxy alcohols, cyclised to give sulphonyl lactones.[76] This sulphonyl loctone was elaborated efficiently to an advanced intermediate for the unusual aminoacid MeBMT as well as to stereo defined cyclopropane derivatives.

The reaction between arenesulphonyl acetic acids, arylglyoxols and isocyanides affords N–substitued-3-aryl-2-aryl-sulphonyl acetoxy-3-oxopropionomides which are

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cyclised to N-substitued oxofuran. Treatment of the oxofurans with diazomethane affords N-substitued-3-aryl-4-arylsulphonyl-5-methoxy furan-2-carboxamides.[77]

1.14 Oxidation studies with hexacyano ferrate(III) ion

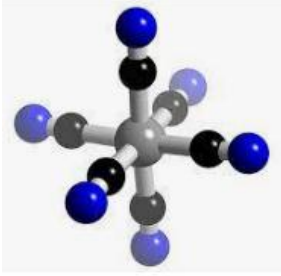

Figure 1.8 *Structure of hexacyanoferrate(III) ion*

Potassium hexacyanoferrate(III) is a potential oxidising agent and large number of reports are available in the literature on the oxidation of a variety of organic and inorganic compounds by it both in acidic and alkaline medium.[78-84] Hexacyanoferrate(III) ion acts as an oxidising agent is more interesting because of its strong ability to abstract a single electron

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from an electron rich site in a molecule. It has a redox potential of +0.4 V.

Eventhough hexacyanoferrate(III) is a weak oxidant, it oxidises aromatic hydro-carbons and benzene derivatives. It oxidises benzene derivatives like nitrotoluenes[85,86] and halotoluenes[87] through a benzylic radical intermediate. Nitrotoluenes are oxidised to benzyl alcohols in alkaline medium and aldehydes in acid medium. Bhattacharjee et al[88] have studied the kinetics of oxidation of naphthalene by hexacyanoferrate(III) in acetic acid-perchloric acid medium. Krishna Pillay et al.[89] have studied the kinetics of oxidation of fluorene by alkaline hexacyanoferrate(III) and proposed a free radical mechanism for the formation of flurenone. A unique instance of the oxidation of an alkyl function, activated by an electron withdrawing group with alkaline hexacyanoferrate(III) was reported by Mahapatra and Radhakrishnamurti.[90]

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In some cases hexacyanoferrate(III) oxidation were found to be very slow, sluggish oxidations have found to catalyse by metal ions like Os(VIII)[91,92], Ru (III)[93] and Cu(II).[94,95]

Platinum metals[96,97] are also used as catalyst in the oxidation of organic compounds by ferricyanide ion in aqueous alkaline medium.

1.14.1 Oxidation of organic sulphur compounds by hexacyanoferrate(III)

Subramanian and co-workers[98,99] have extensively studied the oxidation of phenyl-mercaptoacetic acid and several m-, p- and o-substituted phenylmercaptoacetic acids by hexacyanoferrate(III) in alkaline medium. The mechanism involving removal of α-proton by OH^- followed by the formation of radical intermediate in a slow step has been proposed for the oxidation. The product was identified as α-hydroxyphenylmercaptoacetic acid. Electron-withdrawing substituents in the phenyl ring accelerate the reaction while rate inhibition

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was found with electron releasing-substituents. The study of ortho effect indicates that delocalised effect is more predominant than localised effect and steric effect is found to be insignificant.

A limited number of studies on the oxidation of organic sulphur compounds with hexacyanoferrate(III) have been reported. Agarwal and Mushram studied the oxidation of thiourea and thioacetamide[100] gave the following rate equations.

Rate = k [Thiourea] $[Fe(CN)_6]^{3-}$ $[OH^-]$

Rate = k [Thioacetamide] $[OH^-]$

Lilani et al.[101] have found that the kinetics of thiocurea and N-substituted thioureas with hexacyanoferrate(III) under acid condition proceed via complex formation between protonated species of hexacyano-ferrate(III) and thiourea.

The kinetics of oxidation of thioglycolic acid by hexacyanoferrate(III) has been studied by Kapoor et al.[102] in acid medium. The

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oxidation product was found to be dithiodiglycolic acid.

The kinetics of oxidation of 1,4-thioxane by alkaline $K_3[Fe(CN)_6]$ have been studied in the presence of Os^{VIII} as catalyst.[103] The reaction is first order in hexacyanoferrate(III) and Os^{VIII}. The order in thioxane and OH^- is zero. The added salts and ethanol have a negligible effect on the oxidation rate while $K_4[Fe(CN)_6]$ retards the rate. On the basis of kinetic evidence, a mechanism involves the formation of a free radical α- to sulphur atom has been proposed.

Laloo and Mahanti[104] have reported the kinetics of oxidation of methionine by alkaline hexacyanoferrate(III) to give the sulphide.

Kinetics of oxidation of L-cysteine by one-electron oxidant potassiumhexacyano-ferrate(III) have been investigated spectrophotometrically[105] as a function of temperature, pH and ionic strength. The

reactive species of cysteine are neutral cysteine and protonated cysteine.

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CHAPTER II

SCOPE OF THE PRESENT INVESTIGATION

Micelle catalysed reactions are somewhat similar to enzyme catalysed reactions; the proper choice of surfactant brings about a rate increase of upto 1000 fold. The mechanisms of Micelle-catalysed reactions have been studied not only by anology with Michaelis-Menten equation for enzymatic reactions but also from the perspective of volume fractions of the two part reaction system consisting of the Micelles and the inter miceller bulk solution.

In spite of having numerous applications of micelles in chemical engineering, the preparation of agricultural chemical solutions, recovery of oil etc. very little attempt has been made to use the middle in the kinetics of oxidation of organic sulphur compounds.

Hexacyanoferrate(III) is a versatile oxidising agent and has been widely used both in alkaline and acid medium. Though it

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oxidises a variety of functional groups, literature survey shows that in some organic sulphur compounds, the alkyl function adjacent to sulphur atom undergo oxidation reaction with Hexacyanoferrate(III).

This promoted the author to undertake the present work of oxidation of phenyl sulphonylacetic acid by Hexacyanoferrate(III) in cetyltrimethylammonium bromide micellar medium.

The present investigation is planned with the following objectives.

i) To study the mechanism of oxidation of PSAA in the presence of cationic micelle, CTAB.

ii) To study the effect of CTAB on the above reaction.

iii) To understand the role of electrostatic and hydrophobic interactions between the reactants and the micelles.

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CHAPTER III

EXPERIMENTAL

3.1 Preparation of reagents

3.1.1 Phenylmercaptoacetic acid

The procedure described by Pasto et al.[67,68] was adopted for the preparation of phenylmercaptoacetic acid. A solution containing 4.7 g of chloroacetic acid dissolved in 10 ml of water was added to a cold solution of 10 ml of 20 % sodium hydroxide. 5.5 g of thiophenol (0.05 M) dissolved in 10 ml of 20 % sodium hydroxide was added to the above solution with cooling and constant shaking.

