POST-GRADUATE DEGREE PROGRAMME (CBCS)

IN

ZOOLOGY

(M.Sc. Programme)

SEMESTER-III

HARDCORE THEORY PAPER

Environmental Toxicology and Endocrinology

ZCORT-310

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI-741235, WEST BENGAL

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of Distance and reaching the unreached students are the three fold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self-Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavor. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from **Professor (Dr.) Kallol Paul, Hon'ble Vice-Chancellor, University of Kalyani**, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it with in proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self-Learning Materials are self writing and collected from e-book, journals and websites.

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ZCORT-310:
ENVIRONMENTAL TOXICOLOGY AND ENDOCRINOOGY

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UNIT-I

Toxicology: Scope, division, toxicants and toxicity, LD50, LC50 and ED50, Dose-response relationship.

Objective: In this unit you will learn about basic concept of toxicology. You will also learn about scope and division of toxicology and dose response relationship.In this unit you will also learn about different toxicity testing procedures. You will also knowabout LC50, LD50, ED50, synergism, antagonism and additive Effect.

Introduction to Toxicology:

Introduction to Toxicology

Toxicology is the interdisciplinary science that investigates the adverse effects of chemical and physical agents on living systems. It quantifies how biological systems respond to harmful substances, focusing on dose, exposure duration, and route of entry. Rooted in Paracelsus's principle that *"the dose makes the poison,"* toxicology evolved from early medicinal chemistry into a modern discipline shaped by industrialization, warfare, and environmental health concerns.

Core Concepts in Toxicology

A **toxin** is any substance that causes biological harm at a certain dose. While the field often studies **xenobiotics** (foreign substances), even natural compounds like vitamins or trace elements can become toxic at excessive levels. Toxic responses vary based on:

- **Dose** (amount),
- **Exposure route** (oral, inhalation, dermal, injection),
- **Duration** (acute vs. chronic),
- **Host factors** (age, sex, species, health).

Acute toxicity is commonly assessed using $\mathbf{LD_{50}}$ —the lethal dose for 50% of a test population—while **chronic toxicity** involves long-term effects such as carcinogenesis or reproductive dysfunction. Substances like **aspirin** exemplify this duality: therapeutic at low doses, toxic at high or prolonged exposure.

Toxicology plays a critical role in **public health, pharmacology, occupational safety, and environmental protection**, emphasizing that toxicity is context-dependent and often reversible with appropriate intervention.

Toxicology: Mechanistic Insights and Key Milestones:

Toxicology advanced from identifying harmful effects to understanding **how** and **why** they occur. Toxicity depends on a substance's **chemical properties**, **metabolism**, **exposure conditions**, and the body's **defense mechanisms**.

Major types of toxic damage include:

Inflammation, necrosis, enzyme inhibition, lipid peroxidation, genotoxicity, and **reproductive toxicity**.

Key contributions:

- **Blake (1848):** Linked salt toxicity to **ions**.
- **Crum Brown & Fraser (1869):** Introduced **Structure–Activity Relationship (SAR)**.
- **Overton–Meyer Theory:** Explained how **lipid solubility** affects CNS toxicity.
- **Langley & Ehrlich:** Proposed **receptor theory**, later validated by **Clark**.

World War II emphasized **dose–response relationships** and **chemical–biological interactions**, leading to the classification of **xenobiotic metabolism** into **Phase I** and **Phase II** reactions.

Concept of QSAR:

QSAR and the Interdisciplinary Nature and Scope of Toxicology

1. Concept of QSAR (Quantitative Structure–Activity Relationships):

QSAR refers to the method of predicting a chemical's biological activity or toxicity based on its molecular structure. Originally applied to study the toxicity of **inorganic cations**, QSAR revealed that unlike organic molecules, which act as complete entities, **inorganic compounds dissociate**, and their toxic effects are linked to individual **cations, anions, or un-dissociated molecules**.

These ions can form complexes with various ligands, altering the overall biological activity. The **interactions among these components** depend on their ratios and binding properties. This led to the development of the **molecular connectivity index**, a numerical representation of the molecule's branching pattern, which correlates with toxicity. Thus, QSAR opened a new dimension in predicting toxicity using **chemical structure–function relationships**.

2. Toxicology and Its Relationship to Other Sciences:

Toxicology is closely allied with **pharmacology**, as both study chemical–biological

interactions—though pharmacology focuses on therapeutic effects and toxicology emphasizes adverse outcomes. Given that most drugs are toxic at high doses, toxicology may even be viewed as the broader discipline. It also overlaps significantly with **medicine** (e.g., diagnosis and treatment of poisoning) and **agriculture** (e.g., development and regulation of pesticides and biocides). Additionally, toxicology contributes to **ecology and evolutionary biology** through studies of organismal adaptation to toxic environments.

Chemistry and biochemistry provide vital tools for toxicological research:

- **Chemistry** enables detection and analysis of toxicants, particularly in **forensic and environmental** contexts.
- **Biochemistry** helps elucidate the **metabolic pathways** and **molecular mechanisms** of toxic action.

Conversely, toxicological research has enriched fundamental knowledge in organic chemistry and enzymology by uncovering mechanisms of **detoxification and biochemical toxicity**.

3. Scope of Toxicology:

Toxicology is among the oldest known scientific disciplines. Ancient civilizations, including the **Greeks and Romans**, had practical knowledge of poisons, using substances like **hemlock** and plant toxins for executions and assassinations. Early systematic classification of poisons is credited to **Dioscorides**, while the **scientific foundation** of toxicology began with **Paracelsus** in the 16th century, who advocated for experimentation and dose-based analysis.

The modern discipline was formalized by **Mathieu Orfila (1787–1853)**, who distinguished toxicology as an independent science, introduced chemical methods for poison detection, and emphasized the role of toxicology in legal contexts.

Today, toxicology is broadly categorized into:

- **Environmental Toxicology**: Examines pollutants, residues, and occupational exposure.
- **Economic Toxicology**: Deals with the development and safety of drugs, pesticides, and food additives.
- **Forensic Toxicology**: Focuses on poisoning cases, diagnostics, therapy, and legal evidence.

These categories are often interrelated—for example, pesticide development (economic) may raise concerns about environmental contamination (environmental) or human poisoning (forensic).

Environmental Toxicology:

Environmental toxicology is the most rapidly growing branch of science. Public concern over environmental pollutants and their possible chronic effects, particularly carcinogenicity, has given rise, in the United States, to new research and regulatory agencies and recently to the Toxic Substances Control Act.Similar developments are also taking place in many other countries. The range of environmental-pollutants is enormous, including industrial and domestic effluents, combustion products of fossil fuels, agricultural chemicals, and many other compounds that may be found in food, air, and water. Such compounds as food additives and cosmetics are also subjected to the same scrutiny.Other sub-specialties are frequently mentioned that do not fit into the above divisions. Behavioral toxicology, an area of increasing importance, could be involved in any of these and is usually treated as a separate sub-specialty. Analytical toxicology provides the methods used in essentially every branch of the subject, while biochemical toxicology provides the fundamental basis for all branches of toxicology.

Language of Toxicology:

Key Toxicological Concepts: Language, Distribution, Metabolism, and Site of Action

1. Language of Toxicology:

Toxicology, like all scientific disciplines, uses specific terminology. A “toxic” substance is one capable of causing illness. Toxic effects are generally classified as:

- **Acute toxicity**: Rapid onset, short duration.
- **Chronic toxicity**: Delayed onset, long-lasting or permanent.
- **Sub-acute toxicity**: Mild or less obvious symptoms, often detectable only via tests.

Special categories of toxicants include:

- **Carcinogens** (cancer-causing),
- **Mutagens** (cause genetic mutations), and
- **Teratogens** (cause developmental abnormalities).

Exposure refers to a toxicant entering the body, not merely being near it. Exposures may be acute or chronic and influence the **dose**, which depends on concentration, duration, route, and host factors (e.g., age, health, metabolism).

Measurement units include milligrams (mg), micrograms (µg), parts per million (ppm), parts per billion (ppb), and others. Important phenomena include:

- **Bioaccumulation**: Build-up of toxicants in tissues.
- **Biomagnification**: Increasing concentration up the food chain.

The **threshold** concept assumes a safe exposure level, though modern views suggest that even small doses may cause molecular effects. $\mathbf{LD_{50}}$ (lethal dose for 50% of the population) is commonly used to express acute toxicity.

2. Distribution of Toxicants:

After entry, toxicants bind to **blood proteins** (especially lipoproteins) and distribute throughout the body. This distribution is influenced by interactions such as ionic, covalent, hydrogen bonding, and hydrophobic forces. Toxicokinetics, the mathematical modeling of distribution, helps understand these dynamics.

3. Metabolism of Toxicants:

Most xenobiotics are lipophilic and undergo metabolism to become water-soluble for excretion. Metabolism occurs in two phases:

- **Phase I**: Introduction of reactive groups (e.g., via cytochrome P450).
- **Phase II**: Conjugation with endogenous molecules (e.g., glucuronic acid, glutathione).

Though the **liver** is the primary site of metabolism, other organs like the **lungs, intestines, kidneys, and skin** also participate. Metabolism can activate or detoxify toxicants. Factors such as age, sex, diet, and genetics influence metabolic response. Xenobiotics may act as substrates, inducers, or inhibitors of metabolic enzymes, leading to synergistic or antagonistic effects.

4. Sites of Action of Toxicants:

Toxicants or their metabolites act at specific sites, often targeting:

- **Oxidative metabolism**,
- **Nervous system synapses**, or
- **Neuromuscular junctions**.

Chronic effects often involve **DNA damage**, resulting in cancer or reproductive harm. Organs like the **liver** are commonly affected, though all tissues are potential targets. Toxicants impact critical cellular functions including:

- Glycolysis
- TCA cycle
- Oxidative phosphorylation

- Protein and nucleic acid synthesis

Understanding these mechanisms supports both environmental and clinical toxicology.

Excretion and Nature of Toxic Effects:

Excretion of Toxicants:

Toxicants or their metabolites are primarily excreted via the **urinary and biliary systems**. Secondary excretion routes include the **lungs, sweat glands, hair, nails, milk, and eggs**. Many metabolites are eliminated as **conjugated products** following **Phase II metabolism**, enhancing their water solubility.

Nature of Toxic Effects:

Toxic effects depend on the chemical's **physicochemical properties**, **biotransformation**, **exposure conditions**, and **host defense mechanisms** (e.g., DNA repair, enzyme induction, phagocytosis). Common toxic responses include:

- **Inflammation**: Acute or chronic, possibly leading to **fibrosis**.
- **Necrosis**: Localized tissue death due to membrane disruption or protein synthesis inhibition.
- **Enzyme Inhibition**: Disruption of vital pathways, e.g., organophosphates inhibiting acetylcholinesterase.
- **Biochemical Uncoupling**: Disruption of ATP synthesis, leading to heat generation and cellular stress.
- **Lethal Synthesis**: Metabolic transformation of chemicals into toxic intermediates (e.g., fluoroacetate).
- **Lipid Peroxidation**: Initiated by free radicals, damaging cell membranes.
- **Covalent Binding**: Reactive metabolites binding to macromolecules, often linked to **carcinogenesis**.
- **Receptor Interactions**: Altering cellular signaling, such as Ca^{2+} channel effects.
- **Immune Reactions**: Hypersensitivity (e.g., dermatitis, asthma) or **immunosuppression**.
- **Neoplasia**: Abnormal cell growth, either benign or malignant (carcinogenesis).

Modern Toxicology and Molecular Advances

Modern toxicology integrates classical pathology with molecular biology, focusing

on **signaling pathways** and **gene expression alterations** induced by toxicants. Key molecular mechanisms include:

- **Receptor-mediated toxicity**: Involving AhR, CAR, PXR, and PPAR.
- **Metal-induced toxicity**: Via stress response and metal-specific transcription factors.

Toxicology now encompasses multiple disciplines:

- **Pathology**, **pharmacology**, **biochemistry**, **cell biology**, and **public health**.

Key Technological Frontiers:

i. **Toxicogenomics**: Studies gene and protein expression patterns in response to toxicants. It integrates **genomics**, **functional genomics**, and **proteomics**, offering insight into how environmental stimuli influence disease-related gene expression.

ii. **Metabonomics**: Analyzes biochemical fingerprints in biofluids and tissues using **NMR spectroscopy** and **pattern recognition**, allowing early and precise detection of toxic effects.

iii. **Pharmacogenetics**: Explores how genetic variations (e.g., SNPs) influence individual responses to chemicals, including poor or ultra-rapid metabolism—vital for **personalized medicine and risk assessment**.

iv. **Molecular Toxicology**: Focuses on **apoptosis** and **mitochondrial pathways** in toxicant-induced cell death. It studies oxidative stress, caspase activation, and mitochondrial membrane disruption, aiming to develop therapeutic interventions.

Conclusion

Toxicology today is a **multifaceted science**. It spans from environmental exposure to molecular effects, integrating traditional knowledge with modern tools to address public health, safety, and therapeutic advancements.

v. Concept of Biomarkers:

The emergence of specific biomarkers offer the promise of being able to measure signals and/or events that reflected more accurately the biology associated with exposure, effects, and susceptibility. The 1983 NRC publication formalized human health risk assessment into a four component process namely exposure, assessment, hazard identification, dose response assessment and risk characterization.

vi. Chronotoxicology:

Chronotoxicology studies how **biological rhythms**, particularly **circadian cycles**, influence the **absorption, distribution, metabolism, and elimination** of toxicants. These rhythms can alter drug/toxin binding to plasma proteins, red blood cells, and

affect **blood-brain barrier permeability**. Substances like **mercury** and **cadmium** demonstrate time-dependent toxic effects, and enzymes such as **liver microsomal benzene hydroxylase** show peak activity at specific times of day. Circadian variations also influence the **carcinogenic and teratogenic potential** of chemicals.

Importantly, **fertility and seasonal cycles** can further modulate toxic responses. Neglecting these biological time structures in toxicity assessments may lead to inaccurate predictions and flawed safety evaluations.

Superinteractions:

In real-world scenarios, humans are rarely exposed to a single toxicant. Instead, exposure to **multiple chemicals**, either simultaneously or sequentially, can lead to **complex interactions**. Key terms include:

- **Co-carcinogenesis**: Enhancement of cancer risk by additional carcinogens acting with an initial one.
- **Syn-carcinogenesis**: Additive or synergistic effects of multiple carcinogens.
- **Superinteraction**: When the combined toxic effect far exceeds expected outcomes, as seen with **chlordecone (CD)** and **carbon tetrachloride (CCl_4)**—where prior exposure to CD amplified CCl_4 toxicity by 67-fold.

Recognizing such interactions is vital for **screening protocols** and **risk prediction** in modern toxicology.

Toxicity Testing:

Toxicity testing aims to assess the **potential health and environmental risks** posed by chemical substances. Data from these tests guide:

- **Regulatory standards** (e.g., drinking water safety, workplace exposure limits)
- **Pesticide tolerances** on food
- **Chemical registration and re-evaluation**

With growing public health demands and technological advancements, regulatory bodies like the **U.S. EPA** have initiated strategic reviews of both **traditional and emerging testing methods**. In response, the **National Research Council (NRC)** developed a comprehensive framework to improve testing strategies, incorporating molecular and mechanistic insights.

Toxicity tests:

Toxicity tests have taken on increased importance after scientists realized that many

substances are toxic to living things at levels below chemical detection limits and that there are no methods to analyze for many toxic substances. Toxicity of chemicals is determined in the laboratory. The normal procedure is to expose the test animals to the concerned chemical and measure theeffect.

Route of exposure:

By ingestion (oral), application to the skin (dermal), by inhalation, gavage, or some other method which introduces the material into the body orby placing the test material in the water or air of the test animals 'environment.

Duration of exposure:

Acute: short-term exposure (hours or days) of higher doses of toxicant in a single event or in multiple events over the time period and usually produce immediate effects, depending on absorption time of the toxicant. These tests are generally conducted on organisms during a specific time period of the organism's life cycle, and are considered partial life cycle tests. Acute tests are not valid if mortality in the control sample is greater than 10%. Generally it use lethal endpoints and results are reported inEC50.

Chronic: long-term exposure (weeks, months years) of low, continuous doses of a toxicant, relative to the test organism's life span (>10% of life span), and generally use sub-lethal endpoints. Usually, slowly effects are developed in test organism. Chronic tests are generally considered full life cycle tests and cover an entire generation time or reproductive life cycle ("egg to egg"). Chronic tests are not considered valid if mortality in the control sample is greater than 20%. These results are generally reported in NOECs (No observed effects level) and LOECs (Lowest observed effectslevel).

Sub-chronic: chronic exposure during early, sensitive life stages of an organism that arelessthanacompletereproductivelifecycle.Itisalsocalledasearlylifestage tests, critical life stage, embryo-larval, or egg-fry tests. Early life stage tests are not considered valid if mortality in the control sample is greater than 30%.

Toxicity Endpoints: Toxicity is measured as clinical "endpoints" which include behavioral, physiological, biochemical, histological changes, asfollows,

a. **Mortality(death)**
b. **Teratogenicity (ability to cause birthdefects)**
c. **Carcinogenicity (ability to cause cancer), and**
d. **Mutagenicity or Genotoxicity (ability to cause heritable change in theDNA).**

Measures of Toxicity:

Median Lethal Concentration (LC_{50}): The concentration of a chemical in an environment (generally air or water) which produces death in 50% of an exposed population of test animals in a specified time frame. It is normally expressed as milligrams of substance per litter of air or water (mg/L) or asppm.

- **The Median Lethal Dose (LD_{50}):** The concentration of a chemical that is expected to kill 50% of a group of organisms to which it is administered by any of a variety of methods. It is normally expressed as milligrams of substance per kilogram of animal body weight (mg/kg). One of the more commonly used measures of toxicity is the LD50. Example: LD_{50} of sugar and ethanol are 30,000 mg/kg and 13,700mg/kg.
- **Median Effective Concentration (EC_{50})**: The concentration of a chemical that is expected to have one or more specified effects in 50% of a group of organisms after a specified exposuretime.
- **Median Effective Dose (ED_{50}):** The dose level at which 50 percent of the test organism have turned over is known as the ED_{50}, which means effective dose for 50 percent of the organism tested. The ED_{50} of any toxicant varies depending on the effect measured. In general, the less severe the effect measured, the lower the ED_{50} for that particulareffect.
- **Lowest Observed Effect Concentration (LOEC)**: The lowest test concentration that has a statistically significant effect over a specified exposuretime.
- **No Observed Effect Concentration (NOEC)**: The highest test concentration for which no effect is observed relative to a control over a specified exposure time. In toxicology, residue tolerance levels of poisons that are permitted in food or in drinking water, for instance, are usually set from 100 to 1,000 times less than the NOEL to provide a wide margin of safety forhumans.
- **Maximum Acceptable Toxicant Concentration (MATC)**: An estimated value that represents the highest "no-effect" concentration of a specific substance within the range including the NOEC andLOEC.
- **Application Factor (AF)**: An empirically derived "safe" concentration of a chemical.
- **TLV (threshold limit value)**: The TLV for a chemical is the airborne concentration of the chemical (expressed in ppm) that produces no adverse effects in organism exposed for eight hours per day to five days per week. The TLV is usually set to prevent minor toxic effects like skin or eyeirritation.

- **Model animals:** Obviously toxicity is not tested in humans. Instead, animals are used to predict the toxicity that may occur in humans. Common standard aquatic test species are the fathead minnow (*Pimephalespromelas*), daphnids (*Daphnia magna*, *D. pulex*, *D. pulicaria*, *Ceriodaphnia dubia*), midge (*Chironomus tentans*, C. *ruparius*), rainbow trout (*Oncorhynchus mykiss*), sheepshead minnow (*Cyprinodon variegatus*), mysids (*Mysidopsis*), oyster (*Crassotreas*), scud (*Hyalalla azteca*), grass shrimp (*Palaemonetes pugio*), mussels *(Mytilus sp.*). Common standard mammalian test species include rat and dog. These species are routinely selected on the basis of availability, commercial, recreational, and ecological importance, past successful use, and regulatoryuse.
- **Factors:** Toxicity assessment is quite complex, many factors can affect the results of toxicity tests. Some of these factors include variables like temperature, food, light, and stressful environmental conditions. Other factors related to the animal itself include age, sex, health, and hormonalstatus.
- **Application:** Toxicity tests are usedto,
- provide qualitative and quantitative data on adverse (deleterious) effects on organisms from a toxicant,and
- assess the potential for damage and the risk associated with in a situation for a specific toxicant.

Bioassay:

The foundation of bioassays was laid down by a German physician, Paul Ehrlich. His bioassay on diphtheria antitoxin was the first bioassay to receive recognition.

Definition: A bioassay is an analytical method to determine concentration or potency of a substance by its effect on living cells ortissues.