The mixture was heated at 120-130^{o}C in an oil bath for five hours. The above mixture was cooled and the resultant solution was acidified with 50 % hydrochloric acid (congo red). The Phenylmercaptoacetic acid was precipitated as solid. The solid phenylmercapto

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acetic acid was filtered and recrystallised from hot water.

3.1.2 Phenylsulphonylacetic acid

3 g of phenylmercaptoacetic acid was dissolved in 5 ml of acetic acid. To the above solution, twice the calculated amount of 30 % hydrogen peroxide was added. Some more acetic acid was added if turbidity appeared and warmed the solution for some time. The above solution was kept for two days. Then 5 ml of water was added and the solvent was removed under reduced pressure. The solid obtained was crystallised from water. The recrystallised sample had a melting point – 111^{o}C. The literature[106] melting point is 111.5 - 112.5^{o}C.

3.1.3 Potassium hexacyanoferrate(III)

Potassium hexacyanoferrate(III) (BDH, AR) was used as such for the preparation of standard solution. The purity of the solution was checked by UV-VIS spectrum. The absorption spectrum of potassium

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hexacyanoferrate(III) shows a maximum at 420 nm (Figure 3.1).

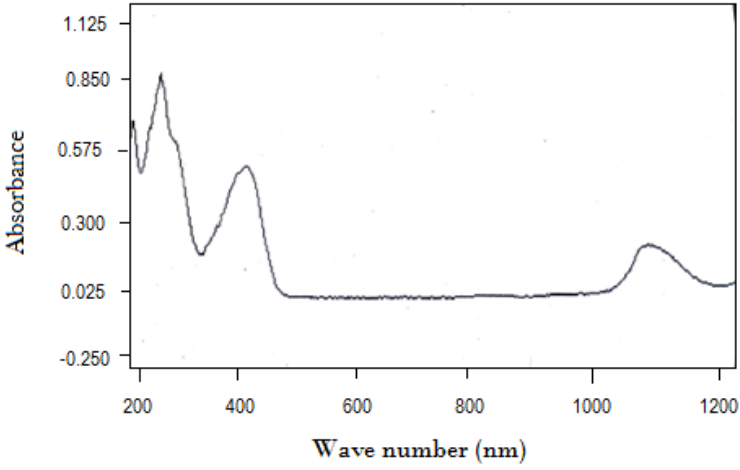

Figure 3.1 *Absorption spectra of hexacyanoferrate(III)*

3.1.4 Sodium hydroxide

The pellets of sodium hydroxide were first washed with double distilled water during the preparation of the stock solution. The strength of sodium hydroxide was determined by titrating against standard oxalic acid.

3.1.5 Cetyltrimethylammonium bromide

N–Cetyl–N,N,N–trimethylammonium bromide (GR, Loba Chemie) was used as such for the preparation of solution. The solutions

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were freshly prepared then and there for the kinetic runs.

3.1.6 Potassium chloride

To keep the ionic strength of the medium constant, potassium chloride (BDH, AR) was used.

3.1.7 Acrylamide

Acrylamide (S.D. fine, Electrophoresis Grade) was used as such without purification.

3.1.8 Other Reagents

The other chemicals viz., oxalic acid, potassium hexacyanoferrate(II) of AR grade were used in the present study.

3.2 Glasswares

'A' certified pipettes, burettes and standard measuring flasks were used throughout the kinetic study.

3.3 Thermostat

For maintaining the temperature of the reaction mixture as constant, the thermostat supplied by Toshniwal and Co., with an accuracy of 0.01^{o}C was employed.

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3.4 UV-VIS Spectrophotometer

"Elico SL164" double beam UV-VIS Spectrophotometer was employed to follow the kinetics of $[Fe(CN)_6]^{3-}$ oxidation with phenylsulphonylacetic acid in CTAB micellar medium.

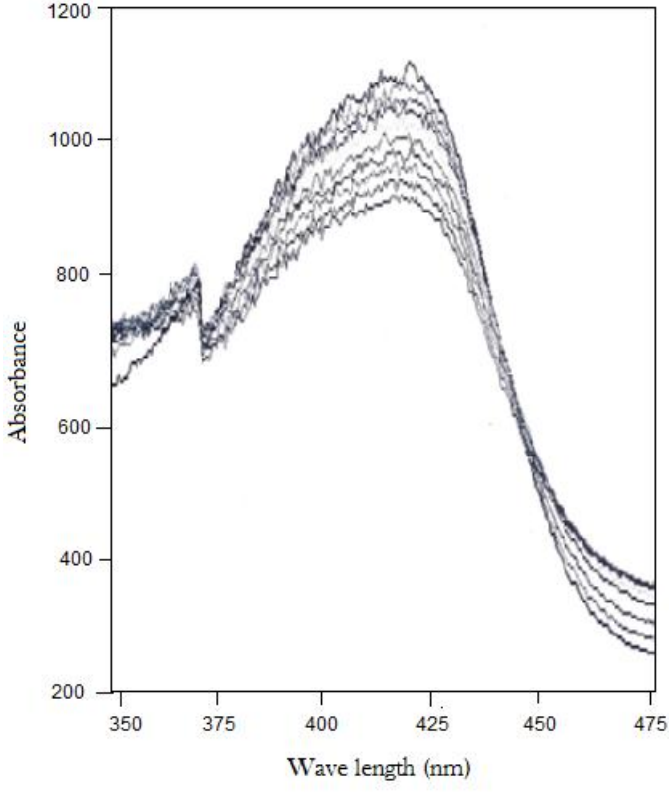

Figure 3.2 *Repetitive spectral scan of reaction mixture*

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3.4.1 Evaluation of rate constants

The pseudo first-order rate constant of each kinetic run was evaluated from the slope of the linear plot of log[absorbance] versus time, by the methods of least square. The success of each fit is expressed in terms of the correlation coefficient (r) and standard deviation (s).

According to the first order rate equation,

$$k_1 = \frac{2.303}{t} \log_{10} \frac{a}{(a-x)}$$

$$t = \frac{2.303}{k_1} \log_{10} \frac{a}{(a-x)}$$

$$k_1 = 2.303 \times \text{slope}$$

Where k_1 is the pseudo first order rate constant, 't' is the time in seconds and 'a' and (a-x) denote the initial concentration and the concentration at time t, respectively of the $[Fe(CN)_6^{3-}]$. Generally 'a' and (a-x) are proportional to the absorbance of $[Fe(CN)_6]^{3-}$ at

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zero time and at different time intervals respectively.

The overall rate constant, k_2 is evaluated as, $k_2 = k_1/[PSAA]$. It is expressed in M^{-1} s^{-1}. The precision of k values in all the kinetic runs is given in terms of 95 % confidence limit of the student's t-test.

3.4.2 Evaluation of activation parameters

According to Eyring's[107] the overall rate constant, k_2 of a reaction is related to the activation parameters as,

$$k_2 = \frac{K_B T}{h} e^{-\Delta H^{\neq}/RT} e^{\Delta S^{\neq}/R}$$

where, K_B is the Boltzmann constant

h is the Planck's constant

T is the temperature in Kelvin

$\Delta H^{\neq}$ and $\Delta S^{\neq}$ are the enthalpy of activation and entropy of activation respectively.

On taking logarithm, the Eyring's equation takes the form.

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$$\log \frac{k_2}{T} = 10.319 + \frac{\Delta S^{\neq}}{4.576} - \frac{\Delta H^{\neq}}{4.576T}$$

$\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated by the least squares analysis, from the linear plot of log (k_2/T) V_s $1/T$ as follows;

$$\Delta H^{\neq} = \frac{4.576 \; x \; slope \; x \; 4.186}{1000} \; kJmol^{-1}$$

$\Delta S^{\neq}$ = 4.576 (Intercept – 10.319) x 4.186 J k^{-1} mol^{-1}. The precision of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated according to the following equations given by Petersen et al.[108]

$$\text{The error in } \Delta H^{\neq} = \delta = \frac{2RT^1T}{(T^1 - T)} \; \alpha$$

Where, α is the maximum fractional error in the rate constants, R is the gas constant in Joules, T and T^1 are the two extreme temperatures at which the reaction is conducted.

$$\text{The error in } \Delta S^{\neq} = \sigma = \delta \frac{1}{T} + \left[\frac{T^1 - T}{2T^1T}\right]$$

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3.4.3 Product Analysis

In a typical experiment 0.4 M of hexacyanoferrate(III) was added to 0.2 M of phenylsulphonylacetic acid in 20 ml of 1M NaOH. The solution was kept at 313 K overnight for completion. The solvent was removed under reduced pressure. The resulting residue was extracted with ether, ether layer dried over anhydrous sodium sulphate and solvent removed. The residue was analysed by IR spectroscopy. The IR Spectrum of the product was not found any stretching frequency corresponds to $\diagdown\!\!\diagup S{=}O$ in the characteristic region 1070-1300 cm^{-1}. Further IR spectrum of the product indicated the presence of secondary hydroxyl group and carbonyl group. The spectral analysis showed the formation of α-hydroxyphenylsulphonyl acetic acid as the only product under present experimental conditions.

The inorganic product of the reaction obtained after the separation of the organic

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product on the completion of the reaction was found to be identified as the corresponding hexacyanoferrate(II). The product, hexacyano ferrate(II) gave two positive tests with ammonium molybdate and thorium nitrate[115].

3.4.4 Stoichiometry

The stoichiometry of the reaction was determined by keeping the reaction mixtures containing known excess of hexacyano ferrate(III) over known amount of phenylsulphonylacetic acid in alkaline medium until the reaction was complete. The amount of unreacted hexcocyanferrate(III) was then determined by measuring their OD spectrophotometrically. The results are reported in Table 1. The review of results clearly indicated that two moles of oxidant was required to one mole phenylsulphonylacetic acid. The overall reaction can be represented as

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$$PhSO_2\,CH_2\,COOH + 2Fe(CN)_6^{3-} + 2\,OH^- \longrightarrow PhSO_2\underset{\displaystyle OH}{\underset{|}{C}}H - COOH + 2Fe(CN)_6^{4-} + H_2O$$

Table 1 : Stoichiometric of the reaction

$[Fe(CN)_6^{3-}]$ x10^3 mol dm^{-3}	[PSAA] x10^4 mol dm^{-3}	$[Fe(CN)_6^{3-}]_{reac}$ x10^4 mol dm^{-3}	$[Fe(CN)_6^{3-}]_{unreac}$ x10^4 mol dm^{-3}	$\frac{[Fe(CN)_6^{3-}]_{react}}{[PSAA]}$
1.0	1.0	1.87	8.13	1.87
2.0	1.0	1.75	18.25	1.75
2.0	2.0	3.63	16.37	1.82

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CHAPTER IV

RESULT AND DISCUSSION

4.1 Effect of [PSAA] and $[Fe(CN)_6]^{3-}$ on reaction rate

By taking excess of phenylsulphonylacetic acid over hexacyano ferrate(III) the kinetic runs were carried out under pseudo first order condition. When log[absorbance] is plotted against time, a linear plot is obtained upto 60 % completion of the reaction. A slight retardation effect has been observed in the linear plot beyond 60 % completion of the reaction.

Figure 4.1 indicates the first-order plot at different concentrations of hexacyanoferrate (III). These linear plots ($r>0.996$) conclude that the reaction is first order with respect to the oxidant, hexacyanoferrate(III). However, the observed rate constant decreases gradually with an increase in the concentration of hexacyanoferrate(III) in the range 3.0-10.0 x 10^{-4} mol.dm^{-3} such an observation is not

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uncommon and many workers[110,111] have come across this anomalous behaviour in different hexacyanoferrate(III) oxidations. This retardation in rate could not be due to the decomposition of hexacyanoferrate(III) in cetyltrimethylammonium bromide medium as the solution of oxidant was found remain intact even after one day under the experimental conditions employed. The reason for this retardation is given in the discussion (vide infra).

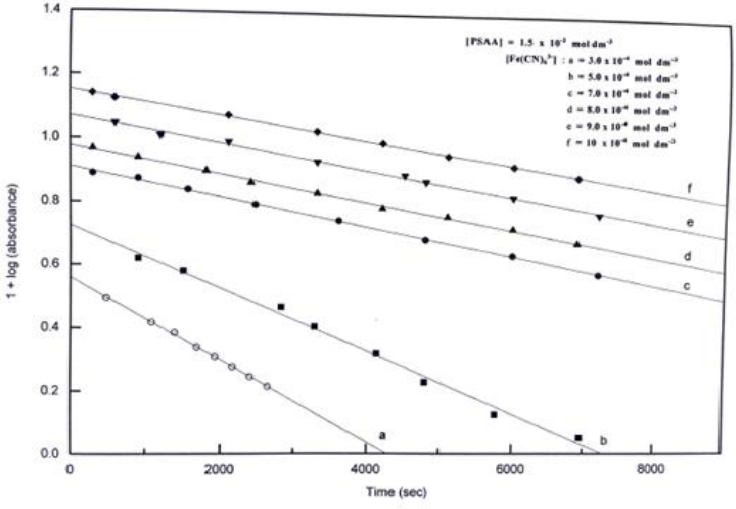

Figure 4.1 *Dependence of rate on hexacyanoferrate(III) at 40°C*

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Table 2: Observed (k_1) and Overall (k_2) rate constants for oxidation of PSAA by [$K_3Fe(CN)_6$]

[NaOH] = 1.0 mol dm^{-3} I = 1.5 mol dm^{-3}

[CTAB] = 1.0 x 10^{-4} mol dm^{-3} T = 40^0C

10^2 [PSAA] mol dm^{-3}	10^4 [$Fe(CN)_6^{3-}$] mol dm^{-3}	10^4 k_{obs} s^{-1}	10^3 k_2 dm^3 mol^{-1} s^{-1}
1.0	5.0	1.59 ± 0.09	4.74 ± 0.27
1.5	5.0	2.25 ± 0.20	4.91 ± 0.43
2.0	5.0	2.79 ± 0.11	4.93 ± 0.19
2.5	5.0	3.18 ± 0.33	4.77 ± 0.49
3.0	5.0	3.62 ± 0.14	4.75 ± 0.18
4.0	5.0	4.28 ± 0.19	4.54 ± 0.20
5.0	5.0	5.50 ± 0.22	4.96 ± 0.20
1.5	3.0	2.99 ± 0.09	6.50 ± 0.20
1.5	7.0	1.05 ± 0.09	2.29 ± 0.20
1.5	8.0	0.983 ± 0.04	2.14 ± 0.87
1.5	9.0	0.937 ± 0.06	2.04 ± 0.13
1.5	10.0	0.861 ± 0.02	1.88 ± 0.04

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The effect of substrate, PSAA was studied in the concentration range 1.0 – 5.0 x 10^{-2} mol dm^{-3} by keeping the others constant. A plot of log(OD) vs time for various concentrations of PSAA is shown in figure 4. The observed rate constant increases with increasing concentration of PSAA, while the second order rate constant calculated using, $k_2 = k_{obs}/[PSAA]^{0.734}$ is not found to be constant. This indicates the reaction is not first order in PSAA.