Principle: Bioassay is a biochemical test to estimate the relative potency of a sample compound to a standard compound. Typical bioassay involves a *stimulus* (e.g. drugs) applied to a *subject* (e.g. animals, tissues, plants) and a *response* (e.g. death) of the subject is triggered and measured. The intensity of stimulus is varied by doses and depending on this intensity of stimulus, a change/response will be followed by asubject.

Classifications:

I. ***In vivo* bioassay**: if assays are used to estimate the potency of agents by observing their effects on living animals, it is called in vivo bioassay. In vivo studies are very important both in the field and laboratory (for validation). They are based on a wide variety of end points, including cell differentiation and enzyme activities.

However,it is not possible to use *in vivo* methods for routine or monitoring studies due to ethical problems, expensive, time consuming, and big installations (aquariums etc.) are needed.

II. ***In vitro* bioassays:** if assays are used to estimate the potency of agents by observing their effects on tissues (in vitro), it is called in vivo bioassay. It can be performed more quickly, and much more cost-effectives than *in vivo* assays. However, *in vitro* assays are not able to explain all themechanisms.

III. **Direct assay:** The stimulus/standard sufficiently produces measurable and specific response. The response must be clear, easily recognized, and directly measured.

VI. **Qualitative bioassay:** If the measured response is binary, the assay is qualitative, if not, it is quantitative.

V. **Indirect assay based on quantitative response**: The relationship between the dose and the response is first ascertained. Then the dose corresponding to a givenresponse is obtained from the relation for each preparation separately.

VI. **Indirect assay based on quantal response:** The assay involves 'all or none'response (ex. life or death). The response is produced by thresholdeffect.

Examples:

1. **Plant and algae bioassay:** Test species, such as marine unicellular algae *Selenastrum capricornutum* or *Dunaliella tertiolecta* are used as indicator species. Inhibition of algal growth is used as the indicator of toxicity. The main disadvantage of algal methods is a lack of reproducibility between consecutiveassays.

2. **Invertebrate bioassays:** Chronic toxicity test using macro invertebrates have been extensively used in aquatic risks assessment studies. The parameters measured are mortality or reproduction. One of the most common invertebrate toxicity tests uses *Daphnia* and *Ceriodaphnia*, both freshwater species pertaining to *Cladocera*. Tests are carried out by exposing the test organisms to toxic substances under control conditions. Acute lethality tests with *Daphnia* conducted for 21 days are well established and standardized.

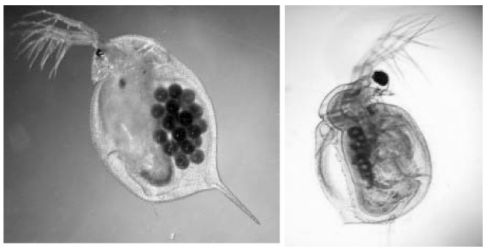


Fig: *Daphnia magna* and *Ceriodaphnia*sp. from left to right.

3. **"In vivo" Fish toxicity bioassays:** Zebrafish, medka, rainbow trout and fathead minnow are generally used in toxicological study. End Points of test includes, mortality (routinely used, 96 hr exposure), larval growth, larval survival, and reproduction. In vivo assays for estrogenicity are widely used. They are based on a wide variety of end points, including cell differentiation and enzyme activities. Vitellogenin (VTG) analysis is done by means of Immunoassay or any other analyticalapproach.

4. **"In vitro" Recombinant yeast assay:** This assay is based on the evaluation of the potential of a compound to interact with oestrogen receptor and activate hormone- regulated genepromoters. Yeast reporter assay is based on a two-hybrid system. Beta-galactosidase, has been used as the most common reporter enzyme. Novel yeast reporter assay are more suitable for high-throughput analysis, employing in the reporter assay luciferase, named CLuc, as a reporterenzyme.

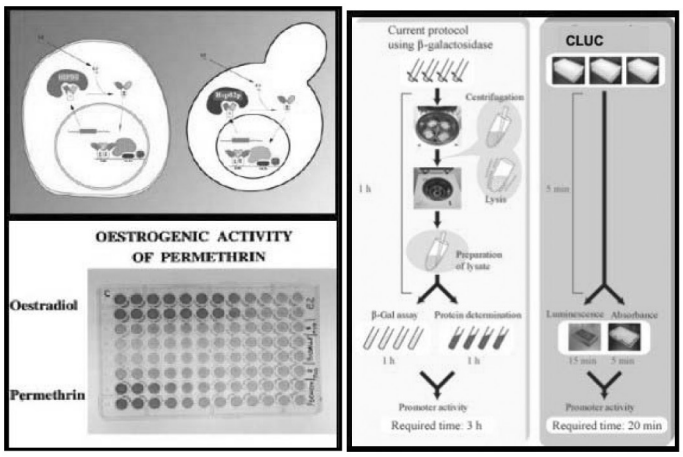


Fig: "In vitro" Recombinant yeast assay.

5. **Bacterial toxicityassays:**

   a) **Bioluminescence inhibition**: The more widely used bioassays in routine laboratories for evaluating toxicity of wastewater effluents and industrial discharges are based on inhibition of the bioluminescence of marine bacteria. The better-known species of luminescent marine bacteria are *Vibrio fischeri* and *Photobacterium phosphoreum*, which naturally emit light due to an enzyme, the bacterial luciferase. This technique allows the easy screening of large numbers of aqueous samples in a quick, reliable, and inexpensive way.

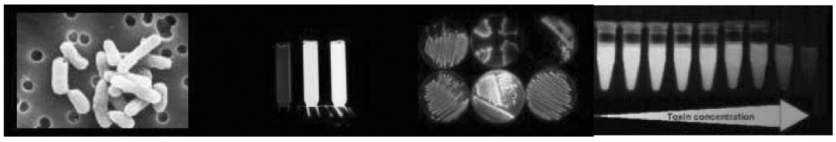


Fig: Bacterial toxicity assays: Bioluminescence inhibition

b) Genotoxicity Ames Test and umu test*:* Genotoxicity is associated with different structures, such as phenols, chlorophenols, polychlorinated biphenyls (PCBs), or polyaromatic hydrocarbons (PAHs), and constitutes an early screening for possible cancer inducing activity of pollution. The most widespread is the Ames test that is based on the retromutation of *Salmonella typhimurium* TA98 (histidine dependent). The umu test is also based on genetically engineered bacteria *Salmonella thyphimurium* TA 1535 pSK1002 (gram negative, facultative anaerobic enterobacteriaceae) and the genotoxicity is detected measuring the activation of the bacterial SOS repair response of genetic damage in the bacterium, through measuring b-galactosidaseactivity.

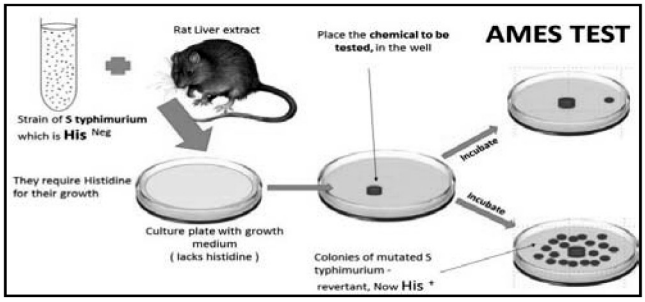


Fig: Bacterial toxicity assays: Genotoxicity Ames Test.

6. **ELISA (Enzyme-linked immunosorbent assay)**: quantitative analytical method that measures absorbance of colour change from antigen-antibody reaction (ex. Direct,indirect, sandwich, competitive). ELISA is used to measure variety of substances in human body from cortisol levels for stress to glucose level fordiabetes.

Uses: Bioassay isused for

1. To test carcinogenicity ofchemicals.
2. To test toxicity and safety of drugs, food additives andpesticides.
3. To detect biological hazards.
4. Give a quality assessment of amixture.
5. To monitor water quality and also sewage discharge and its impact onsurrounding.
6. To assess the environmental impact and safety of new technologies andfacilities.

Reliance on bioassay increased as the public concern for occupational and environmental hazards increased.

Types of Interactions among toxicants:

When toxic chemicals and substances come in contact with each other, chemical reactions occur. These reactions can be divided into one of four categories: additive, synergistic, antagonistic, and potentiating.

I. **Additive effects**: The sum of the effects of the chemicals involved in the reaction. This usually occurs with chemicals that are similar in structure, so they work well as a team. The sum of the additive effects is sum of the effects exposed to each chemicalindividually.

   Example: If you take aspirin and acetaminophen both together, you get the total effect of both pain-killing drugs on your body. Aspirin and acetaminophen, are the active ingredient in drugs like Tylenol.

II. **Synergistic effects** are when the sum of the effects is more than each chemical individually. This can create dangerous situations because each chemical is designed to work well on itsown.

   Example: Alcohol and acetaminophen are a dangerous combination for your body. This is because both are processed in your liver, and each puts a lot of strain on this small but powerful organ. If you put both drugs into your body at the same time, it can overwhelm the liver, sending it into failure.

III. **Antagonistic effects** are when the net effect of the chemical reaction is zero. If one is positive and another is negative, both neutralize each other's effect. Antagonistic effects are important because this is where we get antidotes forpoisons.

   Example: Anti-venom for snakebites and combination of caffeine and alcohol show antagonistic effect.

IV. **Potentiating effects:** This is when one chemical enhances the effect of another chemical. Some chemicals are not toxic on their own, but when they are in the presence of some other chemicals, they become toxic. This is one more less-common type ofinteraction.

Table 1. Types of interactions between toxic chemicals and substances.

Additivity	a combination of two or more chemicals is the sum of the expected individual responses
Antagonism	exposoure to one chemical results in a reduction in the effect of the other chemical
Potentiation	exposure to one chemical results in the other chemical producing an effect greater than if given alone
Synergism	exposure to one chemical causes a dramatic increase in the effect of another chemical

Other Types of ToxicityTests:

1. Bioaccumulation Tests:

Bioaccumulation tests are designed to assess the buildup of **hydrophobic toxicants**—chemicals with low water solubility that tend to accumulate in the **lipid-rich tissues** of organisms. This accumulation can lead to **cumulative toxicity** over time.

A key metric in these tests is the **Bioconcentration Factor (BCF)**, defined as the ratio of the chemical concentration in an organism's tissue (at steady state) to its concentration in the surrounding water. A high BCF indicates a strong potential for bioaccumulation and long-term ecological risk.

2. Effluent Toxicity Tests:

Effluent toxicity tests are regulatory assessments conducted under the **Clean Water Act** via the **NPDES permit program**, aiming to monitor and control pollution from industrial and municipal discharges.

- **Acute Effluent Tests**: Conducted monthly on industrial wastewater using species like *Ceriodaphniadubia* and *Pimephalespromelas*, exposed for 48 hours across five effluent concentrations under static conditions.
- **Chronic Effluent Tests**: Performed quarterly on municipal wastewater, these seven-day tests evaluate **long-term sublethal effects**—including impacts on **growth, reproduction, and survival**—during sensitive life stages of aquatic organisms.

Key endpoints reported include:

- **NOEC (No Observed Effect Concentration)**
- **LOEC (Lowest Observed Effect Concentration)**
- **EC_{50} (Effective Concentration for 50% of population)**

These tests ensure effluents do not pose acute or chronic toxicity risks to aquatic ecosystems, and they follow standardized protocols developed by the **U.S. Environmental Protection Agency (EPA)**.

Probable Questions:

1. Define toxicology. How toxicology was developed.
2. State interrelationship between toxicology and other science disciplines.
3. What are scopes of toxicology.

4. How toxins are metabolized?
5. Briefly state the nature of toxic effects.
6. What is toxico-genomics?
7. What is pharmacogenetics?
8. Define molecular toxicology.
9. What is chronotoxicology?
10. How toxic products are eliminated?
11. What is acute, chronic and sub chronic toxicity?
12. How toxicity is measured?
13. Define bioassay? State the classification of bioassay.
14. How bacterial toxicity is assessed?
15. What is in vitro recombinant yeast assay?
16. What is in vivo fish toxicity bioassay?
17. What are the uses of bioassay?
18. State an experiment on fish by which you can determine LC_{50}
19. State an experiment on fish by which you can determine LD_{50}
20. What is additive effect of toxicity? Give examples.
21. What is synergistic effect of toxicity? Give examples.
22. What is antagonistic effect of toxicity? Give examples.
23. What isPotentiating effects of toxicity? Give examples.
24. What is bioaccumulation test?
25. What isEffluent toxicity tests?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett&Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-II

Carcinogenic, Mutagenic and Teratogenic effects, Method of testing chemicals on insect and evaluation of toxicity

Objective: In this unit we will learn about Carcinogenic, Mutagenic and Teratogenic effects of different chemicals.

Carcinogen:

Carcinogens are substances, radiations, or radionuclides that can initiate or promote cancer—a condition characterized by uncontrolled cell growth and the potential to spread throughout the body. These agents may cause **genetic mutations**, **disrupt metabolic pathways**, or **damage DNA**, ultimately triggering the formation of tumors.

Carcinogens can be:

- **Radioactive**, such as **alpha particles** and **gamma rays**, where the emitted radiation causes cellular damage.
- **Non-radioactive**, such as:
    - **Tobacco smoke** (contains carbon monoxide and cancer-causing chemicals),
    - **Asbestos fibers** (linked to lung cancer),
    - **Dioxins**, and
    - **Polynuclear hydrocarbons** (e.g., benzopyrene), formed by incomplete combustion of coal, petroleum, or tobacco.

They may be of **natural** origin, such as:

- **Aflatoxin B1**, produced by fungi on grains and nuts,
- **Oncogenic viruses** like **Hepatitis B virus** and **Human Papillomavirus (HPV)**.

Carcinogenic substances are often insidious—they may not show immediate toxicity but gradually increase cancer risk by altering genetic material or impairing cellular functions.

Due to their potential to cause serious health consequences, exposure to known carcinogens should be minimized or avoided.

Identifying Chemicals that Cause Cancer:

Carcinogens—substances that cause cancer—are commonly present in our environment, including air, food, water, and even household or workplace materials. However, most are weakly carcinogenic, and exposure to potent ones can often be avoided with informed precautions.

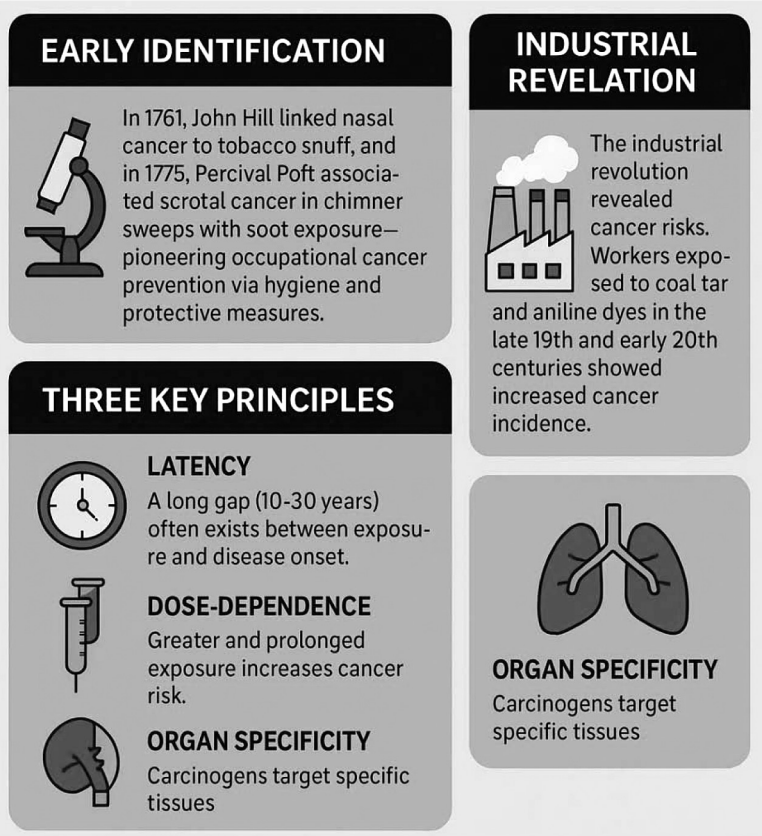
The identification of chemical carcinogens began in the 18th century. In 1761, John Hill linked nasal cancer to tobacco snuff use, and in 1775, Percival Pott associated scrotal cancer in chimney sweeps with soot exposure—pioneering the first occupational cancer prevention campaign through hygiene and protective measures.

The industrial revolution further revealed cancer risks. Workers exposed to coal tar and aniline dyes in the late 19th and early 20th centuries showed increased cancer incidence. A breakthrough case involved **2-naphthylamine**, a dye intermediate that caused bladder cancer among factory workers. This was the first documented example of a specific chemical causing human cancer.

Three key principles emerged:

- **Latency**: A long gap (10–30 years) often exists between exposure and disease onset.
- **Dose-dependence**: Greater and prolonged exposure increases cancer risk.
- **Organ specificity**: Carcinogens target specific tissues; e.g., inhaled substances affect lungs, while urine-concentrated chemicals affect the bladder.

These discoveries laid the foundation for chemical carcinogenesis research and modern regulatory toxicology, emphasizing the need for long-term exposure monitoring and safety regulation in industrial and public health settings.



Asbestos as a Cause of Cancer Deaths:

Asbestos, a mineral used for its fire-resistant properties since the late 1800s, poses severe health risks due to its **microscopic, inhalable fibers**. These fibers lodge in the lungs, causing **asbestosis** and significantly increasing the risk of **lung cancer**—especially in smokers, who face up to **50 times greater risk**.

Uniquely, asbestos is the only confirmed cause of **mesothelioma**, a rare cancer affecting the lining of the lungs and abdomen. This occurs when asbestos fibers**migrate through tissues**, triggering chronic inflammation and malignancy.

Despite global regulations since the 1960s, **mesothelioma cases continue to rise** due to the **long latency period (30+ years)** and residual asbestos in buildings. Asbestos ranks as the **second most lethal commercial carcinogen**, after tobacco.

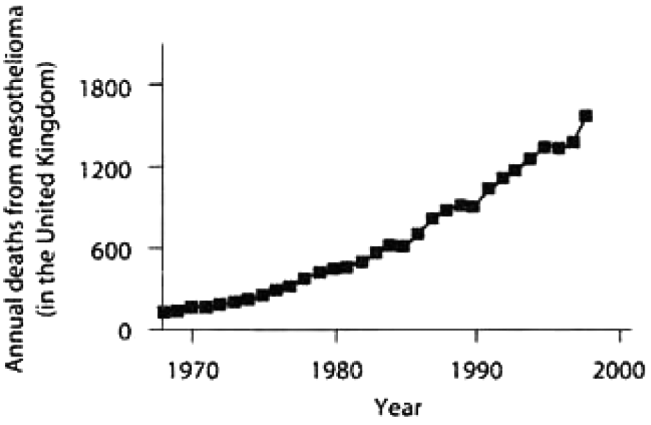


Figure 2 Death Rate Trends for Mesothelioma.
Despite increasing restrictions on the production and use of asbestos, deaths from mesothelioma (a cancer induced almost solely by asbestos exposure) continue to rise. The most likely reasons are that a long delay can intervene between asbestos exposure and developing cancer and that vast reservoirs of asbestos remain behind in existing buildings. Data are for the United Kingdom. [Data from G. Tweedale, *Nature Reviews Cancer* 2 (2002): 311 (Figure 2).]

Workplace Exposure to Chemical Carcinogens as a Cause of Cancer:

Many chemical carcinogens, like 2-naphthylamine and asbestos, were recognized only after cancer cases rose among heavily exposed workers. Throughout the 20th century, industrial sectors—such as dye, rubber, plastic, and mining—revealed cancer patterns that led to the identification of key occupational carcinogens. By the 1970s, most major workplace carcinogens had been discovered. The establishment of OSHA in

1970 helped regulate and reduce industrial exposures, leading to a significant decline in related cancer deaths—now accounting for less than 5% of all cases in developed nations. However, disparities persist. While countries like those in the Nordic region have nearly eliminated asbestos use, exposure remains high in parts of the developing world, where workplace safety regulations are still inadequate.

Environmental vs Occupational Carcinogen Exposure:

Workplace cancers often result from prolonged, high-dose exposure to carcinogens. While traces of these chemicals enter the environment—affecting air, water, and food—the public's exposure is typically thousands of times lower than in industrial settings. For instance, the pesticide ethylene dibromide (EDB) exposed workers to ~150 mg/day, while pre-ban public exposure was ~0.00042 mg/day. Historical cancer trends also challenge the notion of a pollution-driven epidemic. Despite a massive rise in chemical production throughout the 20th century, age-adjusted cancer rates have remained relatively stable, with most common cancers today already prevalent a century ago. Notably, lung cancer—often cited in this context—is mainly linked to smoking, not pollution.