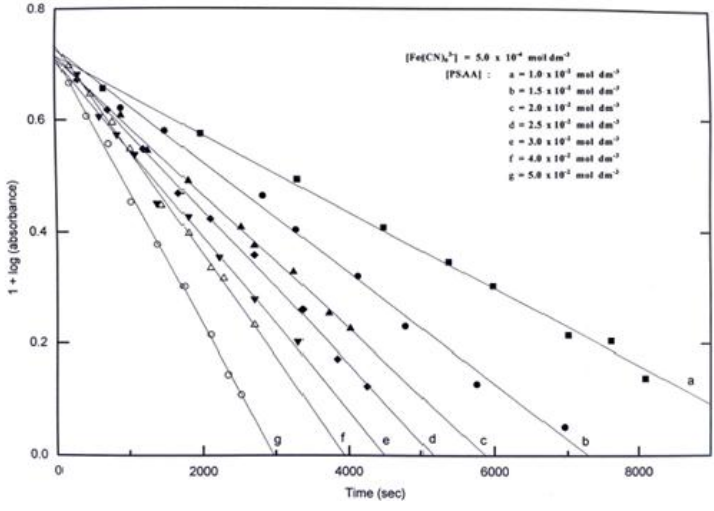

Figure 4.2 *Dependence of rate on [PSAA] at 40°C*

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The plot of logk vs log [PSAA] is a straight line (Figure 4.2; r = 0.997) with the slope of 0.734 ± 0.06 indicates that the reaction is fractional order with respect to PSAA, and the order is 0.734.

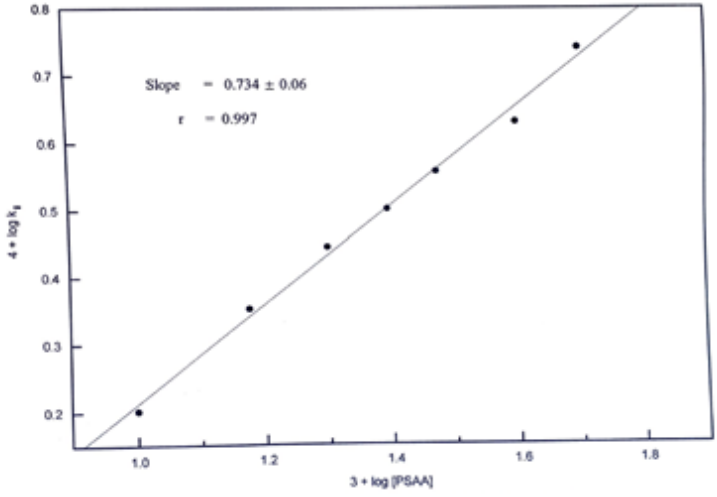

Figure 4.3 *Plot of log log k1 vs log [PSAA]at 40°C*

Further the overall rate constant (k_2) calculated using the formula $k_2 = k_{obs} / [PSAA]^{0.734}$ remains constant in all cases. This confirms the order with respect to the substrate, PSAA is 0.734. The observed rate constants (k_1) and the overall rate constants, (k_2) for the different $[K_4Fe(CN)_6]$ and [PSAA] are

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presented in Table 2. The plot of $1/k_{obs}$ vs 1/[PSAA] at constant $[Fe(CN)_6^{3-}]$ is linear (Figure 6; r = 0.998) which is not passing through the origin and have a definite intercept. This indicates the complex formation between $[Fe(CN)_6]^{3-}$ and PSAA during the course of a reaction.

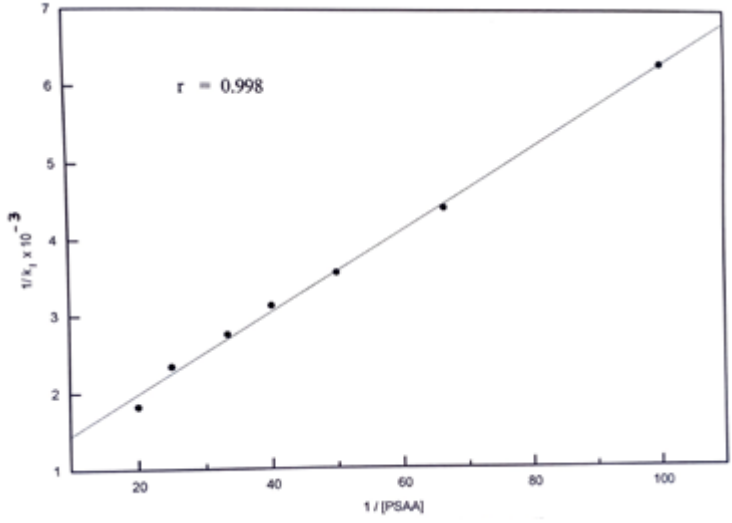

Figure 4.4 *Dependence of rate on [PSAA] at 40°C*

4.2 Effect of NaOH

The effect of $[OH^-]$ on reaction rate has been studied in the range 0.5 – 1.3 mol dm^{-3} and keeping others as constant. The rate

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constants were found to be increased with increasing in $[OH^-]$. The values of k_{obs} and k_3 at various $[OH^-]$ are summarised in Table 3.

The rate constant values of $k_2 = k_{obs} / [PSAA]^{0.734} [OH^-]$ in Table III is not found to be constant. Further, a plot of $\log k_{obs}$ vs log[NaOH] is linear (Figure 4.5; r = 0.993) with slope of 0.727 ± 0.12 confirms that the reaction is fractional order on $[OH^-]$ also.