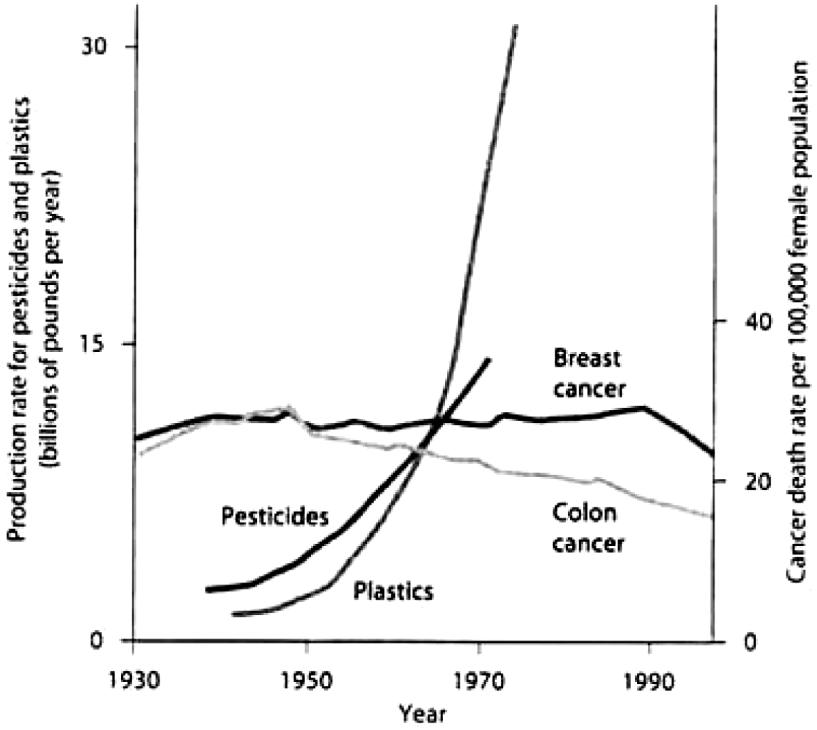


Figure 3 Patterns of Industrial Pollution and Cancer Death Rates in the United States. Industrial use of chemical carcinogens, as occurs in the plastics and pesticide industries, increased dramatically between the 1940s and 1970s. For most types of cancer, there was no corresponding increase in cancer incidence during the following decades. If industrial pollution had a major effect on cancer rates, it should have been evident by now. Mortality rates for breast and colon cancer in women are shown, but many cancers exhibit a similar pattern. The only cancer that has shown a major increase in incidence is lung cancer, which is caused mainly by tobacco smoke rather than industrial pollution. [Based on data from *Cancer Facts & Figures 2002* (Atlanta, GA: American Cancer Society, 2002), p. 3; and R. H. Harris et al. in *Origins of Human Cancers* (H. H. Hiatt et al., eds., Cold Spring Harbor, NY: CSHL Press, 1977), pp. 309–330 (Figure 2).]

Cancer Risk from Low-Dose Carcinogen Exposure:

While high-dose exposure to chemical carcinogens (e.g., in workplaces) poses significant cancer risks, low-dose environmental exposure (via air, water, food) presents a much smaller, often undetectable threat. Chemicals like dioxins and organochlorine pesticides (e.g., DDT) persist in the environment and accumulate in body fat, but their actual carcinogenic impact at low levels remains unclear. Epidemiological studies often fail to establish strong links due to limitations in detecting minimal effects.Air pollution, especially fine-particle soot and indoor contaminants from everyday products, may slightly elevate cancer risk. However, these risks are far lower compared to smoking. Animal testing is often used to estimate risk, but these models—linear (no safe dose), threshold (safe dose exists), or hormetic (low doses may be protective)—may not apply to humans due to species-specific differences. For example, saccharin causes cancer in rats but not in humans, due to physiological differences. The U.S. classifies carcinogens as either "known" or "reasonably anticipated" based on human and/or animal data, acknowledging uncertainty when extrapolating from animal models. High-dose exposure via medications (e.g., DES, immunosuppressants like cyclosporin) can also induce cancer, though benefits may outweigh risks. Rapamycin, a newer drug, shows promise in reducing cancer risk by inhibiting angiogenesis. Despite chemical diversity, most carcinogens fall under a few organic compound categories, sharing common mechanisms such as DNA damage or interference with repair pathways, ultimately leading to uncontrolled cell growth.

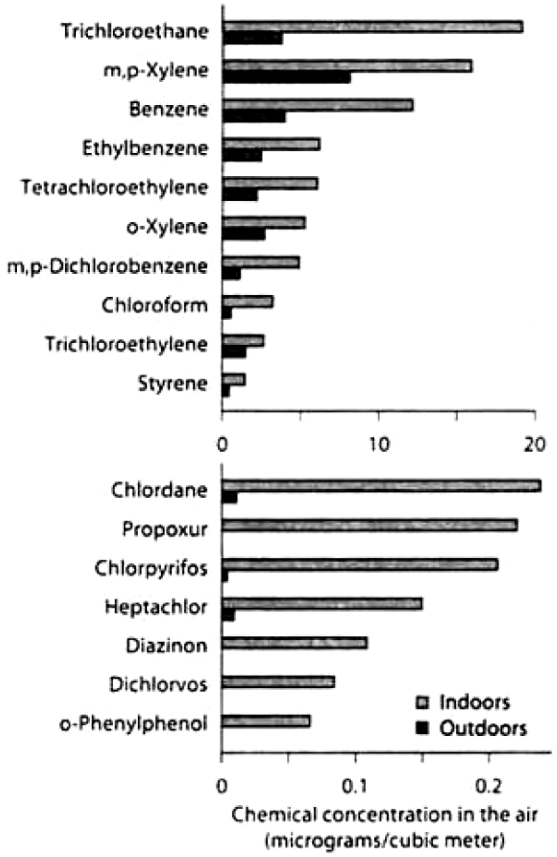


Figure 4 Comparison of Indoor and Outdoor Air Pollution. Pollution data were obtained from people equipped with portable air-quality monitoring devices designed to measure the concentration of toxic volatile organic chemicals (*top*) and pesticides (*bottom*) in indoor and outdoor air. Results from studies involving more than a dozen cities in the United States, including cities with chemical processing plants, have revealed that the air inside a person's home usually contains higher concentrations of potential carcinogens than are present in outdoor air. [Data from W. R. Ott and J. W. Roberts, *Sci. Amer.* 278 (February 1998): 88.]

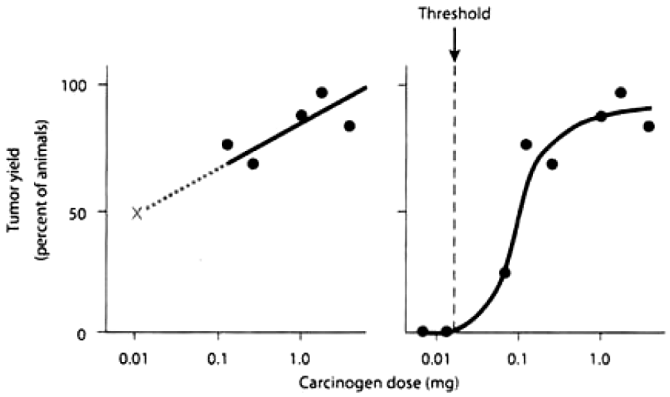


Figure 5 Possibility of Overestimating Cancer Risk When Extrapolating from High-Dose Data. Dose-response curves are illustrated for sarcomas arising in mice after a single injection of benzo[*a*]pyrene. (*Left*) The graph on the left is restricted to high doses of carcinogen (> 0.1 mg). The results appear to be roughly linear, and a straight line can be drawn through the data points to estimate the cancer risk for a lower dose (0.01 mg) of benzo[*a*]pyrene. This estimated cancer risk is indicated by the "X". (*Right*) When actual experiments are carried out that include lower doses of benzo[*a*]pyrene, the shape of the overall curve is seen to exhibit a threshold. Note that the actual cancer risk associated with a 0.01 mg dose of benzo[*a*]pyrene shown in the graph on the right is much lower than the risk estimated by the linear extrapolation derived from the high-dose data shown in the graph on the left. [Based on data from W. R. Bryan and M. B. Shimkin, *J. Natl. Cancer Inst.* 3 (1943): 503.]

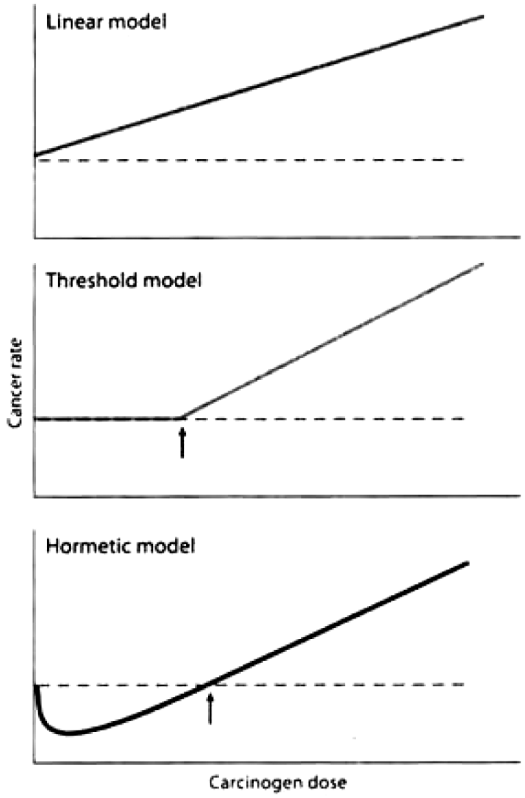


Figure 6 Three Models for the Relationship Between Carcinogen Dose and Cancer Risk. The dashed lines represent the background cancer rate in the absence of carcinogen. Note that the threshold and hormetic models both involve a threshold dose (*arrow*) that must be exceeded before cancer rates begin to rise. [Adapted from E. J. Calabrese, *Mutation Res.* 511 (2002): 181 (Figure 1).]

Table 2 Some Medications That Can Cause Cancer

Medication	Type of Cancer Caused
Analgesic:	
Phenacetin	Kidney
Cancer chemotherapy:	
Chlorambucil	Leukemia
Cyclophosphamide	Bladder, leukemia
Melphalan	Leukemia
Thiotepa	Leukemia
Hormones:	
Estrogens	Breast, uterus, vagina
Oxymetholone	Liver
Immunosuppressive drugs:	
Azathioprine	Lymphoma, skin, liver
Cyclosporin	Lymphoma, skin
Skin treatments:	
Arsenic compounds	Skin, liver, lung
Methoxypsoralen	Skin

Mechanisms of Chemical Carcinogenesis:

As the list of substances known to cause cancer has grown over the years, it has become increasingly apparent that carcinogens exhibit wide variations in structure and potency. At first this variability complicated our thinking about the origins of cancer because it was difficult to envision how such a diverse array of chemical substances could cause the same disease. Through an extensive series of studies, however, a common set of mechanisms and principles has begun to emerge that helps explain how the various kinds of carcinogens work.

Chemical Carcinogens can be grouped into Several Distinct Categories:

Despite their structural diversity, chemical carcinogens can be grouped into a relatively small number of categories (Figure 7). Most are natural or synthetic organic chemicals—that is, carbon-containing compounds. They range from small organic molecules containing only a few carbon atoms to large, complex molecules constructed from multiple carbon-containing rings.

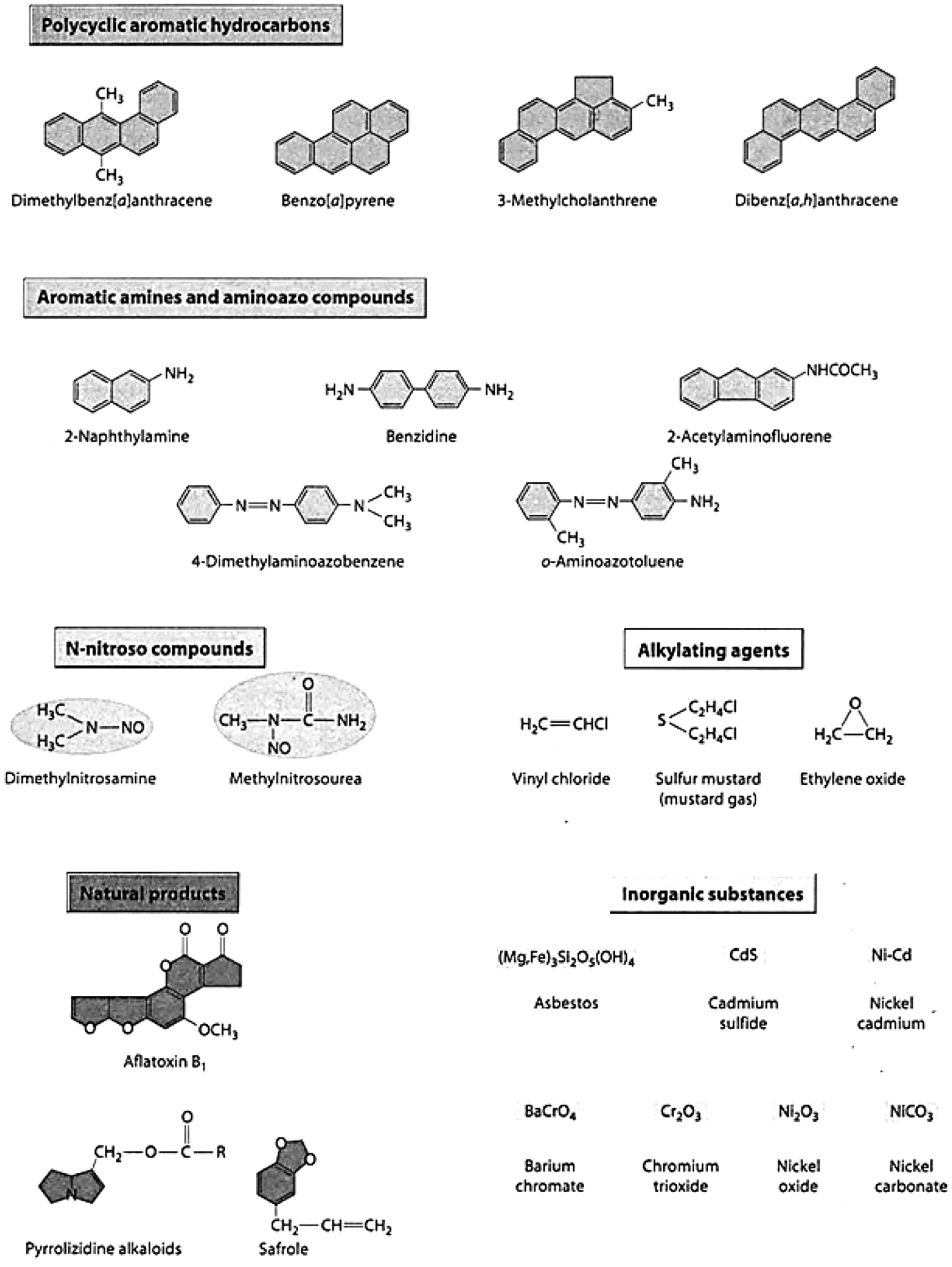


Figure 7 Main Classes of Carcinogenic Chemicals. Selected examples are illustrated for each of the main classes of cancer-causing chemicals. Some of these molecules are precarcinogens that need to be metabolically activated before they can cause cancer, whereas others are direct-acting carcinogens that do not require metabolic activation.

Major Classes and Activation of Chemical Carcinogens

Chemical carcinogens primarily fall into five categories:

1. **Polycyclic Aromatic Hydrocarbons (PAHs):** Found in soot, coal tar, and smoke from burning organic matter; potency varies, with some like benzo[a]pyrene being highly carcinogenic.
2. **Aromatic Amines &Aminoazo Dyes:** Used in dye production and present in tobacco smoke; their carcinogenicity depends on chemical structure.
3. **N-Nitroso Compounds:** Potent in animals, some form in the human stomach from nitrates/nitrites in food, though evidence in humans is limited.
4. **Alkylating Agents:** Defined by their chemical reactivity rather than structure; examples include vinyl chloride, ethylene oxide, and certain chemotherapy drugs.
5. **Natural Products:** Biologically derived carcinogens like aflatoxin (from moldy grains) and plant-based toxins.

Additionally, some inorganic compounds (e.g., cadmium, chromium, asbestos) are carcinogenic due to their physical properties rather than reactivity.

Metabolic Activation of Carcinogens

Many carcinogens, termed pre-carcinogens, require metabolic activation—typically by liver enzymes such as cytochrome P450—to become cancer-causing. This activation can inadvertently convert otherwise harmless substances into carcinogens. For instance, 2-naphthylamine causes bladder cancer only after liver metabolism.

Tobacco smoke not only contains carcinogens but also induces P450 enzymes, heightening cancer risk. Genetic differences in these enzymes (e.g., P450 1A1) further influence individual susceptibility.

This metabolic dependency explains species-specific effects (e.g., AAF is carcinogenic in rats but not guinea pigs) and justifies testing across multiple animal models.

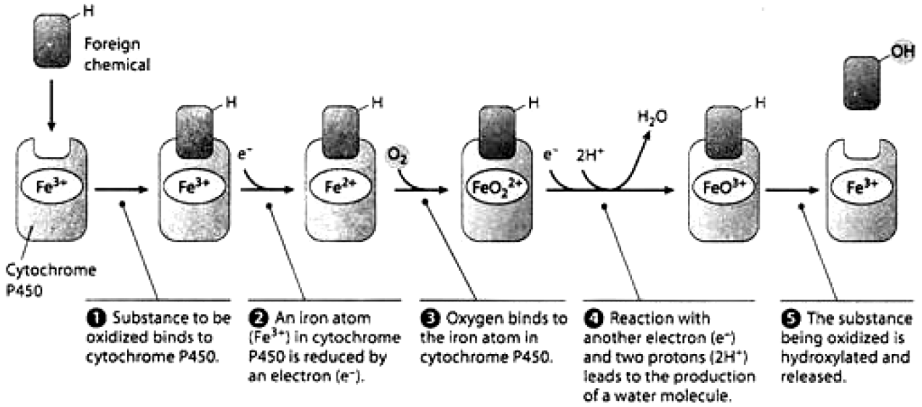


Figure 8 Hydroxylation Reaction Catalyzed by Cytochrome P450. Cytochrome P450 oxidizes chemicals by linking them to a hydroxyl group in a five-step oxidation reaction.

Mechanisms and Multistep Nature of Chemical Carcinogenesis

1. Electrophilic Carcinogens and DNA Adducts:

Many carcinogens, once metabolically activated (often by liver enzymes like cytochrome P450), become electrophilic—electron-seeking compounds that form covalent bonds with DNA. These interactions create DNA adducts, which distort the double helix and cause mutations. Substances like benzo[a]pyrene, aflatoxin, and vinyl chloride undergo such activation, forming epoxides that preferentially bind to DNA bases like guanine. The formation of DNA adducts is a strong indicator of a compound's carcinogenic potential.

2. Multistep Model: Initiation, Promotion, and Progression:

Carcinogenesis occurs through multiple stages:

- **Initiation**: A single exposure to a genotoxic carcinogen (e.g., DMBA) causes a permanent mutation in DNA. If unrepaired before DNA replication, this change becomes fixed.
- **Promotion**: Repeated exposure to non-mutagenic agents (e.g., croton oil, alcohol, or even hormones like estrogen and testosterone) stimulates the proliferation of initiated cells. This stage is reversible in early phases and essential for tumor development.
- **Progression**: Over time, initiated cells undergo further mutations and **epigenetic alterations**, leading to clonal selection of more aggressive, invasive, and therapy-resistant cell populations.

3. Role of Protein Kinase C in Tumor Promotion:

Tumor promoters like TPA mimic diacylglycerol (DAG), persistently activating protein kinase C (PKC), which drives uncontrolled cell division. Other toxins like teleocidin and aplysiatoxin act similarly. Endogenous hormones (e.g., estrogen, testosterone) and dietary components (e.g., fat, alcohol) may also promote tumors by enhancing cell proliferation.

Randomness, Risk, and Potency in Carcinogenesis:

Carcinogens don't guarantee cancer but increase its **probability**. The risk depends on **dose**, **potency**, and **whether the agent is initiating, promoting, or complete**. Potent carcinogens generate more DNA damage or form highly reactive adducts. For instance, smoking increases the risk of lung cancer by elevating random DNA damage, though not every smoker develops cancer—highlighting the probabilistic nature of mutation.

5. Complete vs. Incomplete Carcinogens

- **Initiators** cause mutations (e.g., DMBA).
- **Promoters** drive proliferation without causing mutations (e.g., TPA).
- **Complete carcinogens** perform both functions, especially at high doses.

6. Epigenetics and Tumor Reversibility:

Beyond mutations, **epigenetic changes**—alterations in gene expression without DNA sequence change—contribute to malignancy. These are more reversible and can be reprogrammed under certain conditions, as seen in experimental nuclear transplantation models.

7. Tumor Evolution Through Clonal Selection:

With continued mutations and selective advantages (e.g., faster growth, metastasis, drug resistance), tumor cells undergo clonal evolution. This explains delayed onset of cancer after exposure and variability in cancer outcomes.