Table 3: Rate constants for the variation of $[OH^-]$ at 40°C

[PSAA] = 1.5 x 10^{-2} mol dm^{-3} I = 1.5 mol dm^3

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4} mol dm^{-3}

[CTAB] = 1.0 X 10^{-4} mol dm^{-3}

[NaOH] mol dm^{-3}	**10^4 k_{obs} s^{-1}**	**10^2 k_3 dm^6 mol^{-6} s^{-1}**
0.5	1.40 ± 0.09	0.611 ± 0.30
0.7	1.64 ± 0.10	0.511 ± 0.31
0.8	1.88 ± 0.09	0.513 ± 0.24
1.0	2.25 ± 0.20	0.491 ± 0.44
1.1	2.46 ± 0.11	0.488 ± 0.22
1.3	2.71 ± 0.08	0.455 ± 0.13

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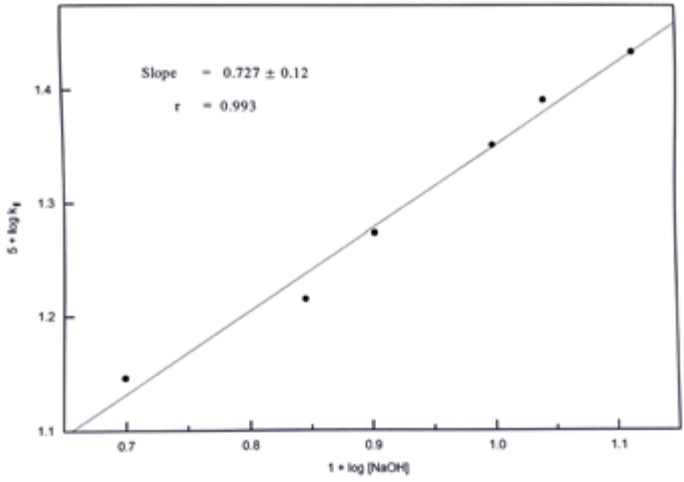

Figure 4.5 *Plot for [OH-] at T 40°C*

4.3 Effect of CTAB

The effect of cationic micelle, CTAB, on the reaction rate was studied in concentration range 0.3 – 500 x 10^{-4} mol dm^{-3}. The reactions can be conducted only at these concentrations, because beyond this range precipitation of the oxidant occurs. Similarly, the reaction could not be conducted in between the concentration range 1 x 10^{-4} mol dm^{-3} and 100 x 10^{-4} mol dm^{-3} due to precipitation problem. The kinetic data obtained for the oxidation of PSAA by

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$[Fe(CN)_6]^{3-}$ in aqueous medium and at CTAB micellar medium at various [CTAB] are collected in Table 4.

The values of observed rate constants with the change in [CTAB] for the oxidation are shown in the figure 4.6. The kinetic data show that the reaction between $[Fe(CN)_6]^{3-}$ and phenylsulphonylacetate ion is catalysed in CTAB medium at low concentrations of CTAB while rate retardation is observed at higher concentrations of CTAB.

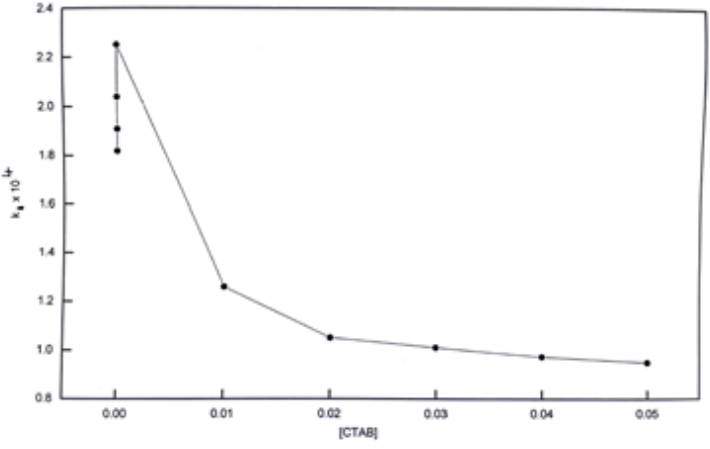

Figure 4.6 *Variation of rate on [CTAB]at 40°C*

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Table 4: Effect of CTAB on reaction rate at 40^{0}C

[PSAA] = 1.5 x 10^{-2} mol dm^{-3} I = 1.5 mol dm^{-3}

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4}mol dm^{-3}

[NaOH] = 1.0 mol dm^{-3}

10^4 [CTAB] mol dm^{-3}	**10^4 k_{obs} s^{-1}**
0	1.82 ± 0.08
0.3	1.91 ± 0.09
0.5	2.04 ± 0.10
1.0	2.25 ± 0.20
100	1.26 ± 0.08
200	1.05 ± 0.08
300	1.01 ± 0.05
400	0.974 ± 0.08
500	0.951 ± 0.11

The catalysis of the reaction at low [CTAB] indicates that negatively charged reactants, $[Fe(CN)_6]^{3-}$ and phenysulphonyl acetate ions are bring closer on the cationic

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micellar surface by electrostatic forces of attraction and the major part of the reaction may takes place in the stern layer. These results also demonstrate the important of electrostatic interactions over the hydrophobic interactions in the binding of reactants to micelles.

At low concentrations of CTAB, as [CTAB] increases number of molecular of reactants binding on the micellar surface increases thereby acceleration in rate. But at higher [CTAB], the number of reactant molecules per micelle decreases with increasing CTAB concentration. This may be the reason for the decrease in rate constant with increase in [CTAB]. Such type of explanation was given in the literature[112] for the retardation observed when reactants and micellar surface have opposite charges.

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4.4 Effect of ionic strength

The effect of ionic strength on the rate was studied in the concentration range from 1.1 to 1.7 mol dm^{-3} using potassium chloride in the presence of CTAB. The rate constants given in Table 5 show that the ionic strength affects the rate of the reaction.

Table 5: Effect of ionic strength on reaction rate at 40°C

[PSAA] = 1.5 x 10^{-2} mol dm^{-3}

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4} mol dm^{-3}

[NaOH] = 1.0 mol dm^{-3}

[CTAB] = 1.0 x 10^{-4} mol dm^{-3}

I **mol dm^{-3}**	**10^4 k_{obs}** **s^{-1}**
1.1	0.778 ± 0.07
1.2	1.02 ± 0.05
1.4	1.73 ± 0.16
1.5	2.25 ± 0.20
1.6	2.64 ± 0.12
1.7	2.97 ± 0.1

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A plot of log k_{obs} vs $\sqrt{\mu}$ has been found to be linear (Figure 4.7; r = 0.995) with a positive slope, +2.36 ± 0.33, indicating thereby involvement of two ionic species of like charges in the reaction.

In the electron transfer reactions involving hexacyanoferrate(III), it has been proposed that addition of the added salt serves to reduce the repulsion between the highly charged ions[113-115], thereby increasing the rate.