8. Immune Suppression and Cancer Risk:

Unlike chemical carcinogens, **immunosuppressive drugs** raise cancer risk indirectly by weakening immune surveillance, allowing abnormal cells to escape destruction.

Teratogen:

A teratogen is any agent that causes an abnormality following fetal exposure during pregnancy. Teratogens are usually discovered after an increased prevalence of a particular birth defect. For example, in the early 1960's, a drug known as thalidomide was used to treat morning sickness. Exposure of the fetus during this early stage of development resulted in cases of phocomelia, a congenital malformation in which the hands and feet are attached to abbreviated arms and legs.

Teratogens can also be found at home or the workplace. The effect is related to type of agent, dose and duration and time of exposure. The first half of pregnancy is the most vulnerable. Teratogenic agents include infectious agents (rubella, cytomegalovirus, varicella, herpes simplex, toxoplasma, syphilis, etc.); physical agents (ionizing agents, hyperthermia); maternal health factors (diabetes, maternal PKU); environmental chemicals (organic mercury compounds, polychlorinated biphenyl or PCB, herbicides and industrial solvents); and drugs (prescription, over- the-counter, or recreational). In general, if medication is required, the lowest dose possible should be used and combination drug therapies and first trimester exposures should be avoided.

Causes of Teratogenicity:

The toxicants which cause teratogenesis are known as teratogenic agents.A gestating-embryo exhibits great dynamicity of the living cells. The embryonic cells multiply and differentiate at a tremendous rate making the embryo more susceptible to the drugs.

Stage Sensitivity for Teratogenicity:

i. Pre-Differentiation Stage:

During this stage the embryo is not susceptible to teratogenic agents. These agents either cause death to the embryo by killing all or most of the cells, or have no apparent effect on the embryo. Even when some widely harmful effects have been produced, the surviving cells can compensate and form a normal embryo. This resistant stage varies from 5-9 days depending on the species.

ii. Embryonic Stage:

In fact this is the period when the cells undergo intensive differentiation, mobilization and organization. It is during this period that most of the organogenesis takes place. As a result, the embryo becomes most susceptible to the effects of various teratogens.

This period generally ends sometimes from the 10th-14th day in rodents and in the 14th week of the gestation period in humans. All organs are, however, not susceptible in the same period of the pregnancy. Rat embryo is most susceptible between days 8 and 12 for most organs, but the palate and urinogenital organs are more susceptible at a later stage for teratogens.

J. G. Wilson (1965) observed teratogenic treatment on the 10th day of gestation which resulted in the following incidences of malformations in rat:

Brain defects – 35%

Eye defects – 33%

Heart defects – 24%

Skeletal defects – 18%

Urinogential defects – 6%

iii. Fetal Stage:

This stage is characterized by growth and functional maturation. Teratogens are thus unlikely to cause morphological defects during this stage, but they may induce functional abnormalities. Whereas, morphologic defects are, in general, readily detected at birth or shortly thereafter functional abnormalities, viz., CNS impairment, may not be diagnosed for some time even after birth.

Mode of Action of Teratogens:

Various mechanisms are involved in teratogenic effects:

i. Interference with Nucleic Acids:

Various teratogenic agents interfere with nucleic acid replication, transcription, or RNA translation. These include alkylating agents, antimetabolites, intercalating agents and amino acid antagonists.

ii. Inhibition of Enzymes:

Inhibitors of enzymes, e.g. 5-flourouracil, may induce malformation through interference with differentiation or growth by inhibiting thymidylate synthatase. Other examples include 6-aminonicotinamide, which inhibits glucose-6-phosphate dehydrogenase, and folate antagonists which inhibit dihydrofolate reductase.

iii. Deficiency of Energy Supply and Osmolarity:

Certain teratogens can affect the energy supply for the metabolism by restricting the availability of substrates either directly (e.g., dietary deficiencies) or through the presence of analogs for antagonists of vitamins, essential amino acids, and others.

In addition, hypoxia and agents i.e., CO and CO_2, can be teratogenic by depriving the metabolic process of the required O_2 and probably also by the production of osmolar imbalances. These can induce edema, which, in turn, cause mechanical distortion and tissue ischemia. Physical agents that can cause malformations include radiation, hypothermia, hyperthermia and mechanical trauma. It shall not be out of place to mention that the mode of action of many teratogens is yet uncertain. Furthermore, a potential teratogen may or may not exert teratogenic effects depending on such factors as bio-activating mechanism, stability and detoxifying capability of the embryonic tissues. Appropriate experimental testing for the teratogenicity of toxicants is, therefore, essential.

Observations:

The Pregnant Animals:

The animals should be examined daily for gross signs of toxicity and many females that show signs of impending abortion or premature delivery (e.g., vaginal bleeding) should be examined.

The Fetuses:

Fetuses are usually surgically removed from the mother about one day prior to the expected delivery. This procedure is intended to avoid cannibalism and permit counting of resorption sites and dead fetuses.

Following observations are to be made and recorded:

i. Number of corpora lutea

ii. Number and position of implantations

iii. Number and position of resorptions

iv. Number and position of dead fetuses

v. Number and position of live fetuses

vi. Sex of each live fetus

vii. Weight of each live fetus

viii. Length of each live fetus, and

ix. Abnormalities of each fetus.

Detailed Examinations:

To determine the different types of abnormalities, each fetus is examined for external defects. In addition, about 2/3rd of random sampled fetusesare closely examined for skeletal abnormalities after staining with Alizarin Red. The remaining one-third of the fetuses are examined for visceral defects after fixations in Bowin's fluid and sectioned by microtome. With larger animals, e.g., dogs, pigs, and non-human primates, the skeletal structure is generally examined with X-ray instead of staining.

Delayed Effects:

With toxicants that are suspected of having effects on the central nervous system or genitourinary system, a sufficient number of pregnant females are allowed to deliver their pups. These pups are nursed either by their biological mothers — thus possibly being exposed to the toxicants via the milk — or by foster mothers. In the latter case, the potential effects of postnatal exposure are eliminated.

Neuromotor and behavioural tests may be applied to detect CNS effects. These include posture, mother activity, coordination, endurance, vision, hearing, learning ability, response to foreign environment, mating behaviour and maternal behaviour.

Evaluation of Teratogenic Effects:

1. Categories of Aberrations

Teratogenic effects range from minor anomalies (e.g., supernumerary ribs, malrotated limbs) to major malformations (e.g., spina bifida, hydrocephalus) that impair survival or development. The severity and impact determine their significance.

2. Key Parameters in Assessment

Resorptions: Indicate embryonic death; calculated by subtracting live offspring from total implantations. Elevated rates may require dose adjustments to differentiate between embryotoxicity and teratogenicity.

Fetal Toxicity: Evident through low birth weight or non-viability. Viability in rabbits may require 24-hour postnatal observation.

3. Sources of Error

- Genetic susceptibility or resistance in test animals
- Poor husbandry or maternal health
- Nutritional deficits due to toxicant exposure
- Inappropriate dosing (too high: increased resorptions; too low: no effect)

4. Data Analysis

The litter is the experimental unit. Statistical significance is evaluated by the number of litters with defects, not individual fetuses.Dose–response trends strengthen teratogenicity claims; historical control data may support conclusions.

5. Human Extrapolation Challenges

Species differences limit direct extrapolation. Example: thalidomide is highly teratogenic in humans but not in rodents; aspirin shows the reverse. Mechanisms remain unclear, demanding more research and caution in interpretation.

6. Regulatory Consideration

If multiple animal models show positive results, chemicals should be restricted for women of childbearing age. Both incidence and severity of effects are critical for assessment.

7. In Vitro Approaches

Not yet standard but valuable for understanding mechanisms. Techniques include cell and organ cultures.

Mutagen:

A mutagen is a substance or agent that causes DNA impairment that results in the alteration of the DNA sequence. This alteration of the DNA sequence is known as mutation. Any agent causing mutation is called mutagen. Mutagens can be physical mutagens, chemical mutagens, or biological mutagens.

1. Chemical Mutagens:

Singer and Kusmierek (1982) have published an excellent review on chemical mutagenesis.

Some of the chemical mutagens and mutagenesis are given in Table 9.3, and described below:

Table 9.3: Different types of chemical mutagens

Class of Chemical	*Chemical Mutagens*
Acridines	Ethyleneimine (El)
Mustard	Nitrogen mustard Sulphur mustard
Nitrosamines	Diethylnitrosamine (DMN) Diethylsulphonate (DES) Nitrosomethylurea (NMU)
Epoxide	Ethyleneoxide (EO) Diepoxybutane (DEB)
Alkyl sulphonates	Diethylsulphonate (DES) Methylmethanesulphonate (MMS) Ethylmethanesulphonate (EMS)
Others	Nitrous acid Maleic hydrazide Hydyazide

i. Base Analogues:

A base analogue is a chemical compound similar to one of the four bases of DNA. It can be incorporated into a growing polynucleotide chain when normal process of replication occurs.' These compounds have base pairing properties different from the bases. They replace the bases and cause stable mutation.

A very common and widely used base analogue is 5-bromouracil (5-BU) which is an analogue of thymine. The 5-BU functions like thymine and pairs with adenine (Fig. 9.6A).

The 5-BU undergoes tautomeric shift from keto form to enol form caused by bromine atom. The enol form can exist for a long time for 5-BU than for thymine (Fig. 9.6B). If 5-BU replaces a thymine, it generates a guanine during replication which in turn specifies cytosine causing G: C pair (Fig. 9.6A).

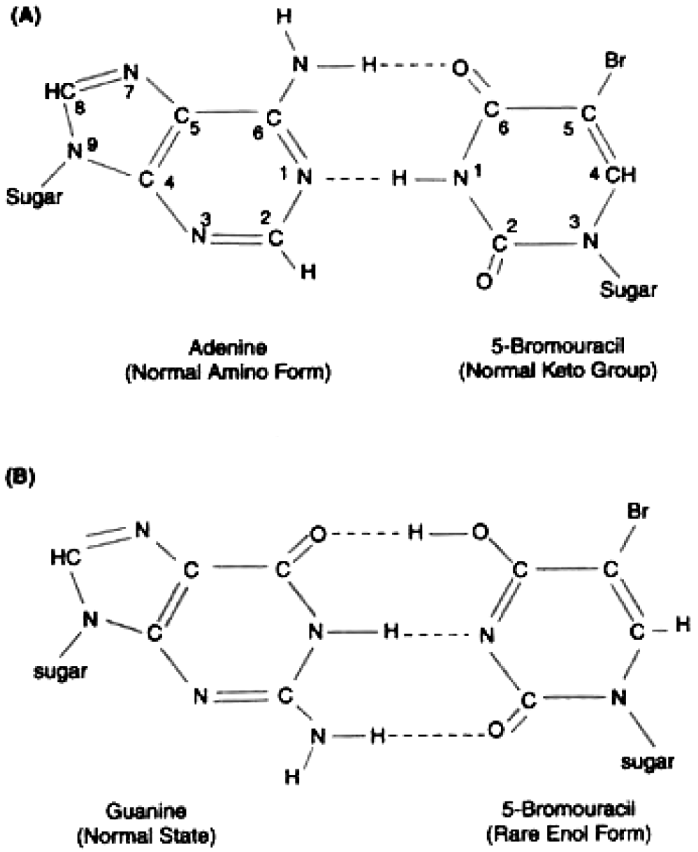


Fig. 9.6 : Mutagenesis by base analogue 5-bromouracil. A, the keto form of 5-BU pairs with adenine; B, 5-BU is tautomarised to enol form and pairs with guanine rather than adenine.

During the replication, keto form of 5-BU substitutes for T and the replication of an initial AT pair becomes an A: BU pair (Fig. 9.7A). The rare enol form of 5-BU that pairs with G is the first mutagenic step of replication. In the next round of replication G pairs with C. Thus, the transition is completed from AT'!GC pair.

The 5-BU can also induce the conversion of GC to AT. The enol form infrequently acts as an analogue of cytosine rather than thymine. Due to error, GC pair is converted into a G: BU pair which in turn becomes an AT pair (Fig. 9.7B). Due to such pairing properties 5-BU is used in chemotherapy of viruses and cancer. Because of pairing with guanine it disturbs the normal replication process in microorganisms.

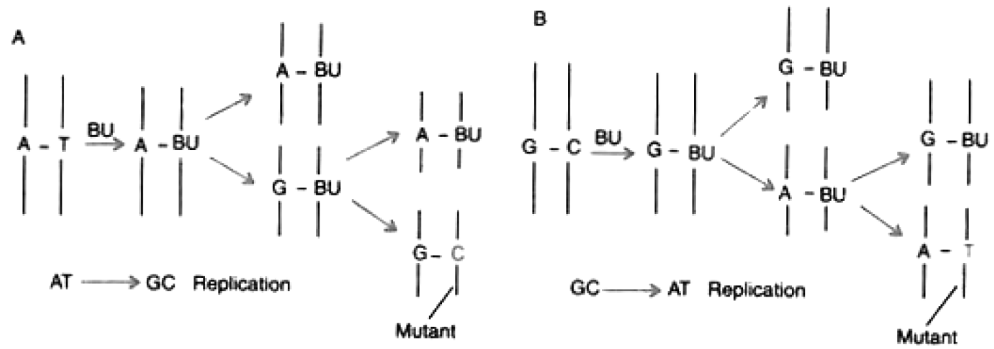


Fig. 9.7 : Mechanism of 5-bromouracil (BU)-induced mutagenesis. A, AT→GC replication; B, GC→AT replication.

The 5-bromodeoxyuridine (5-BDU) can replace thymidine in DNA molecule. The 2-amino-purine (2-AP) and 2, 6-di-amino-purine (2, 6-DAP) are the purine analogues. The 2-AP normally pairs with thymine but it is able to form a single hydrogen bond with cytosine resulting in transition of AT to GC. The 2-AP and 2, 6-DAP are not as effective as 5-BU and 5-BDU.

ii. Chemicals Changing the Specificity of Hydrogen Bonding:

There are many chemicals that after incorporation into DNA change the specificity of hydrogen-bonding. Those which are used as mutagens are nitrous oxide (HNO_2), hydroxylamine (HA) and ethyl-methane-sulphonate (EMS).

(a) Nitrous Oxide (HNO_2):

Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The order of frequency of deamination (removal of amino group) is adenine > cytosine > guanine.

(b) Deamination of Adenine:

Deamination of adenine results in formation of hypoxanthine, the pairing behaviour of which is like guanine. Hence, it pairs with cytosine instead of thymine replacing AT pairing by GC pairing (Fig. 9.8A).

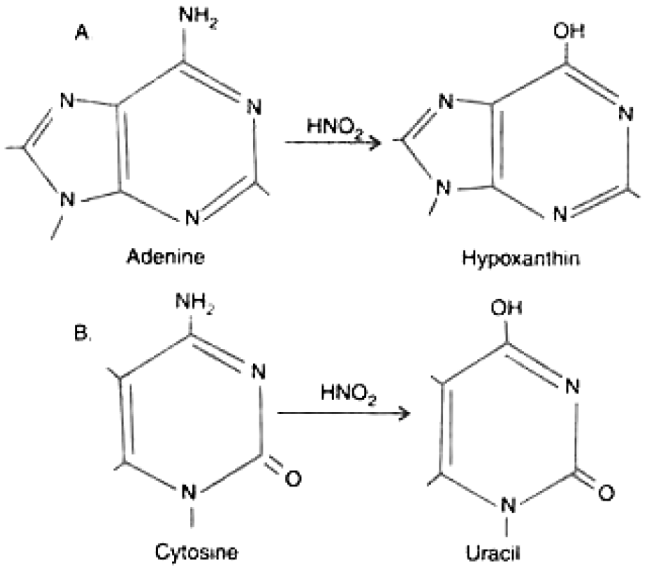


Fig. 9.8 : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

(c) Deamination of Cytosine:

Deamination of cytosine results in formation of uracil by replacing – NH_2 group with -OH group. The affinity for hydrogen bonding of uracil is like thymine; therefore, C-G pairing is replaced by U-A pairing (Fig. 9.8B).

(d) Deamination of Guanine:

Deamination of guanine results in formation of xanthine, the later is not mutagenic. Xanthine behaves like guanine because there is no change in pairing behaviour. Xanthine pairs with cytosine. Therefore, G-C pairing is replaced by X-C pairing.

(e) Hydroxylamine (NH_2OH):

It hydroxylates the C_4 nitrogen of cytosine and converts into a modified base via deamination which causes to base pairs like thyamine. Therefore, GC pairs are changed into AT pairs.

iii. Alkylating Agents:

Addition of an alkyl group to the hydrogen bonding oxygen of guanine (N_7 position) and adenine (at N_3 position) residues of DNA is done by alkylating agents. As a result of alkylation, possibility of ionization is increased with the introduction of pairing errors. Hydrolysis of linkage of base-sugar occurs resulting in gap in one chain.This phenomenon of loss of alkylated base from the DNA molecule (by breakage of bond joining the nitrogen of purine and deoxyribose) is called depurination. Depurination is not always mutagenic. The gap created by loss of a purine can effectively be repaired.

Following are some of the important widely used alkylating agents:

(a) Dimethyl sulphate (DMS)

(b) Ethyl methane sulphonate (EMS) -$CH_3CH_2SO_3CH_3$

(c) Ethyl ethane sulphonate (EES) -$CH_3CH_2SO_3CH_2CH_3$

EMS has the specificity to remove guanine and cytosine from the chain and results in gap formation. Any base (A,T,G,C) may be inserted in the gap. During replication chain without gap will result in normal DNA. In the second round of replication gap is filled by suitable base.

If the correct base is inserted, normal DNA sequence will be produced. Insertion of incorrect bases results in transversion or transition mutation. Another example is methyl nitrosoguanidine that adds methyl group to guanine causing it to mispair with thyamine. After subsequent replication, GC is converted into AT transition.

iv. Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs (Fig. 9.9). In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation.

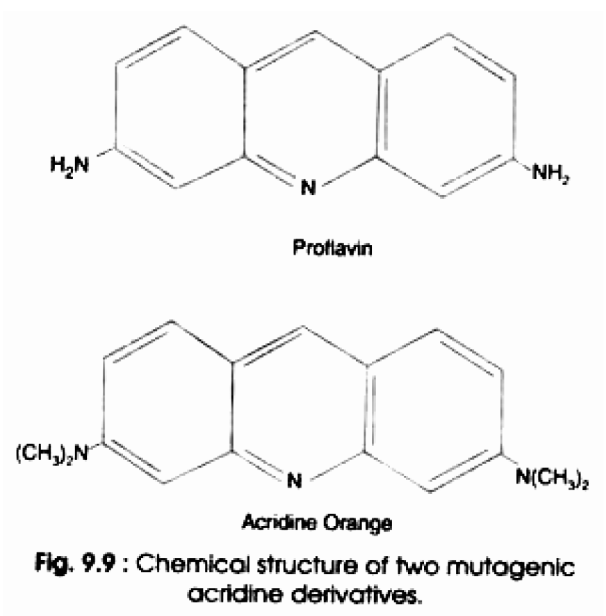


Fig. 9.9 : Chemical structure of two mutagenic acridine derivatives.

Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion or insertion after replication of DNA molecule. Due to deletion or insertion of intercalating agents, there occur frameshift mutations (Fig. 9.10).

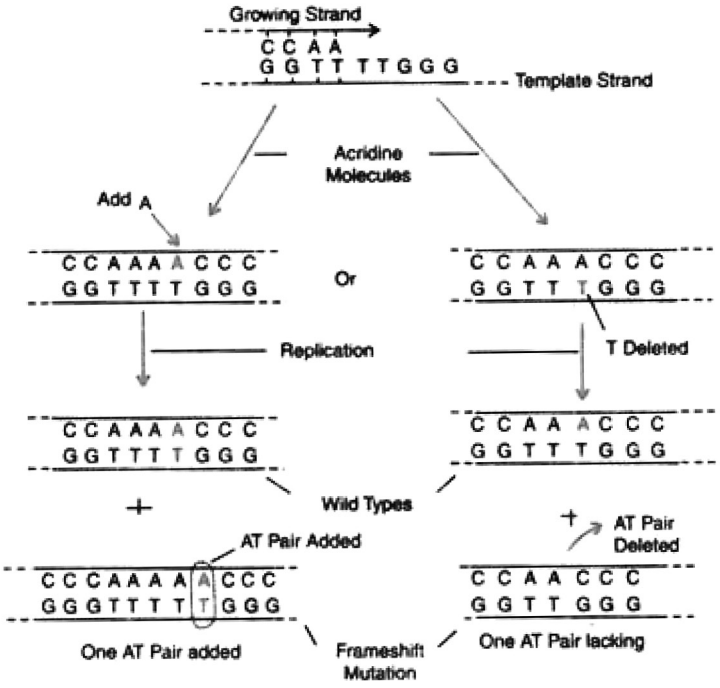


Fig. 9.10 : Mechanism of intercalation of an acridine molecule in the replication fork.

1. Physical Mutagens:

i. Radiations as Mutagens:

Radiation is the most important among the physical mutagens. Radiations damaging the DNA molecules fall in the wavelength range below 340 nm and photon energy above 1 electro-volt (eV). The destructive radiation consists of ultraviolet (UV) rays, X-rays, γ-rays, alpha (α) rays, beta (β) rays, cosmic rays, neutrons, etc. (Fig. 9.11).

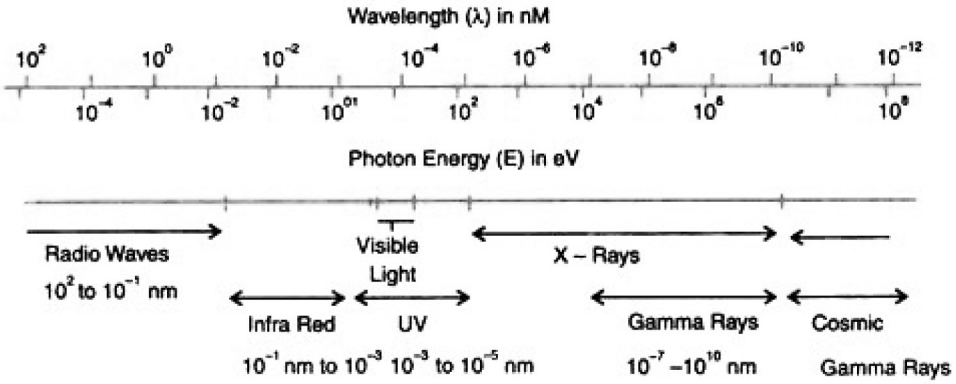


Fig. 9.11 : Wavelengths and photon energy of various radiations.

Radiation induced damage can be categorized into the three broad types: lethal damage (killing the organisms), potentially lethal damage (can be lethal under certain

ordinary conditions) and sub-lethal damage (cells do not die unless radiation reaches to a certain threshold value). The effect of damage is at molecular level.

In a live cell radiation damage to proteins, lipoproteins, DNA, carbohydrates, etc. is caused directly by ionization/excitation, or indirectly through highly reactive free radicals produced by radiolysis of cellular water. DNA stores genetic information's so a damage to it assumes great dimension. It can perpetuate genetic effects and, therefore, the cellular repair system is largely devoted to its welfare.

When the bacteria are exposed to radiation they gradually lose the ability to develop colonies. This gradual loss of viability can be expressed graphically by plotting the surviving colonies against the gradually increasing exposure time. This dose-response graph is called survival curve. The survival curve of bacteria is given in Fig. 9.12. The survival curve is analysed by a simple mathematical theory called hit theory.

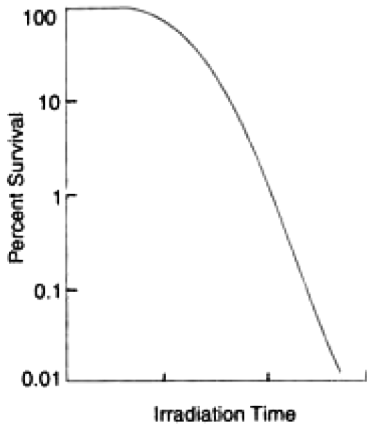


Fig. 9.12 : A typical ultra violet light survival curve for a bacterium.

Hit Theory:

Each organism possesses at least one sensitive site which is known as target site. Radiation photons (particles of light) damage or hit the target site and inactivate the organisms. One can derive the equation based on this theory.

The equations help to calculate the survival curve for many kinds of populations of N identical organisms exposed to dose D of radiation causing damage. The number dN damaged by a dose dD is proportional to the initial population that has not received radiation; hence $dN = KN$

where,

K is the constant which measures the effectiveness of dose.

Integrating this equation from $N = No$ at $D = O$ we get

$N = No^{e-KD}$ ...(1)

The surviving fraction $S = N/N_o$ is

$S = N/N_o = e^{-KD}$ ...(2)

A plot of S virus D gives a straight line with a slope of -K (Fig. 9.12). This type of curves are called exponential or single hit curve. The exponential curve is obtained when the phages are irradiated with X-rays.

If there is a population of different organisms, and each organism consists of at least n sites, each site must be hit to inactivate an organism. Therefore, each organism is hit by n times. The probability of one unit being hit by a dose D is, $P = 1-e^{-KD}$), so the probability of Pn will be $Pn = (1-e^{-KD})^n$

The surviving fraction S of the population is 1-pn or $S = 1-(1-e^{-KD})n$ ...(3)

This equation can be expanded as:

$S = 1- (1-ne^{-KD} + e^{-nKD})$

At the large value of D, the higher order terms become negligible as compared to Therefore, at high dose D,

$S = ne^{-KD}$ or

In S = In n – KD ...(4)

When the equation 3 is plotted for K – 1, various values of n reveals that for small valuesof D, In S gradually changes (Fig. 9.13). At large value of D, equation 4 dominates and curve becomes linear.

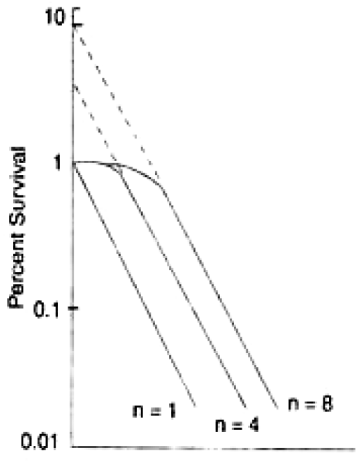


Fig. 9.13 : Survival curve for different values of n (hits' time).

ii. Ultraviolet (UV) Radiation:

UV radiation causes damage in the DNA duplex of the bacteria and phages. The UV rays are absorbed and cause excitation of macromolecules. The absorption maxima

of nucleic acid = (280 nm) and protein (260 nm) are more or less similar. The DNA molecule is the target molecule for UV rays but not the proteins. However, absorption spectrum of RNA is quite similar to that of DNA.

The excited DNA leads to cross-linking, single strand breaks and base damage as minor lesion and generation of nucleotide dimer as a major one. Purines are generally more radio – resistant than the pyrimidine of the latter, thymine is more reactive than cytosine.

Hence, the ratio of thymine-thymine (TT), thymine-cytosine (TC), cytosine-cytosine (CC) dimer (Fig. 9.14) is 10:3:3, respectively. A few dimers of TU and UU also appear. The initial step in pyrimidine dimerization is known to be hydration of their 4: 5 bonds.

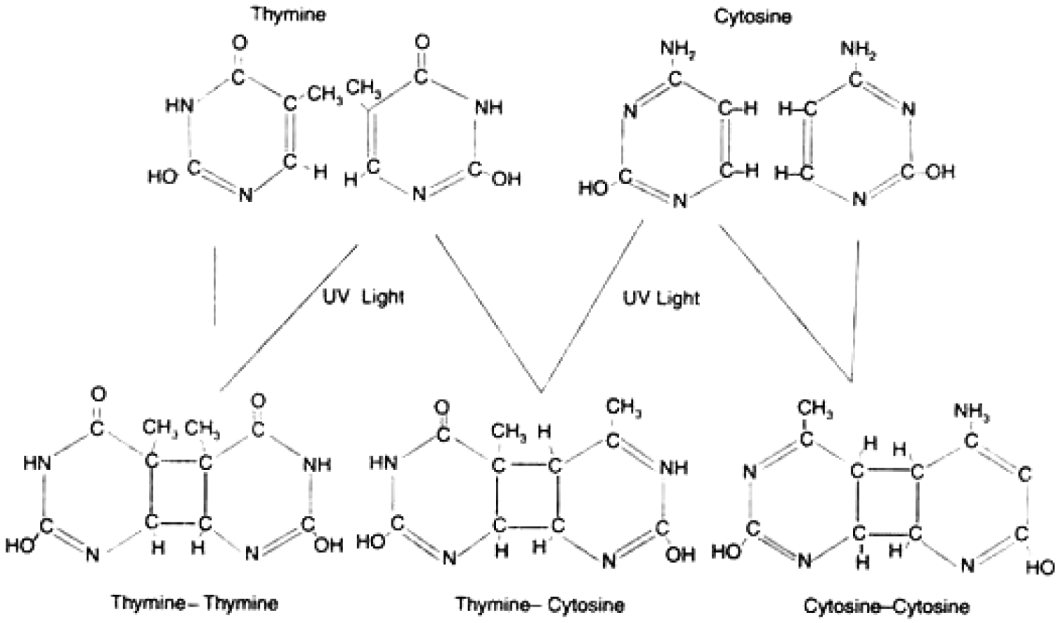


Fig. 9.14 : Formation of pyrimidine dimer induced by UV radiation.

Formation of thymine-thymine (TT) dimer causes distortion of DNA helix because the thymines are pulled towards one another. The distortion results in weakening of hydrogen-bonding to adenines in the opposing strand. This structural distortion inhibits the advance of replication fork.

iii. The X-Rays:

The X-rays cause breaking of phosphate ester linkages in the DNA. This breakage occurs at one or more points. Consequently, a large number of bases are deleted or rearranged in the DNA molecule.

The X-rays may break the DNA either in one or both strands. If breaks occur in both strands, it becomes lethal. The DNA segment between the two breaks is removed

resulting in deletion. Since both the X-rays and UV rays bring about damage in DNA molecule, they are used in sterilization of bacteria and viruses.

Radiation Exposure and Genetic Effects:

1. Genetic Impact of Ionizing Radiation:

Even low doses of ionizing radiation (e.g., 100R X-rays) can damage germ cells—especially spermatogonia—leading to sterility and increased genetic risks if fertilization occurs shortly after exposure. Both immediate somatic damage and delayed genetic mutations may result.

2. Germ Cell Sensitivity:

- **Females** are more sensitive than males; mature oocytes are particularly vulnerable near fertilization.
- **Extended low-dose exposure** is less harmful than brief high-dose exposure due to DNA repair mechanisms.

3. Influencing Factors:

- **Oxygen** presence during exposure amplifies mutation rates.
- **Low oxygen tension** or **hypoxia** protects germ cells.
- **Temperature** also modulates radiation-induced mutagenesis.

4. Consequences of Radiation

- Long-term germ cell damage
- Increased **recessive and dominant mutations**
- Occurrence of **chromosomal aberrations**
- Most mutations are recessive and manifest in later generations
- Post-exposure conception significantly raises risk

5. Mutation Induction Thresholds in Mice (as Human Reference)

- **Dominant morphological mutations**: 16–26 R
- **Recessive mutations**: 32 R
- **Autosomal recessive lethals**: 51 R
- **Chromosomal aberrations**: 31 R

Conclusion: There is no truly “safe” dose of ionizing radiation. Genetic damage is probabilistic and cumulative, influenced by dose, exposure rate, oxygen, and biological context.

Probable Questions:

1. Define carcinogen. How workplace exposure can cause cancer?
2. How asbestos can cause cancer?
3. Can low dose exposure of carcinogen can cause cancer? Discuss.
4. Medications and hormones can cause cancer, discuss.
5. State five categories of chemical carcinogens.
6. How liver can transform compounds more carcinogenic?
7. How electrophile DNA can cause cancer?
8. Discuss different steps of chemical carcinogenesis in brief.
9. How dose and potency of carcinogen chemicals affect carcinogenesis.
10. Define teratogen? Discuss different stages of teratogenicity.
11. Discuss mode of action of teratogens.
12. Define mutagen. How base analogues cause DNA mutation?
13. How intercalating agents can cause DNA mutation?
14. How ionizing radiation can cause DNA mutation?
15. How UV ray can cause DNA mutation?
16. How X ray can cause DNA mutation?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett&Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-III

Properties of few individual insecticides i.e. DDT, HCH(BHC), Lindane, Endosulfan, Parathion, Malathion, Carbaryl, Cypermethrin.

Objective: In this unit we will discuss properties and toxicity of some selected insecticides such as DDT, HCH(BHC), Lindane, Endosulfan, Parathion, Malathion, Carbaryl, Cypermethrin.

Insecticides: Definition and Classification:

Insecticides are chemical agents designed to kill or manage insect populations, widely used in agriculture, medicine, and public health. While effective in pest control, they can adversely affect ecosystems, harm non-target species, and bioaccumulate through food chains.

Classification of Insecticides:

- **By Chemical Nature:** Organic, inorganic, synthetic, and miscellaneous.
- **By Mode of Entry:** Stomach poisons (ingested), contact poisons (skin absorption), fumigants (inhaled), and systemic (absorbed by plants).
- **By Mode of Action:** Nerve, respiratory, physical, general, protoplasmic poisons, and chitin synthesis inhibitors.
- **By Toxicity:**
- *Extremely toxic* (**Red**, LD50: 1–50 mg/kg)
    - *Highly toxic* (**Yellow**, LD50: 51–500 mg/kg)
    - *Moderately toxic* (**Blue**, LD50: 501–5000 mg/kg)
    - *Slightly toxic* (**Green**, LD50: >5000 mg/kg)
    - **By Developmental Stage Targeted:** Ovicides, larvicides, pupicides, adulticides.

Modes of Action:

- **Stomach poisons:** Effective against chewing insects (e.g., caterpillars); include arsenicals and fluorides.

- **Contact poisons:** Absorbed through insect cuticle; examples include nicotine, pyrethrum, rotenone.
- **Fumigants:** Inhaled via spiracles; used in stored products (e.g., methyl bromide).
- **Systemic:** Absorbed by plants, offering long-term protection against sap-sucking and root-boring insects.

Major Insecticides and Their Characteristics:

- **DDT:** A persistent organochlorine used against malaria vectors; banned globally due to bioaccumulation, endocrine disruption, and possible carcinogenicity.
- **Lindane (γ-BHC):** Organochlorine compound with neurotoxic and hepatotoxic effects; banned in most countries except for restricted pharmaceutical use.
- **Endosulfan:** A phased-out organochlorine due to neurotoxicity, endocrine disruption, and environmental persistence; linked to reproductive issues and birth defects, especially in Kerala, India.
- **Parathion:** A highly toxic organophosphate cholinesterase inhibitor; banned due to risks of acute poisoning and environmental persistence.
- **Malathion:** A moderately toxic organophosphate used in agriculture and public health; acts on the nervous system and degrades relatively quickly in the environment.
- **Carbaryl:** A carbamate insecticide and plant growth regulator; associated with endocrine, developmental, and neurological effects. Toxic to aquatic organisms and pollinators.
- **Cypermethrin:** A synthetic pyrethroid; neurotoxic to insects, highly toxic to fish and bees, but persistent under anaerobic conditions. Preharvest intervals are critical to minimize residue levels in crops.

Environmental and Health Hazards:

- Non-target toxicity to humans, wildlife, and aquatic systems.
- Resistance development due to repeated use.
- Bioaccumulation and long-term ecological consequences.

Conclusion:

Insecticides, though vital for pest control, must be used judiciously considering their toxicological profiles, environmental persistence, and regulatory status. Safer alternatives

and integrated pest management (IPM) approaches are encouraged to minimize health and ecological risks.

Probable Questions:

1. What is the primary function of insecticides?
2. Name any two fields where insecticides are commonly used.
3. What colour label is used for extremely toxic insecticides?
4. Which mode of insecticide entry targets insects through ingestion?
5. Give one example of a systemic insecticide mode of action.
6. Which organochlorine insecticide was banned for its persistence and bioaccumulation?
7. Which insecticide is known to act as a cholinesterase inhibitor and is highly toxic?
8. Name one hazard associated with repeated use of insecticides.
9. What type of poison is absorbed through the insect cuticle?
10. Which synthetic pyrethroid is highly toxic to fish and bees?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett&Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-IV

Toxico-kinetics and toxico-dynamics: Absorption, distribution, Metabolism, elimination, organ toxicity

Objective: In this unit we will learn about Toxico-kinetics and toxico-dynamics. We will discuss how toxins are absorbed, distributed and how its metabolism and elimination occur. We will also learn about organ toxicity.

Introduction:

The biological response to chemical exposure involves two key processes: **toxicokinetics**, which describes how a chemical is absorbed, distributed, metabolized, and eliminated (ADME), and **toxicodynamics**, which involves molecular interactions at the target site that lead to adverse effects. This distinction is essential in understanding toxicity and conducting risk assessments.

Toxicokinetics assesses how chemicals move through the body and are cleared. It involves measuring plasma or blood concentrations over time to derive parameters such as absorption rates, distribution volumes, and elimination half-lives. While radiolabelled compounds (e.g., using ^{3}H, ^{1}t C, or ^{3}u S) provide valuable data on the fate of a chemical and its metabolites, they lack specificity in distinguishing parent compounds from metabolites. Nonetheless, radiochromatographic techniques enhance metabolite identification and help trace compound pathways.

Plasma concentration–time profiles are used to compute the **area under the curve (AUC)**, a key metric of systemic exposure, especially in chronic studies. Comparison of intravenous and extravascular doses helps delineate absorption characteristics. With advanced analytical tools, low-dose toxicokinetics in humans is now possible, improving interspecies extrapolation and enhancing the relevance of animal data to human risk evaluation.

Toxicodynamics, in contrast, is harder to assess in humans due to ethical constraints. Information is limited to accidental exposures, reversible biomarkers, or in vitro studies.

Quantitative interpretation of toxicokinetic data employs mathematical modeling. **Compartmental models** simplify chemical movement into discrete kinetic compartments, enabling prediction of unmeasured data points. **Physiologically based pharmacokinetic (PBPK) models**, though data-intensive, integrate biological parameters and can bridge species gaps and support biologically based dose-response assessments.

Together, toxicokinetics and toxicodynamics provide a scientific framework for predicting chemical behavior in organisms and evaluating potential health risks.

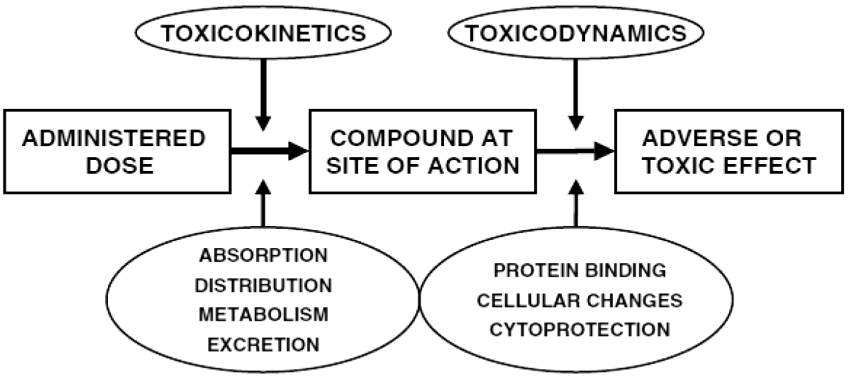


Figure 3.1 *The relationship between delivery of the administered dose to the target site and the generation of the adverse or toxic response*

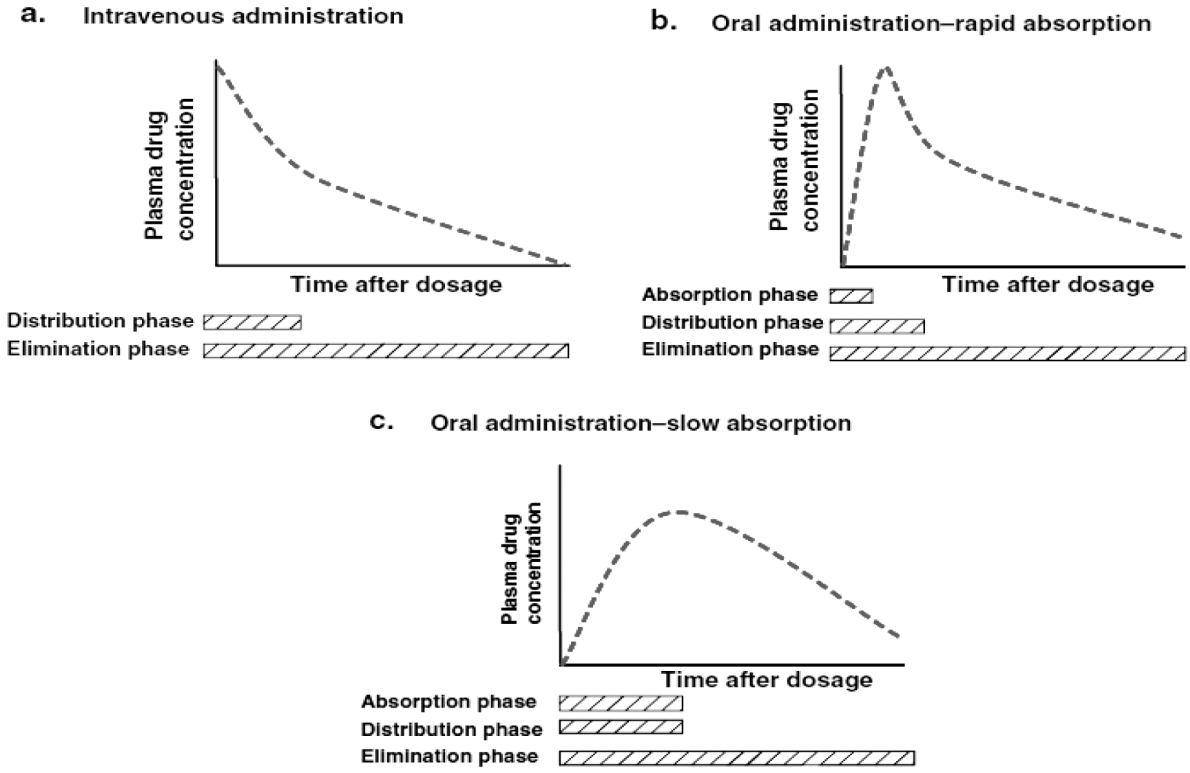


Figure 3.2 *The plasma concentration–time profiles of a chemical following intravenous and oral dosage*