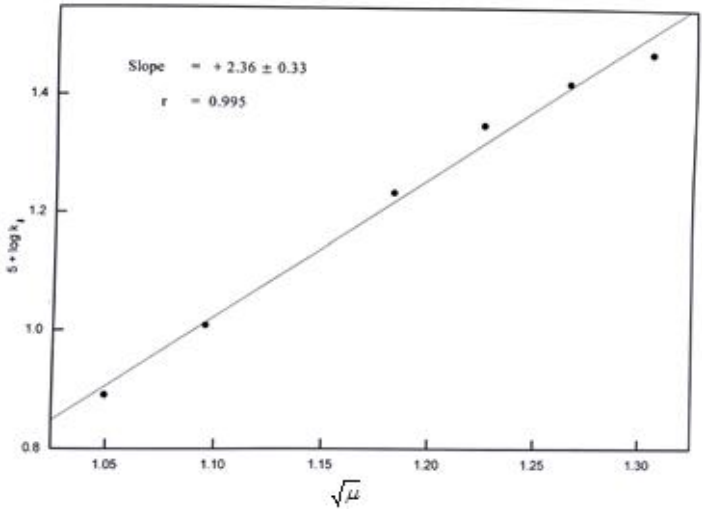

Figure 4.7 *Plot of* $logk_1$ *vs* $\sqrt{\mu}$

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4.5 Effect of Acrylamide

The observed rate constant increases in the presence of acrylamide, a radical scavenger, in the range 0.5 – 10.0 x 10^{-2} mol dm^{-3}

Table 6: Rate constants at different [acrylamide] at 40°C

[PSAA] = 1.5 x 10^{-2} mol dm^{-3}

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4} mol dm^{-3}

[NaOH] = 1.0 mol dm^{-3}

[CTAB] = 1.0 x 10^{-4} mol dm^{-3}

I = 1.5 mol dm^{-3}

10^2 [Acrylamide] mol dm^{-3}	10^4 k_{obs} s^{-1}
0.0	2.25 ± 0.20
0.5	2.37 ± 0.21
2.0	2.92 ± 0.24
5.0	3.55 ± 0.26
10.0	4.03 ± 0.20

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This rate acceleration (Table 6) has been taken as evidence for the reaction to proceed through a radical intermediate (vide infra – mechanism, scheme 1)

4.6 Effect of hexacyanoferrate(II) on the rate of oxidation

Table 7: Rate constants for oxidation at varying hexacyanoferrate(II) at 40^{0}C

[PSAA] = 1.5 x 10^{-2} mol dm^{-3} I = 1.5 mol dm^{-3}

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4} mol dm^{-3}

[NaOH] = 1.0 mol dm^{-3}

[CTAB] = 1.0 x 10^{-4} mol dm^{-3}

10^3 $[Fe(CN)_6^{4-}]$ mol dm^{-3}	10^4 k_{obs} s^{-1}
0.0	2.25 ± 0.20
1.0	1.8 ± 0.16
3.0	1.71 ± 0.20
5.0	1.65 ± 0.36
7.0	1.52 ± 0.24
9.0	1.40 ± 0.22
10.0	1.26 ± 0.18

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The influence of added potassium hexacyanoferrate(II) on the rate can be seen from the data in Table 7. The observed retardation of rate by added hexacyano ferrate(II) indicates that it is formed in a slow reversible step. The slight retardation after ~ 60 % completion of the oxidation in the first–order plot may be due to the hexacyano ferrate(II) formed in the reaction

4.7 Variation of rate constant with temperature

The oxidation reaction was carried out at five different temperatures in the range of 30-50^{0}C. The reaction rate is greatly enhanced by increasing temperature. The observed rate constants fit the Arrhenius equation nicely.

The activation parameters, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ calculated, respectively, from the slope and intercept of the linear Eyring's plot (Figure 4.8) of log (k_2/T) vs 1/T are presented in Table 8.

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Table 8: Effect of temperature on reaction rate

[PSAA] = 1.5 x 10^{-2} mol.dm^{-3} I = 1.5 mol dm^{-3}

$[Fe(CN)_6]^{3-}$ = 5 x 10^{-4} mol dm^{-3}

[NaOH] = 1.0 mol dm^{-3}

[CTAB] = 1.0 x 10^{-4} mol dm^{-3}

Temp. ^0C	**$10^2 k_2$ $M^{-1} s^{-1}$**	**$\Delta H^{\neq}$ $kJmol^{-1}$**	**$\Delta S^{\neq}$ $JK^{-1}mol^{-1}$**
30	0.49 ± 0.06	67.07 ± 11.1	-76.87 ± 36.8
35	0.81 ± 0.17		
40	1.51 ± 0.20		
45	1.75 ± 0.27		
50	2.81 ± 0.20		

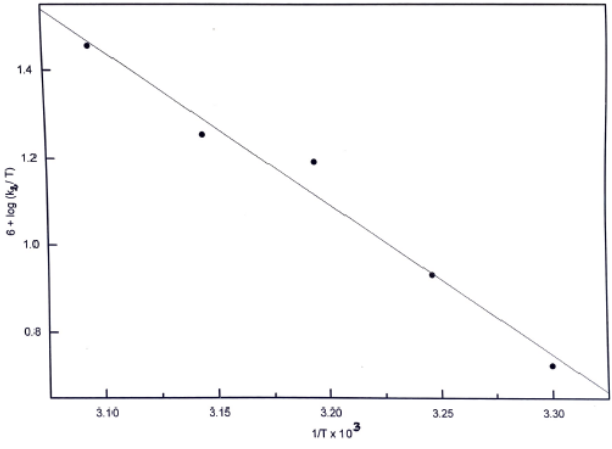

Figure 4.8 *Eyring's Plot*

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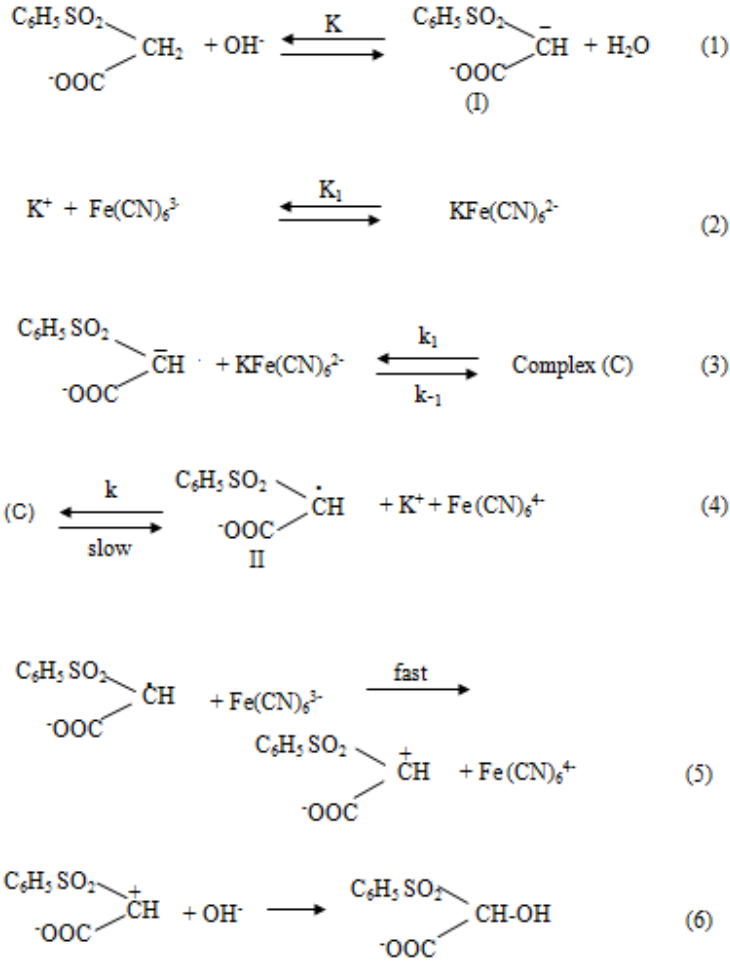
4.8 Mechanism and rate law



Scheme 1

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Any mechanism proposed for this oxidation should explain all the foregoing kinetic features. Taking into account all the above kinetic facts, the following mechanism (scheme 1) has been proposed.