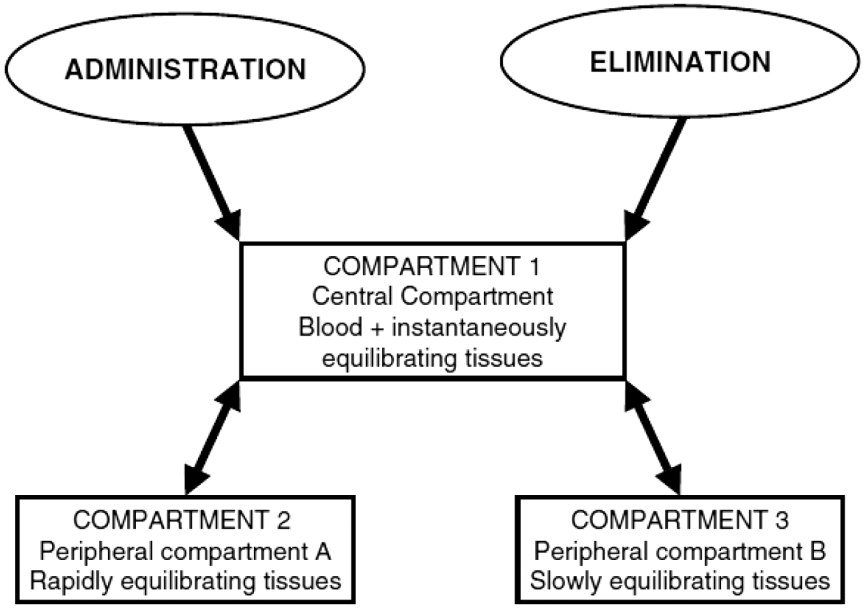
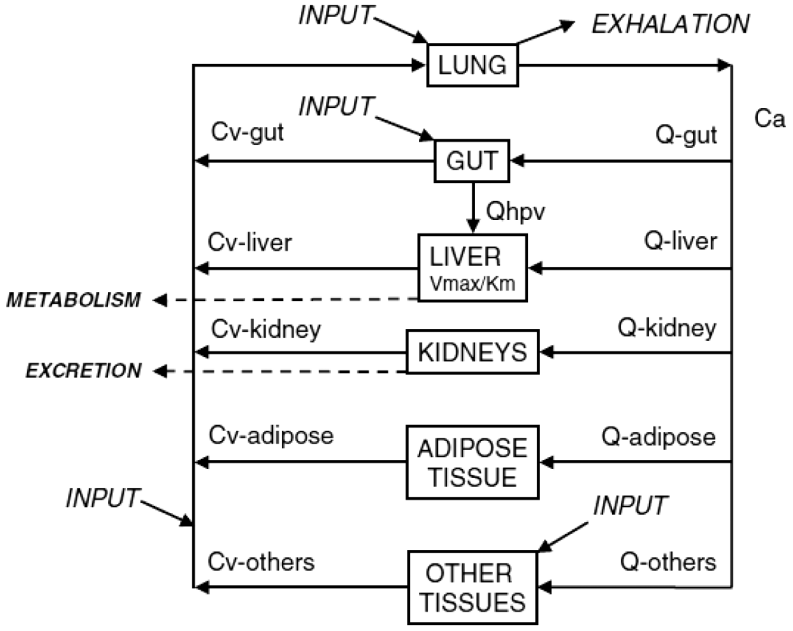


Figure 3.3 *Compartmental analysis. In the example shown, the body is considered to consist of two peripheral compartments that equilibrate with the central compartment. Strictly speaking the only property that links tissues that are part of the same "compartment" is the rate of transfer into and out of the tissue. The central compartment usually comprises blood and well-perfused tissues and equilibrates instantaneously. In the example shown, the compound is eliminated from the central compartment, for example by extraction by the liver or kidneys. The number of compartments necessary in the mathematical model fitted to the data depends on the number of exponential terms necessary to describe the plasma concentration–time curve. The mathematical model can be used to estimate the concentration in plasma or blood at any time after dosage*



I. Absorption

Absorption refers to the transfer of a chemical from the site of administration (e.g., gut, skin, lungs) into systemic circulation. This process is influenced by chemical properties like lipid solubility and ionization, as well as the route and formulation. Lipid-soluble, unionized molecules absorb rapidly, particularly through the gastrointestinal tract or lungs; skin acts as a more restrictive barrier. The **rate of absorption** determines the peak plasma concentration, which is critical for acute toxicity. The **extent of absorption**—or **bioavailability (F)**—is the proportion of the administered parent compound that reaches the bloodstream intact. It is commonly evaluated by comparing the **area under the plasma concentration–time curve (AUC)** after oral and intravenous dosing. Bioavailability can be reduced by **first-pass metabolism** in the gut or liver, enzymatic degradation, or poor solubility.

II. Distribution

Distribution describes the reversible movement of chemicals between blood and tissues. It depends on **membrane permeability**, **blood flow**, and **chemical affinity for tissues**. Lipid-soluble compounds distribute rapidly into fatty tissues and may show biphasic kinetics: fast distribution to well-perfused tissues and slower accumulation in fat. Water-soluble compounds tend to remain in extracellular fluid or total body water, depending on their ability to cross membranes.

Barriers like the **blood–brain barrier** restrict entry of water-soluble molecules into the CNS, while lipid-soluble agents cross more easily, often causing neurotoxicity. The **apparent volume of distribution (V)** quantifies the extent of tissue distribution, calculated as the total body burden divided by plasma concentration. A high V suggests extensive tissue binding and often correlates with longer **elimination half-lives**.

III. Elimination

Elimination removes the compound from the body through **metabolism** (mainly liver) and/or **excretion** (urine, bile, breath). The **rate of elimination** is usually a first-order process, described by the **half-life (t½)** and the **elimination rate constant (k)**. Clearance (**CL**) reflects the functional ability of eliminating organs (liver, kidney, lungs) and is calculated as the volume of plasma cleared per unit time.

Renal clearance is influenced by protein binding, secretion, and reabsorption. **Total plasma clearance** (CL) is best assessed after intravenous dosing and is independent of the route but affected by **bioavailability (F)** in non-IV administration. The relationship:

$$t_{\frac{1}{2}} = \frac{0.693V}{CL}$$

shows that a long half-life may result from low clearance or high distribution. Lipophilic

compounds stored in adipose tissue (e.g., TCDD) have prolonged half-lives due to both extensive distribution and metabolic resistance.

Organ toxicity: Organ toxicity means the capacity of substances (xenobiotics) to damage various organs such as kidneys, liver, heart, lungs, nerves etc.

Hepatotoxicity

Hepatotoxicity: It is a toxicants-induced damage of liver, bile duct and gall bladder. The liver is exposed to high very high amount of xenobiotics or its metabolites because of extensive blood supply for the metabolic process of xenobiotics and other substances. The liver is plays a central role in transforming and clearing xenobiotics from the body. The over use of some medicinal drugs or other substances (e.g., natural, agricultural, industrial, herbal, chemical etc.) can induce hepatotoxicity.

Forms of hepatotoxicity: The various forms of hepatotoxicity are-

1. **Steatosis (Fatty liver):** Lipid accumulation in the adipocytes.
2. **Necrosis:** Death of hepatocytes.
3. **Cirrhosis:** Chronic fibrosis due to alcohol intake.
4. **Cholestasis:** Backup of bile salts into the liver.
5. **Hypersensitivity:** Hepatic necrosis due to immune response.
6. **Cancer:** Liver cancer.

Hepatotoxins:

The substances that cause liver injury is called hepatotoxins. There are more than 900 hepatotoxins causing liver damage. Some most familiar hepatotoxins are-

1. **Antipyretic-analgesics (Paracetamol or Acetaminophen):** The overdoseof paracetamol causes acute liver failure worldwide.Actually damage to the liver is not due to paracetamol but to its metabolites (NAPQI = N-acetyl benzoquinone imine) of paracetamol produced by the action of cytochrome p450 enzyme in the liver. Normally NAPQI is detoxified in conjugation with glutathione, but its overuse produces a large amount of NAPQI that inhibits the detoxification process and causes liver damage.
2. **NSAIDs (Non steroidal anti-inflammatory drugs):** The NSAIDs (e.g., Aspirin, Ibuprofen, diclofenac, Aceclofenac, nimesulide, piroxicam etc.) are nonnarcotic, nonopoidanalgesic, antipyretic and anti-inflammatory drugs. The individual analgesic rarely induces liver damage. But, worldwide over use of these drugs is showing hepatotoxicity. It is actually dose-dependent hepatotoxic drugs.

3. **Glucocorticoids (corticosterone, cortisone, cortisol):** They are so named due to their effect on carbohydrate metabolism. They cause enlargement of liver due to storage of glycogen that may causes side effect in children. But the prolong use of these drugs causes fattyliver.
4. **Ioniazid (Anti-tuberculosis drugs):** It is associated with upto 20% elevation of liver enzymes (SGOT & SGPT) and severe hepatotoxicity to 1-2% of patients.
5. **Natural products:** The natural hepatotoxins are amanita mushrooms, aflatoxins, alkaloids & green tea extract.
6. **Industrial toxins (Arsenic, CCl_4, vinyl chloride):** They may cause liverdamage.

Nephrotoxicity

The kidneys are the highly susceptible to the toxicants because a high volume of blood with toxicants flows through these organs for the filtration of all kind of toxins from the blood. The toxicants (especially lipid soluble) are concentrated within the cell during this process that can cause nephrotoxicity.

Forms of nephrotoxicity:-

1. Decrease rate of excretion of bodywaste.
2. Inability to maintain body fluid &electrolytes.
3. Decrease synthesis of essential hormone like erythropoietin (Function: It promotes the production of bloodcells).

Nephrotoxic agents & their function:

i) **Cardiovascular drugs as nephrotoxins:** Diuretics (thiazides, furosemide), vasodilators, beta blockers, ACE inhibitors etc.

ii) **NSAIDs:**Aspirin, ibuprofen, diclofenacetc.

iii) **Antibiotics:** Gentamicin, amphotericin B, cisplatin, ciprofloxacin, rifampicinetc.

iv) **Antacids (PPI & H_2 antagonists):** Ranitidine, cemetidineetc.

v) **Heavy metals:** Lead, mercury &cadmium

vi) **Others:** Lithium salt, gold salt, Heroin, fluorideetc.

Respiratory toxicity

Respiratory toxicity: The toxicants-induced damage of respiratory system (Upper respiratory system e.g., nose, pharynx, larynx & trachea & lower respiratory system e.g., bronchi, bronchioles & lungs &alveoli).

Forms of respiratory toxicity:

1. Asthma
2. Bronchitis
3. Emphysema
4. Laryngitis /pharyngitis
5. Allergic reactions
6. Pneumoconiosis
7. Lung cancer

Respiratory toxicants: There are numerous respiratory toxicants in environment-

i. SO_x, CO_x, NO_x, Ammonia, Chlorine, Fluorine, bromine.

ii. Arsenic & arsenic compounds, cadmium, lead, mercury, nickel, pyrethrum

iii. Aldrin, Dieldrin, Endrin, formaldehyde, kerosene, methane, ethanol, methanol, phenol, xylene, benzene, caffeine, colchicines, DDT

iv. Hydrogen, hydrogen peroxide, HCN, ozone

Reproductive toxicity

The toxicants which are involved in the damage of male or female reproductive systems is called reproductive toxicity.

Forms of toxic effects:

1. Infertility
2. Impotence
3. Interruption of pregnancy: Abortion, fetal death, premature delivery etc.
4. Infant death
5. Altered sexratio

6. Chromosomal abnormalities & birth defects
7. Childhood cancer

Reproductive toxicants:

i. Steroids
ii. Colchicine
iii. DDT
iv. Etodolac
v. Gemfibrozil
vi. Lead & its compounds, cadmium,benzene
vii. Levonorgerserol
viii. Nifedipine, Rifampicin,streptozocin
ix. Cocaine

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Probable Questions:

1. Define toxicokinetics. Which parameters affect it?
2. How a toxic chemical is absorbed. Give suitable examples.
3. How a toxic chemical is eliminated. Give suitable examples.
4. How a toxic chemical is distributed. Give suitable examples.

5. What is organ toxicity?
6. Define hepatotoxicity? Describe different forms of hepatotoxicity.
7. Describe different kinds of hepatotoxins.
8. What is nephrotoxicity?
9. Discuss about Nephrotoxic agents & their functions.
10. Define Respiratory toxicity? Describe different forms of respiratory toxicity.
11. Describe different kinds of respiratory toxicants.
12. What is reproductive toxicity? What are toxic effects of toxins on reproductive system?
13. Name some chemicals which act as toxins to reproductive system.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen.

Unit-V

Toxicants of public health hazards: Pesticides, Heavy Metals, Radiation, Food and Additives

Objective: In this unit you will learn about toxicants related to public health hazards such as pesticides, heavy metals, radiation, food and additives.

Introduction:

Environmental toxicology studies the harmful effects of chemical, biological, and physical agents on organisms. Its subfield, **ecotoxicology**, examines toxic impacts at population and ecosystem levels.

The field emerged with **Rachel Carson's** *Silent Spring* (1962), which exposed pesticide hazards, notably DDT.

Toxicant effects vary with life stage and food web position. **Bioaccumulation** in tissues can lead to **biomagnification** across trophic levels. Though **biodegradation** breaks down toxicants into harmless products, it is often limited in polluted environments.

I. PESTICIDES:

According to World Health Organization (WHO), Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly. In general, a pesticide is a chemical or biological agent (such as a virus, bacterium, or fungus) that deters, incapacitates, kills, or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other species.

A. Classification of pesticides by target organisms:

There are many different types of pesticides, each is meant to be effective against specific pests. The term "**-**cide" comes from the Latin word "to kill."

a. ***Algaecides***are used for killing and/or slowing the growth of algae.

b. **Antimicrobials**control germs and microbes such as bacteria and viruses.

c. ***Acaricides*** are used against mites and ticks, members of Acaridae.

d. ***Herbicides*** kill or inhibit the growth of unwanted plants, aka weeds.

e. **Fungicides** are used to control fungal problems like molds, mildew, and rust.

f. ***Insecticides*** are used to control insects.

g. **Molluscicides** are designed to control slugs, snails and other mollusks.

h. **Rodenticides** are used to kills rodents like mice, rats, and gophers.

i. ***Ovicides*** are used to control eggs of insects and mites.

j. ***Pisicides*** used to reduce the population of rough fish in a water body.

k. ***Nematicides*** are used against nematodes.

l. ***Mothballs*** are insecticides used to kill fabric pests by fumigation in sealed containers.

B. Classification of pesticides by mode of action:

Pesticides can be classifyon the basis of the following ways-

a. **Cell toxicants:** inhibit different important steps of cell metabolism.

b. **Neurotoxicants or nerve Poison*:*** interferes with nervous system function.

c. **Chemosterilants*:*** sterilize males of insects or pest vertebrates, classical mode of biological pest control.

d. **Disinfectant (Eradicant**): effective against pathogen that has already infected the crop.

e. **Defoliants*:*** removes the leaves of plants.

f. **Germination Inhibitor:** inhibits germination of weed seeds, fungus spores and bacterial spores.

g. **Nonselective:** kills broad range of pests and/or crop plants, usually used in reference to herbicides.

h. **Protectants:** protects crop if applied before pathogens infect the crop.

i. **Repellents:** deters or repels pest from crop or interferes with pest's ability to locate crop.

j. **Systemic pesticides*:*** absorbed and translocated throughout the plant to provide protection.

k. **Stomach Poison:** kills after ingestion by an animal.

l. **Pheromones:** are biologically active chemicals used to attract insects or disrupt their mating behavior.

Benefits of Pesticides:

Pesticides are used for the following purposes:

1. In agriculture, the protection of crops from variouspests.
2. In public health programmes for the control of vectors of variousdiseases.
3. To control household and garden pests.
4. In control of ectoparasites of domestic animals and even the humanbeings.
5. In industry and commercial establishment.
6. In weed control.
7. Invasive species control.

Effects of Pesticides:

a. Health Effects:

Pesticides may cause acute and delayed health effects in people who are exposed. Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer.

A 2007 systematic review found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" and thus concluded that cosmetic use of pesticides should be decreased. There is substantial evidence of associations between organophosphate insecticide exposures and neurobehavioral alterations. Limited evidence also exists for other negative outcomes from pesticide exposure including neurological, birth defects, and fetal death.

b. Environmental Effects:

Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

In addition, pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat (especially for birds), and threatens endangered species. Pests can develop a resistance to the pesticide (pesticide resistance), necessitating a new pesticide. Alternatively a greater dose of the pesticide can be used to counteract the resistance, although this will cause a worsening of the ambient pollution problem.

c. Economic aspects:

In one study, the human health and environmental costs due to pesticides in the United States was estimated to be $9.6 billion: offset by about $40 billion in increased agricultural production.

Additional costs include the registration process and the cost of purchasing pesticides: which are typically borne by agrichemical companies and farmers respectively. The registration process can take several years to complete (there are 70 different types of field test) and can cost $50–70 million for a single pesticide. At the beginning of the 21st century, the United States spent approximately $10 billion on pesticides annually.

Control of Pesticide Pollution:

1. The non-selective persistent pesticides such as DDT must be phased out of use.
2. Only selective pesticides must be used.
3. Measurement of pesticides to be applied is so important.
4. Repeated pesticides application should be stopped.
5. Proper knowledge about pesticides should be given to public and farmers.
6. Research on pesticides should progress.

2. Heavy Metals: Sources, Toxicity, and Effects

Heavy metals are naturally occurring elements with high density (>5 g/cm^3) and atomic weight (>40.04), including lead, mercury, cadmium, arsenic, and others. While some, like iron, zinc, and copper, are essential micronutrients, excessive exposure can lead to acute or chronic toxicity.

These metals are distributed through both natural processes (volcanic activity, erosion, microbial action) and anthropogenic sources (mining, fossil fuel combustion, industrial discharge, pesticides, cosmetics). During mining, metals like arsenic, lead, and copper—often present as sulfides or oxides—are released into the environment and spread via air, soil, and water.

In humans, heavy metals bioaccumulate, bind to macromolecules (proteins, DNA), and disrupt cellular functions, leading to organ damage, neurotoxicity, immune dysfunction, hormonal imbalance, and carcinogenesis. Long-term exposure may contribute to diseases resembling Parkinson's or Alzheimer's. Some compounds also damage nucleic acids and alter gene expression.

This section explores sources, exposure routes, bioaccumulation mechanisms, and cellular and molecular consequences of heavy metal toxicity, with emphasis on oxidative damage, neurodegeneration, endocrine disruption, and cancer risk.

a. Arsenic:

Arsenic is the 20th most abundant element on earth and the 33rd on the periodic table. The inorganic forms such as arsenite and arsenate compounds are lethal to humans and other organisms in the environment. Humans get in contact with arsenic through several means which include industrial sources such as smelting and microelectronic industries. Drinking water may be contaminated with arsenic which is present in wood preservatives, herbicides, pesticides, fungicides and paints.

b. Lead:

Lead is a slightly bluish, bright silvery metal in a dry atmosphere. The main sources of lead exposure include drinking water, food, cigarette, industrial processes and domestic sources. The industrial sources of lead include gasoline, house paint, plumbing pipes, lead bullets, storage batteries, pewter pitchers, toys and faucets. Lead is released into the atmosphere from industrial processes as well as from vehicle exhausts. Therefore, it may get into the soil and flow into water bodies which can be taken up by plants and hence human exposure of lead may also be through food or drinking water.

c. Mercury:

The metallic mercury is a shiny silver-white, odorless liquid metal which becomes colorless and odorless gas upon heating. Mercury is used in producing dental amalgams, thermometers and some batteries. Also, it can be found in some chemical, electrical-equipment, automotive, metal-processing, and building industries. Mercury can exist in a gaseous form thus it can be inhaled. Other forms of mercury contamination in humans may be through anthropogenic activities such as municipal wastewater discharges, agriculture, incineration, mining, and discharges of industrial wastewater.

d. Cadmium:

This metal is mostly used in industries for the production of paints, pigments alloys, coatings, batteries as well as plastics. Majority of cadmium, about three-fourths is used as electrode component in producing alkaline batteries. Cadmium is emitted through industrial processes and from cadmium smelters into sewage sludge, fertilizers, and groundwater which can remain in soils and sediments for several decades and taken up by plants. Therefore, significant human exposure to cadmium can be by the ingestion of contaminated foodstuffs especially cereals, grains, fruits and leafy vegetables as well as contaminated beverages. Also, humans may get exposed to cadmium by inhalation through incineration of municipal waste.

e. Chromium:

Chromium is a metal that is present in petroleum and coal, chromium steel, pigment oxidants, fertilizers, catalyst, oil well drilling and metal plating tanneries. Chromium is extensively used in industries such as wood preservation, electroplating, metallurgy, production of paints and pigments, chemical production, tanning, and pulp and paper production. These industries play a major role in chromium pollution with an adverse effect on biological and ecological species. Following the anthropogenic activities by humans, disposal of sewage and use of fertilizers may lead to the release of chromium into the environment. Therefore, these industrial and agricultural practices increase the environmental contamination of chromium. Environmental pollution by chromium has been mostly by the hexavalent chromium in recent years.

f. Copper:

This is a heavy metal which is used in industries to produce copper pipes, cables, wires, copper cookware, etc. It is also used to make copper intrauterine devices and birth control pills. Copper in the form of copper sulfate is added to drinking water and swimming pools. Due to man's anthropogenic and industrial activities, it can accumulate in the soil and up taken by plants. As such, copper is present in some nuts, avocado, wheat germ and bran etc.

g. Manganese:

This metal is added to gasoline as methyl cyclopentadienyl manganese tricarbonyl (MMT) and thus, gasoline fumes contain a very toxic form of manganese.

h. Nickel:

It is used in the production of batteries, nickel-plated jewelry, machine parts, nickel plating on metallic objects, manufacture of steel, cigarette smoking, wire, electrical parts, etc. Also, it can be found in food stuff such as imitation whip cream, unrefined grains and cereals, commercial peanut butter, hydrogenated vegetable oils, as well as contaminated alcoholic beverages [19]. The various sources of heavy metals are summarized in Figure 1.

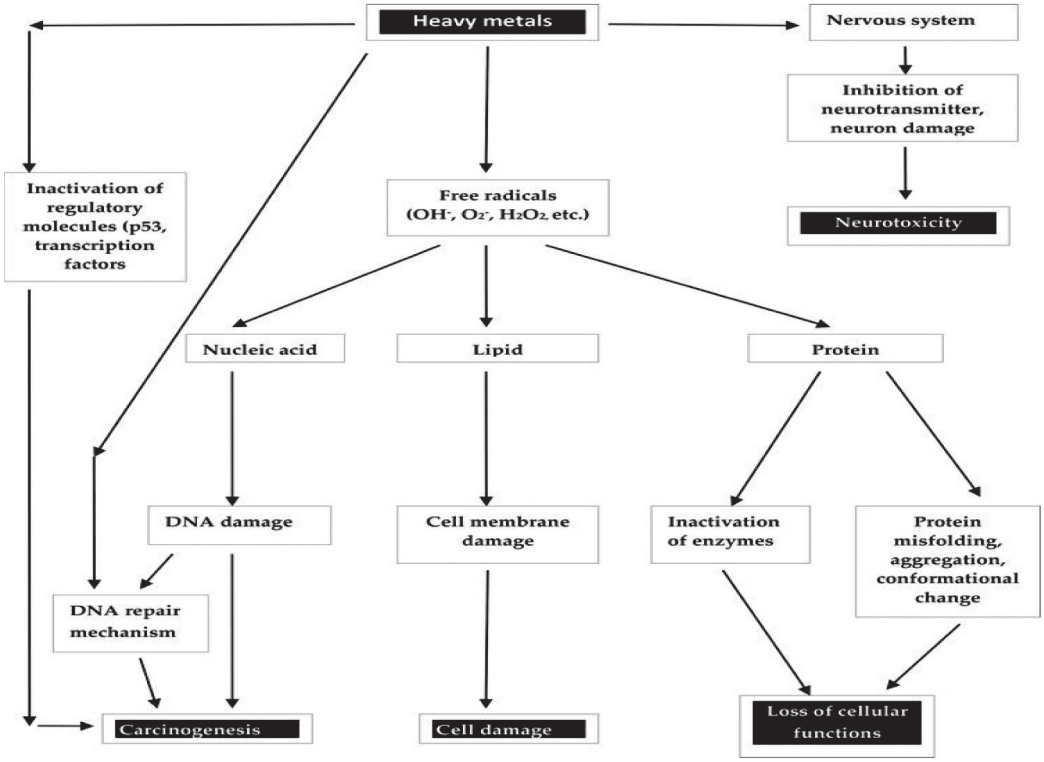


Figure 1. Pathway of heavy metals sources and exposure to human

Heavy Metals	Acute exposure	Chronic exposure
Cadmium	Pneumonitis (lung inflammation)	Lung cancer; Osteomalacia (bones dysfunction); Proteinuria (excessprotein in urine).
Mercury	Diarrhea; Fever; Vomiting.	Stomatitis (inflammation of gums and mouth); Nausea; Nephritic syndrome (nonspecific kidney disorder); Paraguesia (metallic disorder); Neurasthenia (neurotic disorder); Pink disease (pink coloration and pain of hands andfeet).
Lead	Encephalopathy (brain dysfunction); Nausea; Vomiting.	Anemia; Foot drop/wrist drop (palsy); Neuropathy (kidney disease).
Chromium	Gastrointestinal hemorrhage; Hemolysis; renalfailure.	Pulmonary fibrosis; Lung cancer.
Arsenic	Arrhythmia; Nausea; Vomiting; painful neuropathy.	Diabetes; Hypopigmentation; Cancer.

3. Route of exposure, bio-uptake and bioaccumulation of heavy metals in humans:

Humans may directly get in contact with heavy metals by consuming contaminated food stuffs, sea animals, and drinking of water, through inhalation of polluted air as dust fumes, or through occupational exposure at workplace. The contamination chain of heavy metals almost usually follows this cyclic order: from industry, to the atmosphere, soil, water and foods thenhuman. These heavy metals can be taken up through several routes. Some heavy metals such as lead, cadmium, manganese, arsenic can enter the body through the gastrointestinal route; that is, through the mouth when eating food, fruits, vegetables or drinking water or other beverages. Others can enter the body by inhalation while others such as lead can be absorbed through the skin.

Most heavy metals are distributed in the body through blood to tissues. Lead is carried by red blood cells to the liver and kidney and subsequently redistributed to the teeth, bone and hair mostly as phosphate salt. Cadmium initially binds to blood cells and albumin, and subsequently binds to metallothionein in kidney and liver tissue. Following its distribution from blood to the lungs, manganese vapor diffuses across the lung membrane to the Central nervous system (CNS). Organic salts of manganese which are lipid soluble are distributed in the intestine for fecal elimination while inorganic manganese salts which are water soluble are distributed in plasma and kidney for renal elimination. Arsenic is distributed in blood and accumulates in heart, lung, liver, kidney, muscle and neural tissues and also in the skin, nails and hair.

A. Mechanism of heavy metal toxicity:

a. Iron: Iron is a useful heavy metal in the human body as it is a constituent of certain biological molecules like the hemoglobin and involved in various physiological activities. However, in its free state, iron is one of the heavy metals generally known to generate hydroxyl radical (OH•) as shown below by the Fenton reaction.

$Fe^{3+} + O_2 \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH–$ (Fenton reaction)

Net reaction (Haber-Weiss reaction):

$O^{2-} + H_2O_2 \rightarrow OH^{-} + OH^{\cdot} + O_2$

In addition to the above reactions, the following reactions below can also occur:

$OH. + H_2O_2 \rightarrow H_2O + H^{+} + O_2^{\cdot -}$

$OH. + Fe^{2+} \rightarrow Fe^{3+} + OH^{-}$

$LOOH + Fe^{2+} \rightarrow Fe^{3+} + LO^{-} + OH^{\cdot}$

Hydroxyl radical (OH•) is the most common free radical generated by the oxidation of iron. OH• is capable of reacting with biological molecules such as proteins, lipids and DNA damaging them. When OH• reacts with guanine, a nitrogenous base of nucleic acids, it leads to the generation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxo-dG) and 2,6-diamino-5-formamido-4-hydroxypyrimidine (FAPy-G), in which the former is a good marker for oxidative damage.

It is well documented that metal-induced generation of oxygen reactive species can attack polyunsaturated fatty acid such as phospholipids. The first of such observation was first presented by Bucher et al. who showed that iron-generated OH• can oxidize lipid membranes through a process known as lipid peroxidation. Following his experimental observations, he proposed the following mechanism:

Steps of lipid peroxidation:

Initiation:Lipid+R./OH. $\rightarrow$ Lipid.

Propagation:Lipid.+O_2 $\rightarrow$ Lipid–OO.

Lipid–OO+Lipid. $\rightarrow$ Lipid–OOH+Lipid.

Termination:Lipid.+Lipid. $\rightarrow$ Lipid–Lipid

Lipid–OO.+Lipid. $\rightarrow$ Lipid–OO–Lipid

At the initiation stage, the radical (R•)/OH• attacks the lipid membrane to form a radial lipid. This radical lipid further propagates the formation of peroxyl lipid radical by reacting with dioxygen molecule or with a lipid. This reaction further promotes damage of the lipid molecule. At the termination stage, two radical lipid molecules and/or with a peroxyl lipid radical reacts to form a stable lipid molecule. The major aldehyde product of lipid peroxidation is malondialdehyde and it serves as a marker for lipid peroxidation.

Generally, proteins are not easily damaged by H_2O_2 and other simple oxidants unless transition metals are present. Thus, protein damaged are usually metal-catalyzed and involves oxidative scission, bityrosine cross links, loss of histidine residues, the introduction of carbonyl groups, and the formation of protein-centered alkyl (R•), alkoxyl (RO•) and alkylperoxyl (ROO•) radicals.

b. Copper:

Copper ions have been identified to participate in the formation of reactive oxygen species (ROS) as cupric (Cu^{2+}) and cuprous (Cu^{1+}) which can participate in oxidation and reduction reactions. The Cu^{2+} in the presence of biological reductants such as glutathione (GSH) or ascorbic acid can be reduced to Cu+ which is capable of catalyzing the decomposition of H_2O_2 to form OH• via the Fenton reaction as shown below.

$Cu+ + H_2O_2 \rightarrow Cu^{2+} + OH^{\cdot} + OH–$

The OH• radical formed is capable of reacting with several biomolecules. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases via oxygen free radicals. Though in vivo studies have not revealed copper-induced oxidation of low density lipoprotein (LDL), in vitro studies clearly demonstrated LDL oxidation induced by copper.

c. Chromium:

Chromium (Cr), particularly Cr^{4+} has been shown in in vitro studies to generate free radicals from H_2O_2. Also, in vivostudies were able to show the detection of free radicals due to chromium in the liver and blood of animals. It was observed that Cr^{5+} intermediates were generated as a result of one-electron reduction.

d. Cobalt:

Cobalt (Co), particularly Co^{2+} has been shown to generate superoxide ($\bullet O^{2-}$) from the decomposition of H_2O_2.

$Co^{2+} + O_2 \rightarrow Co^{+} + O^{\cdot 2-} \rightarrow Co^{+} – O^{\cdot 2-}$

e. Vanadium:

Vanadium is a heavy metal that occurs in various oxidative states and has been shown to generate free radical. In the plasma, vanadium (V) is rapidly reduced to vanadium (IV) by NADPH and ascorbic acid antioxidants which bind to plasma proteins for transportation.

$V_5 + NADPH \rightarrow V_4 + NADP + H^{+}$

$V_4 + O_2 \rightarrow V_5 + O^{2\cdot -}$

$V_5 + O_2^{\cdot} \rightarrow V_5 + OO^{\cdot}$

More so under physiological conditions at approximately pH of 7, V(IV) can generate OH• from the decomposition of H_2O_2 according to the Fenton reaction.

$V_4 + H_2O_2 \rightarrow V_5 + OH + OH^{\cdot}$

f. Arsenic:

Arsenic has also been shown to generate free radicals such as superoxide ($O^{2\bullet -}$), singlet oxygen ($1O_2$), nitric oxide (NO•), hydrogen peroxide (H_2O_2), the peroxyl radical (ROO•), dimethylarsinic peroxyl radicals ($(CH_3)_2AsOO\bullet$) and also the dimethylarsinic radical ($(CH_3)_2As\bullet$) in some studies though the mechanism for the generation of all these reactive species remains unclear.

B. Heavy metal-induced carcinogenesis:

Some heavy metals are known to have carcinogenic effect. Several signaling proteins or cellular regulatory proteins that participate in apoptosis, cell cycle regulation, DNA repair, DNA methylation, cell growth and differentiation are targets of heavy metals. Thus, heavy metals may induce carcinogenic effect by targeting a number of these proteins. More so, the carcinogenic effects of certain heavy metals have been related to the activation of redox-sensitive transcription factors such as AP-1, NF-κB and p53 through the recycling of electrons by antioxidant network. These transcription factors control the expression of protective genes that induce apoptosis, arrest the proliferation of damaged cells, repair damaged DNA and power the immune system. Metal signalization of transcription factor AP-1 and NF-κB has been observed in the mitogen-activated protein (MAP) kinase pathways where the nuclear transcription factor NF-κB, is involved in controlling inflammatory responses while AP-1 is involved in cell growth and differentiation. The p53 protein is an important protein in cell division as it guards a cell-cycle checkpoint and control cell division. Inactivation of p53 allows uncontrolled cell division and thus p53 gene disruption has been associated with most human cancers. Also, AP-1 and NF-κB family of transcription factors are involved in both cell proliferation and apoptosis, and also regulate p53. Heavy metals generated free radicals inside the cell selectively activates these transcription factors and thus, may suggest that cell proliferation or cell death may be related to the exposure to carcinogenic metals. There exist various mechanisms of heavy metal-induced carcinogenesis.

a. Arsenic:

Arsenic-induced carcinogenic mechanisms include epigenetic alterations, damage to the dynamic DNA maintenance system and generation of ROS. Alterations of histones, DNA methylation, and miRNA are the key epigenetic changes induced by arsenic which have shown to possess potentials to cause malignant growth. In vitro studies have shown arsenic to alter the expression of p53 protein which also led to decreased expression of p21, one downstream target. Arsenic compounds have been shown in an in vitro cell line study to promote genotoxicity in humans and mice leucocytes. Also, a methylated form of arsenic was shown to inhibit DNA repair processes and also generate ROS in liver and spleen as metabolic products. Arsenic can bind DNA-binding proteins and disrupt the DNA repair processes thereby increasing the risk of carcinogenesis. For example, the tumor suppressor gene-coded DNA was suppressed when arsenic was bound to methyl-transferase. Also, cancers of the liver, skin, prostate and Kupffer cell were associated with Arsenic poisoning.

b. Lead:

The mechanism of lead-induced carcinogenic process is postulated to induce DNA damage, disrupt DNA repair system and cellular tumor regulatory genes through the generation of ROS. Studies have supported with evidence that ROS generation by lead is key in altering chromosomal structure and sequence. Lead can disrupt transcription processes by replacing zinc in certain regulatory proteins.

c. Mercury:

Little is known on the potential of mercury to act as a mutagen or carcinogen. However, the proposed mechanism of mercury-induced cancer is through the generation of free radicals inducing oxidative stress thereby damaging biomolecules. Mercury has been shown to induce malignant growth through the generation of free radicals as well as disruption of DNA molecular structure, the repair and maintenance system.

d. Nickel:

Nickel has an extensive range of carcinogenic mechanisms which include regulation of transcription factors, controlled expression of certain genes and generation of free radicals. Nickel has been shown to be implicated in regulating the expression of specific long non-coding RNAs, certain mRNAs and microRNAs. Nickel can promote methylation of promoter and induce the down regulation of maternally expressed gene 3 (MEG3) thereby upregulating hypoxia-inducible factor-1$\propto$, two proteins which are known to be implicated in carcinogenesis. It has also been demonstrated that nickel can generate free radicals, which contributes to carcinogenic processes.

e. Cadmium:

Cadmium has been implicated in promoting apoptosis, oxidative stress, DNA methylation and DNA damage.

f. Iron:

The main cause of cancer due to iron intoxication is through the generation of free radicals. A school of thought produced a mechanism for iron-induced cancer whereby bile acids (deoxycholic acid), iron(II) complexes, vitamins K and oxygen interact to generate free radicals which induced oncogenic effect in the colon.

C. Heavy metal-induced neurotoxicity:

Some heavy metals such as lead and manganese may affect the brain and cause neurological toxicity as reviewed from.

a. Lead:

Lead toxicity is targeted towards the memory and learning processes of the brain and can be mediated through three processes. Lead can impair learning and memory in the brain by inhibiting the N-methyl-d-aspartate receptor (NMDAR) and can block neurotransmission by inhibit neurotransmitter release, block the neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs) and reduce the expression of brain-derived neurotrophic factor (BDNF).

b. Manganese:

Manganese is known to accumulate in the mitochondria of neurons, astrocytes and oligodendrocytes cells and disrupts ATP synthesis by inhibiting the F1/F0 ATP synthase or complex 1 (NADH dehydrogenase) of the mitochondrial respiration chain. More so, it has recently been shown that manganese inhibits ATP synthesis at two sites in the brain mitochondria which are either the glutamate/aspartate exchanger or the complex II (succinate dehydrogenase) depending on the mitochondrial energy source. The disruption of ATP synthesis by manganese leads to decreased intracellular ATP levels and generation of free radicals thereby increasing oxidative stress which may contribute to manganese cellular toxicity. Furthermore, manganese can oxidize dopamine (DA) to react with quinone species thereby disrupting the dopaminergic system. This has been shown in animal studies were manganese exposure has led to specific deficits in the dopaminergic system. The DA reactive species are taken up by the dopamine transporter (DAT1) thus causing dopaminergic neurotoxicity.

Biochemical mechanism of heavy metal toxicity:

Heavy metals ingested through food or water are converted to reactive ionic forms (e.g., Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}) in the stomach's acidic environment. These ions bind strongly to sulfhydryl (–SH) groups in proteins (cysteine, methionine), disrupting enzyme activity. For example, cadmium inhibits key thiol enzymes like thioredoxin reductase by binding to active-site cysteines, while methylmercury impairs yeast metabolic enzymes.

Heavy metal–protein complexes can block enzyme function, leading to oxidative stress, cellular damage, and accumulation in tissues. Arsenic disrupts enzyme activity by targeting –SH groups, and cadmium displaces essential metals like Zn^{2+} in metalloenzymes, rendering them inactive.

Additionally, heavy metals interfere with proper protein folding and promote aggregation. Metals such as Hg^{2+}, Cd^{2+}, and As^{3+} inhibit protein refolding, with mercury being most potent. Aggregated proteins, often involved in metabolism and stability, accumulate in cells, particularly in yeast exposed to cadmium, arsenic, and chromium. The severity of toxicity correlates with metal uptake efficiency and their chemical reactivity.

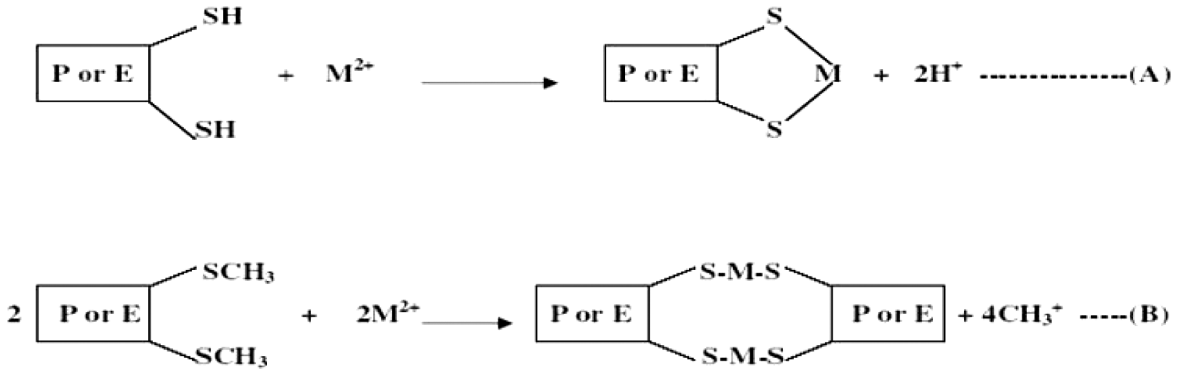


Figure 2.Reactions of Heavy metals with sulphydryl groups of proteins or enzymes (A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal.