The first step in the mechanism is the abstraction of hydrogen atom from the α-carbon atom to the sulphonyl group in a reversible step (Eq.1). It has been reported that the reactions of organic sulphur compounds with hexacyanoferrate(III) are not facile and require the presence of a removable proton on the α-carbon atom.[116-118] The removal of a proton from an alkyl function activated by electron withdrawing groups has been also reported in a variety of hexacyanoferrate(III) oxidation.[119,120,91,92] Such type of α-sulphonyl carbanions have been identified and used as a synthon unit by many workers during the synthesis of complex organic molecules.[71]

As the reaction is carried out in the presence of excess of K^+ ion, it is assumed that

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most of the hexacyanoferrate may exist as $KFe(CN)_6^{2-}$ and is proposed as a active species in this oxidation (Eq.2) Several workers[121-124] have also reported that $K_4Fe(CN)_6^{2-}$ formed in step - 2 lies well towards right.

α-sulphonyl carbanion(I) then combine with $KFe(CN)_6^{2-}$ to form a complex. The complex formation is proved from the linear Michaelis-Menten plot. Electron transfer may take place within the complex to yield α-sulphonyl free radical and hexacyanoferrate(II) in a slow reversible step (Eq.4). The retardation with $[Fe(CN)_6]^{4-}$ is due to the shifting of the equlibrium (4) towards left and increase in rate with acrylamide is due to removal of free radical (II) by acrylamide thus shifting the equilibrium (4) towards right.

The more reactive radical intermediate then combines with another oxidant to form α-sulphonyl carbonium ion in a fast step. Irreversible fast reactions then follow, giving the product.

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Now considering the steady state conditions and taking total hexacyanoferrate (III) as

$$[Fe(CN)_6^{3-}]_{Total} = [Fe(CN)_6^{3-}] + [KFe(CN)_6^{2-}] + Complex$$

The rate law in terms of decreasing [hexacyanoferrate(III)] would be given by the equation.

$$\frac{-d\,[Fe(CN)_6^{3-}]}{dt} = \frac{2kk_1\,KK_1\,[K^+]\,[PSAA]\,[OH^-]\,[Fe(CN)_6^{3-}]_{Total}}{(k+k_{-1})\,\{1+k_1[K^+]\} + k_1\,KK_1\,[K^+]\,[OH^-]}$$

The above equation is apparently consistent with the observed kinetics. It shows first order kinetics with respect to hexacyanoferrate(III) and explains the observed fractional order if the reaction rate with respect to substrate and OH^- concentrations.

4.9 Effect of CTAB on reactivity

The kinetics of micellar solutions is generated by electrostatic and hydrophobic interactions between micelles and reactants, transition complexes and product. If any of the

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reaction species interacts with micelles, then the presence of micelles will affect the reaction rate. The micellar effect in this oxidation can be explained by pseudo-phase ion exchange model. According to this model, the reactants are distributed between bulk solvent and the micellar assemblies and consider the bulk solvent and the micelles as distinct reaction regions.

The observed dependence of rate constant on [CTAB] is typical for micellar catalysis involving two reacting species in the middle. Therefore, with the reasonable assumption that $[Fe(CN)_6]^{3-}$ and phenyl-sulphonylacetate ions are strongly associated with the cationic micelle and the reaction occurs in the aqueous as well as in the micellar pseudophases. The observed rate constants in the presence of CTAB can be explained by scheme 2.

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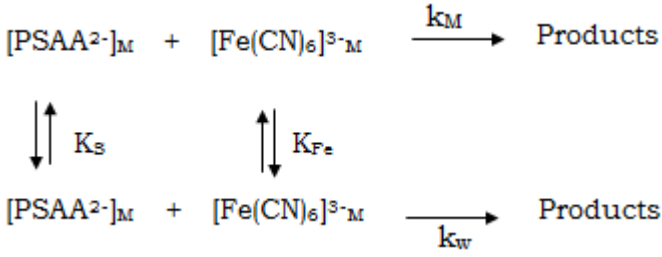

Scheme 2: *Oxidation in CTAB Medium*

In the above scheme the subscripts M and W stand for micellar and aqueous phases respectively. The observed reaction rates are described in terms of distribution constants of the reactants (Ks and K_{Fe}) and the specific rate constants for the aqueous and micellar pseudo-phases (K_W and K_M).

The kinetic data collected in Table IV and Figure 8 show that the rate constant increases with increase in [CTAB], reaches a maximum at 1 x 10^{-4} mol dm^{-3} and further increase in CTAB concentration decreases the rate.

The positive potential at the micellar surface can attract negatively charged reactants. Thus electrostatic attraction brings

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the reactant molecules closer in micellar surface. As the reactants are move closer in micellar surface, the reaction rate at micellar phase is expected to be higher. As [CTAB] increases, at low concentrations region, the number of reagent molecules per micelle increases followed by miceller catalysis. Beyond 100 x 10^{-4} mol dm^{-3}, an increase in the micellar concentration dilutes the reagents in the micelle and thus reduces the observed reaction rate.

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CHAPTER V

SUMMARY

The effect of cationic middle, cetyltrimethylammonium bromide, oxidation of phenylsulphonylacetic acid by potassium hexocyantoferrate (III) has been studied in alkaline medium. The reactions were studied under pseudo first order conditions by maintaining large excess of PSAA over $[Fe(CN)_6]^{3-}$. The reactions were carried out at a constant temperature and followed upto 60 % reaction by monitoring the decrease in absorption due to $[Fe(CN)_6]^{3-}$ at 420 nm. The results obtained are summarised below.

i) The reaction is first order with respect to hexocyanoferrate(III). However the observed rate constant is found to decrease with an increase in $[Fe(CN)_6]^{3-}$ concentration.

ii) The oxidation follows a fractional order in phenylsulphonylacetic acid. Michaelis-

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Menten type of kinetics was observed with respect to PSAA.

iii) The rate of the reaction increases with an increase in the concentration of OH^- and the order is found to be fractional on $[OH^-]$.

iv) Oxidation rate increases with increase in ionic strength of the medium.

v) Addition of potassium hexacyanoferrate(II) retards the oxidation rate.

vi) The addition of acrylamide, a radical scavenger, has found to increase the rate of the reaction.

vii) The effect of cetyltrimethylammonium bromide has been studied over a wide range. Micellar catalysis is noticed at low concentrations, while rate retardation is observed at higher concentrations of CTAB.

viii) The rate acceleration may be due to increase in the number of reactants which come closer on the micellar surface. At higher concentration region, the increase in [CTAB]

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dilutes the reagents on the micelles followed by rate retardation.

ix) The stoichiometric results show that each molecule of phenylsulphonylacetic acid requires two molecules of hyxocyanoferrate for complete reaction.

x) α–hydroxyphenylsulphonylacetic acid is identified as the final product in the oxidation.

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Table of Kinetic Runs

S. No.	10^2 [PSAA] mol dm^{-3}	10^4 [$Fe(CN)_6^{3-}$] mol dm^{-3}	[NaOH] mol dm^{-3}	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^0C	10^4 k_{obs} s^{-1}	r	s	n
1	1.0	5.0	1.0	1.0	1.5	40	1.59 ± 0.09	0.998	0.013	9
2	1.5	5.0	1.0	1.0	1.5	40	2.25 ± 0.20	0.996	0.019	8
3	2.0	5.0	1.0	1.0	1.5	40	2.79 ± 0.11	0.999	0.008	9
4	2.5	5.0	1.0	1.0	1.5	40	3.18 ± 0.33	0.995	0.018	9
5	3.0	5.0	1.0	1.0	1.5	40	3.62 ± 0.14	0.999	0.010	9
6	4.0	5.0	1.0	1.0	1.5	40	4.28 ± 0.19	0.999	0.008	9
7	5.0	5.0	1.0	1.0	1.5	40	5.50 ± 0.22	0.999	0.010	9
8	1.5	3.0	1.0	1.0	1.5	40	2.99 ± 0.09	0.999	0.003	8

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S. No.	10^2 [PSAA] mol dm^{-3}	10^4 $[Fe(CN)_6^{3-}]$ mol dm^{-3}	[NaOH] mol $dm^{-\beta}$	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^0C	10^4 k_{obs} s^{-1}	r	s	n
9	1.5	7.0	1.0	1.0	1.5	40	1.05 ± 0.04	0.999	0.004	8
10	1.5	8.0	1.0	1.0	1.5	40	0.983 ± 0.04	0.999	0.005	9
11	1.5	9.0	1.0	1.0	1.5	40	0.937 ± 0.06	0.998	0.007	8
12	1.5	10.0	1.0	1.0	1.5	40	0.861 ± 0.02	0.999	0.003	8
13	1.5	5.0	0.5	1.0	1.5	40	1.40 ± 0.09	0.998	0.010	8
14	1.5	5.0	0.7	1.0	1.5	40	1.64 ± 0.10	0.997	0.009	9
15	1.5	5.0	0.8	1.0	1.5	40	1.88 ± 0.10	0.998	0.010	9
16	1.5	5.0	1.1	1.0	1.5	40	2.46 ± 0.11	0.998	0.008	7
17	1.5	5.0	1.3	1.0	1.5	40	2.71 ± 0.08	0.997	0.011	7

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S. No.	10^2 [PSAA] mol dm^{-3}	10^4 [$Fe(CN)_6^{3-}$] mol dm^{-3}	[NaOH] mol dm^{-3}	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^{0}C	10^4 k_{obs} s^{-1}	r	s	n
18	1.5	5.0	1.0	0.0	1.5	40	1.82 ± 0.20	0.998	0.009	8
19	1.5	5.0	1.0	0.3	1.5	40	1.91 ± 0.09	0.999	0.010	7
20	1.5	5.0	1.0	0.5	1.5	40	2.04 ± 0.10	0.999	0.006	9
21	1.5	5.0	1.0	1.0	1.5	40	2.25 ± 0.07	0.998	0.007	7
22	1.5	5.0	1.0	100	1.5	40	1.26 ± 0.08	0.999	0.009	7
23	1.5	5.0	1.0	200	1.5	40	1.05 ± 0.08	0.996	0.015	10
24	1.5	5.0	1.0	300	1.5	40	1.01 ± 0.05	0.999	0.004	7
25	1.5	5.0	1.0	400	1.5	40	0.974 ± 0.08	0.998	0.006	7
26	1.5	5.0	1.0	500	1.5	40	0.951 ± 0.11	0.997	0.007	6

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S. No.	10^2 [PSAA] mol dm^{-3}	10^4 [$Fe(CN)_6^{3-}$] mol dm^{-3}	[NaOH] mol dm^{-3}	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^{0}C	10^4 k_{obs} s^{-1}	r	s	n
27	1.5	5.0	1.0	1.0	1.1	40	0.778 ± 0.07	0.998	0.009	7
28	1.5	5.0	1.0	1.0	1.2	40	1.02 ± 0.05	0.999	0.006	8
29	1.5	5.0	1.0	1.0	1.4	40	1.73 ± 0.16	0.994	0.017	9
30	1.5	5.0	1.0	1.0	1.6	40	2.64 ± 0.12	0.997	0.009	9
31	1.5	5.0	1.0	1.0	1.7	40	2.97 ± 0.11	0.999	0.019	8
32	1.5	5.0	1.0	1.0	1.4	30	0.49 ± 0.06	0.996	0.014	9
33	1.5	5.0	1.0	1.0	1.5	35	0.81 ± 0.17	0.997	0.023	8
34	1.5	5.0	1.0	1.0	1.5	45	1.75 ± 0.27	0.994	0.024	9
35	1.5	5.0	1.0	1.0	1.5	50	2.81 ± 0.20	0.996	0.017	8

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S. No.	10^2 [PSAA] mol dm^{-3}	10^4 $[Fe(CN)_6^{3-}]$ mol dm^{-3}	[NaOH] mol dm^{-3}	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^{0}C	10^4 k_{obs} s^{-1}	r	s	n
	10^2 [Acrylamide]			$[Fe(CN)_6^{3-}] = 5.0 \times 10^{-4}$ mol dm^{-3}						
36	1.5	0.0	1.0	1.0	1.5	40	2.25 ± 0.20	0.996	0.019	8
37	1.5	0.5	1.0	1.0	1.5	40	2.37 ± 0.21	0.996	0.018	8
38	1.5	2.0	1.0	1.0	1.5	40	2.92 ± 0.24	0.996	0.016	9
39	1.5	5.0	1.0	1.0	1.5	40	3.55 ± 0.26	0.997	0.016	8
40	1.5	10.0	1.0	1.0	1.5	40	4.03 ± 0.20	0.997	0.017	9

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S. No.	10^2 [PSAA] mol dm^{-3}	10^4 [Fe(CN)$_6^{3-}$] mol dm^{-3}	[NaOH] mol dm^{-3}	10^4 [CTAB] mol dm^{-3}	I mol dm^{-3}	Temp ^{0}C	10^4 k_{obs} s^{-1}	r	s	n
		10^3 K$_4$[Fe(CN)$_6$]		[Fe(CN)$_6^{3-}$] = 5.0 x 10^{-4} mol dm^{-3}						
41	1.5	0.0	1.0	1.0	1.5	40	2.25 ± 0.20	0.996	0.019	8
42	1.5	1.0	1.0	1.0	1.5	40	1.8 ± 0.16	0.995	0.019	9
43	1.5	3.0	1.0	1.0	1.5	40	1.71 ± 0.20	0.996	0.018	7
44	1.5	5.0	1.0	1.0	1.5	40	1.65 ± 0.36	0.997	0.028	7
45	1.5	7.0	1.0	1.0	1.5	40	1.52 ± 0.24	0.994	0.022	8
46	1.5	9.0	1.0	1.0	1.5	40	1.40 ± 0.22	0.995	0.021	9
47	1.5	10.0	1.0	1.0	1.5	40	1.26 ± 0.18	0.998	0.023	8

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CHAPTER VI

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Kinetic And Mechanistic Approach To Oxidation Reaction

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