Figure 3. Reaction of arsenic with the thio group of enzymes.

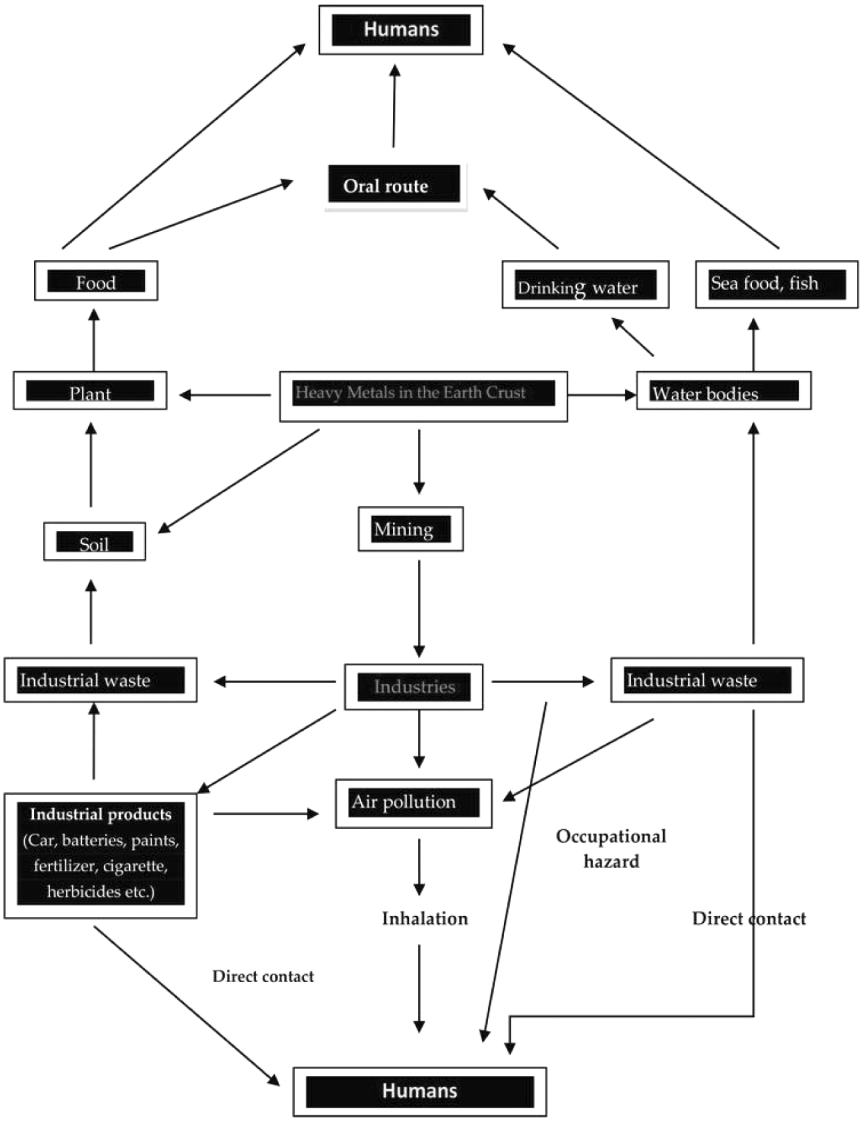


Figure 4. Mechanisms of heavy metal intoxication in humans.

Health effects of heavy metal toxicity in humans:

Heavy metals exert toxic effects through accumulation in vital organs such as the brain, liver, kidney, lungs, and blood. Exposure may be **acute** or **chronic**, potentially causing **neurodegeneration**, **organ dysfunction**, **carcinogenesis**, and **metabolic disorders**.

a. Arsenic

Chronic exposure leads to **arsenicosis**, marked by skin lesions, neurological issues, cardiovascular diseases, diabetes, and cancer (skin, lung, bladder, liver, etc.). Acute exposure damages vasculature, GI tract, and CNS. Arsenic binds to thiol groups, inhibiting

enzyme activity. Regulatory limits (e.g., 50 µg/L in U.S. drinking water) are under revision due to cancer risk.

b. Lead

Lead disrupts **neurological and renal functions**, especially in children. Acute effects include headache, fatigue, and GI distress; chronic exposure causes cognitive deficits, developmental delays, psychosis, paralysis, and even death. Lead alters cell signaling and disrupts the blood-brain barrier. Neurodevelopmental toxicity is well-documented at blood levels as low as 5–10 µg/dL.

c. Mercury

Mercury, especially **methyl mercury**, is neurotoxic and carcinogenic. It impairs memory, coordination, and sensory function. Organic forms cross cell membranes easily due to their lipophilicity. Chronic exposure leads to **erethism** (emotional lability, tremors, memory loss). Methylmercury exposure during pregnancy can result in fetal neural defects and cognitive impairment.

d. Cadmium

Cadmium accumulates in the **kidneys and bones**, causing **osteoporosis**, renal dysfunction, and reproductive toxicity. Itai-itai disease in Japan exemplifies cadmium-induced skeletal damage. It is a **Group 1 carcinogen**, with smoking as a primary exposure source. Cadmium disrupts calcium metabolism and may contribute to prostate and testicular disorders.

e. Chromium (VI)

Hexavalent chromium is highly toxic and **carcinogenic**. It causes ulcers, anemia, respiratory and gastrointestinal distress, and DNA damage through formation of adducts and chromosomal aberrations. Workers exposed to Cr(VI) show elevated risks of lung and stomach cancers. Trivalent chromium is relatively nontoxic.

f. Iron

While essential, excess iron can be toxic. Acute overdose progresses through GI upset to systemic toxicity (e.g., **hepatic necrosis**, **shock**, **acidosis**). Chronic overload promotes **carcinogenesis** via **free radical-induced DNA damage**. Occupational exposure (e.g., asbestos) increases lung cancer risk.

g. Manganese

An essential trace metal, manganese becomes neurotoxic in excess. Exposure (e.g., from MMT additives) can cause **manganism**, a Parkinson-like disorder with tremors, gait

disturbance, and cognitive decline. Unlike Parkinson's disease, manganism is unresponsive to L-DOPA and follows a distinct clinical course.

Conclusion:

Heavy metals pose significant risks to human health through diverse mechanisms including enzyme inhibition, oxidative stress, and genotoxicity. Chronic exposure is associated with serious neurological, renal, skeletal, and carcinogenic outcomes, necessitating strict monitoring and regulatory control.

Management and Remediation of Heavy Metal Contamination

In humans, heavy metal poisoning is primarily treated using **chelating agents** like *CaNa, EDTA*, which bind toxic metal ions and render them inert for excretion. However, chelation may also remove essential trace elements, necessitating the co-administration of **vitamin and mineral supplements** to mitigate side effects.

In soils, remediation strategies include:

- **Isolation**: Using caps, liners, or barriers to prevent contaminant spread.
- **Immobilization**: Chemically stabilizing metals to reduce their mobility.
- **Toxicity Reduction**: Converting toxic metal ions into less harmful forms via redox reactions (chemical or biological).
- **Physical Separation**: Mechanically isolating contaminated fractions.
- **Extraction**: Removing metals using chemical leaching, thermal volatilization, or electrokinetic methods, either on-site or off-site.

The choice of method depends on the metal type, concentration, and specific site conditions.

III. RADIATION HAZARD:

The term 'radiation' can refer to a wide variety of forms of energy moving around as waves or particles. It can mean x-rays, or it can mean microwaves. It can also refer to infrared light and even visible light. But when we say 'radioactive pollution,' we're being more specific. **Radioactive pollution** refers to the release of ionizing radiation into the environment as a result of human activity.

Types and Measurement of Radiation

1. Ionizing Radiation:

Ionizing radiation has **short wavelengths and high frequencies**, carrying enough energy to remove electrons from atoms. It includes **X-rays, gamma rays**, and particles like **alpha (α), beta (β), and neutrons**, all of which are by-products of **radioactive decay** or cosmic interactions. These radiations are harmful to biological tissues, capable of causing **mutations, cancer, and cellular damage**. Natural sources include **cosmic rays** and decaying **radioisotopes (e.g., carbon-14)**, while artificial sources include **X-ray machines, particle accelerators, and nuclear reactors**. Ionizing radiation is classified into:

- **Electromagnetic radiation** (e.g., X-rays, gamma rays)
- **Particulate radiation** (e.g., alpha, beta, neutrons)

Measurement Units:

- **Roentgen (R):** Measures ionization in air.
- **Rad:** Measures absorbed radiation dose (1 rad = 100 ergs/g).
- **Gray (Gy):** SI unit; 1 Gy = 100 rads.
- **Rem/Sievert (Sv):** Measure biological effects (1 Sv = 100 rems).
- Radiation detection is typically done using a **Geiger-Müller counter**, which detects ionization in a gas-filled tube.

2. Non-Ionizing Radiation:

Non-ionizing radiation lacks sufficient energy to ionize atoms, instead causing **excitation** of electrons. It includes **ultraviolet (UV), visible light, infrared, microwaves, and radio waves**. Although generally safer, excessive exposure (e.g., UV radiation) can still lead to health risks. Non-ionizing radiation **does not require extensive shielding** unlike ionizing radiation.

Radiation Pollution:

Radiation pollution primarily results from **alpha, beta, and gamma radiation**:

- **Alpha particles (α):** Heavily charged, cause strong damage but low penetration.
- **Beta particles (β):** High-speed electrons with moderate penetration and tissue interaction.

- **Gamma rays (γ):** High-energy photons with deep penetration and significant biological impact.

Sources of Radiation Pollution:

a. Natural sources of radiation:

1. **Radioactive minerals:**

   The minerals containing Uranium- 235 (U^{235}), Uranium-238 (U^{238}), Thorium-232 (Th^{232}), Plutonium- 239 (Pu^{239}) etc. are capable of emitting energetic radiations causing pollution.

2. **Cosmic rays:**

   The cosmic rays containing highly energetic particles reach the surface of the earth causing pollution. The intensity of cosmic rays depends on latitudes and altitude of the place. The intensity is maximum at the poles and minimum at the equator.

3. **Radionuclides:**

   The unstable radio-nuclides in the atmosphere can be splitted up into smaller parts emitting energetic radiation. The smaller radio-nuclidesenter into the body of organism along with air during respiration.

b. Anthropogenic or Man-made radiation:

1. **Nuclear power plants:**

   Nuclear power plants emit radiation to a very smaller extent except accidental leaks (Chernobyl accident of undivided USSR).

2. **Radio-activeWastes:**

   The nuclear power plants produce a lot of nuclear radio-activewastes. The disposal of these wastes has become a global problem. Some countries producing large quantity of nuclear wastes dump them in ocean near other countries.

3. **Nuclear Explosion:**

   During nuclear explosion, a large number of radio-nuclides are generated in the atmosphere. The radio nuclides settle down with rain contaminating the soil and water bodies. Finally, these enter into food chain causing serious problemto the living organisms.

4. **Radio-isotopes:**

   Radio-isotopes are also prepared artificially either by nuclear fusion or by nuclear

fission. If these radio-isotopes are not properly handled, these emit radiations causing pollution.

5. **TelevisionSet:**

   Television sets produce radiations which can also cause cancer.

Mechanism of Radiation Toxicity:

Mechanism of Radiation Toxicity:

Ionizing radiation (α, β particles, γ rays) causes cellular damage by generating **ion pairs** and **reactive oxygen species (ROS)**, such as superoxide anions and hydrogen peroxide, upon interaction with biological tissues. These ROS disrupt essential biomolecules—**proteins, nucleic acids, lipids, and carbohydrates**—leading to **DNA damage, mutations, chromosomal aberrations**, and ultimately **cell death**. The extent of damage is **dose-dependent**.

Radiation Toxicity and Susceptibility:

Radiation sensitivity varies among **species, organs, and life stages**. Rapidly dividing cells (e.g., in the **GIT, bone marrow, skin**) are most vulnerable. **Young animals and fetuses** are more radiosensitive than adults. Toxicity may be:

a. Acute Radiation Syndrome (ARS):

High-dose exposure causes severe **gastrointestinal symptoms** (diarrhea, dehydration, mucosal ulceration), **hematopoietic suppression** (anemia, leukopenia, thrombocytopenia), **skin and eye lesions**, and may lead to **mutations, secondary infections, or leukemia**. Death typically occurs within **1–4 weeks**.

b. Sub-Acute Toxicity:

Results from **moderate, prolonged exposure** over weeks. Symptoms include **vomiting, depression, leg swelling, dysentery**, and eventually **anemia, sepsis**, and death within **3–4 weeks**.

c. Chronic Radiation Toxicity:

Arises from long-term ingestion of **contaminated food or water**. Clinical outcomes include **growth retardation, infertility, alopecia**, and **cancers** of the **blood, thyroid, breast, lung, liver, colon**, and **reproductive organs**, along with **teratogenic effects**.

Pathological Lesions:

Include **dermal edema**, **GI ulceration**, **pulmonary fibrosis**, **adrenal hypertrophy**, **bone marrow and lymphoid atrophy**, **testicular degeneration**, **hepatomegaly**, **ascites**, and **jaundice**.

Diagnosis: Diagnosis is made on the basis of history, clinical signs and pathological lesions.

Effect of Radiation Pollution:

When radiation passes through different living organisms the following disorders takes place:

- Radiation splits the molecules of the tissues into ions and free radicals and causes mutation by breaking DNA (Deoxy ribonucleic acid) molecules in the nucleus.
- Radiation in bone marrow may cause leukaemia.
- Radiation may cause skin burns which may lead to skin cancer.
- Radiation at pelvic regions of pregnant ladies, cause damage to the foetus.

Effects of Ionising Radiation on DNA:

Ionizing radiation (e.g., X-rays, γ-rays) damages DNA through **direct interaction** or **indirect effects via free radicals (e.g., OH•)** generated from water ionization. This leads to:

- **Base modifications or deletions** (thymine is most sensitive),
- **Breakage of hydrogen bonds**,
- **Single/double-strand breaks**,
- **Cross-linking**, and
- **Oxidation of sugar moieties**.

These disruptions inhibit **DNA replication**, especially during the **S phase**, arresting **cell division** and potentially causing **cell death**. Damage during **G1 or mitosis** may delay division but still permit replication. Chromosomal aberrations like **inversions, translocations, and deletions** result from mis-repaired double-strand breaks. **Microorganisms** exhibit higher resistance to radiation compared to human cells. The **Df ‡ dose** (dose reducing survival to 37%) ranges from **2000–30,000 rads** for bacteria, but only **~120 rads** for human cells.

Protective agents like aminothiols (–SH and –NH, groups) mitigate radiation effects, measured by the **Dose Reduction Factor (DRF)**—the ratio of LD_{50} (30) in protected vs. unprotected animals. For example:

- LD_{50} (30): Dog – 350 rads, Mouse – 550 rads, Goldfish – 2300 rads.

Natural background radiation, from cosmic rays and terrestrial radioisotopes (e.g., ^{1}t C, t p K), averages **~0.8 mSv/year at sea level**. Studies in high-background regions like coastal Kerala show elevated risks of **chromosomal anomalies and Down syndrome**, suggesting potential genetic impacts even from low-dose exposure.

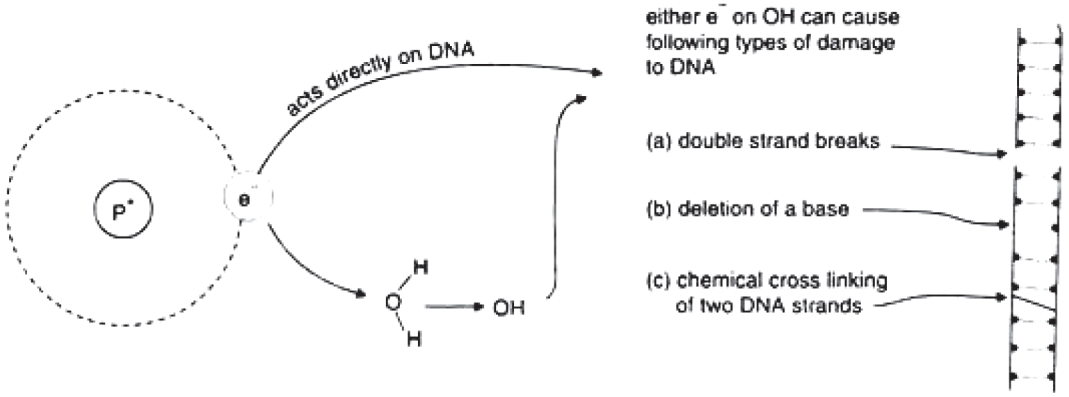


Fig. 20.2 Diagram showing types of damage to DNA by ionising radiation.

Control of Radiation Pollution:

Radiation pollution can be controlled in the following ways:

1. Care should be taken to check manmade radiation pollution at source.
2. Nuclear reactor should be perfectly maintained to avoid accidental leakage.
3. Nuclear tests should be banned.

IV. FOOD AND ADDITIVES

Substances that are added to food to maintain or improve the safety, freshness, taste, texture, or appearance of food are known as food additives. Some food additives have been in use for centuries for preservation – such as salt (in meats such as bacon or dried fish), sugar (in marmalade), or sulfur dioxide (in wine).Many different food additives have been developed over time to meet the needs of food production, as making food on a large scale is very different from making them on a small scale at home. Additives are needed to ensure processed food remains safe and in good condition throughout its journey from factories or industrial kitchens, during transportation to warehouses and shops, and finally to consumers.

The use of food additives is only justified when their use has a technological need, does not mislead consumers, and serves a well-defined technological function, such as to preserve the nutritional quality of the food or enhance the stability of the food.Food additives can be derived from plants, animals, or minerals, or they can be synthetic.

They are added intentionally to food to perform certain technological purposes which consumers often take for granted. There are several thousand food additives used, all of which are designed to do a specific job in making food safer or more appealing. WHO, together with FAO, groups food additives into 3 broad categories based on their function.

Types of Food Additives

Food additives can be divided into several groups, although there is some overlap because some additives exert more than one effect. For example, salt is both a preservative as well as a flavor.

1. **Acidulants:** Acidulants confer sour or acid taste. Common acidulants include vinegar, citric acid, tartaric acid, malic acid, fumaric acid, and lactic acid.
2. **Acidity regulators:** Acidity regulators are used for controlling the pH of foods for stability or to affect activity of enzymes.
3. **Anticaking agents:** Anticaking agents keep powders such as milk powder from caking or sticking.
4. **Antifoaming and foaming agents:** Antifoaming agents reduce or prevent foaming in foods. Foaming agents do the reverse.
5. **Antioxidants:** Antioxidants such as vitamin C are preservatives by inhibiting the degradation of food by oxygen.
6. **Bulking agents**: Bulking agents such as starch are additives that increase the bulk of a food without affecting its taste.
7. **Food colouring agents**: Colourings are added to food to replace colors lost during preparation or to make food look more attractive.
8. **Fortifying agents**:Vitamins, minerals, and dietary supplements to increase the nutritional value
9. **Colour retention agents**: In contrast to colourings, colour retention agents are used to preserve a food's existing colour.
10. **Emulsifiers:**Emulsifiers allow water and oils to remain mixed together in an emulsion, as in mayonnaise, ice cream, and homogenized milk.
11. **Flavours**: Flavours are additives that give food a particular taste or smell, and may be derived from natural ingredients or created artificially.
12. **Flavour enhancers**: Flavour enhancers enhance a food's existing flavors. A popular example is monosodium glutamate. Some flavor enhancers have their own flavors that are independent of the food.

13. **Flour treatment agents:**Flour treatment agents are added to flour to improve its color or its use in baking.
14. **Glazing agents:** Glazing agents provide a shiny appearance or protective coating to foods.
15. **Humectants**: Humectants prevent foods from drying out.
16. **Tracer gas:**Tracer gas allow for package integrity testing to prevent foods from being exposed to atmosphere, thus guaranteeing shelf life.
17. **Preservatives:**Preservatives prevent or inhibit spoilage of food due to fungi, bacteria and other microorganisms.
18. **Stabilizers**: Stabilizers, thickeners and gelling agents, like agar or pectin (used in jam for example) give food a firmer texture. While they are not true emulsifiers, they help to stabilize emulsions.
19. **Sweeteners**: Sweeteners are added to foods for flavouring. Sweeteners other than sugar are added to keep the food energy (calories) low, or because they have beneficial effects regarding diabetes mellitus, tooth decay, or diarrhoea.
20. **Thickeners**: Thickening agents are substances which, when added to the mixture, increase its viscosity without substantially modifying its other properties.

Since the 19th century, food additives have raised safety concerns. Boric acid and saccharin, once widely used, were linked to toxicity or cancer in animals. This led to strict regulations like the U.S. Delaney clause, banning carcinogenic additives, though exceptions like saccharin persisted due to public pressure and later scientific clarification.

Probable Questions:

1. What is environmental toxicology?
2. Classify pesticides on the basis of target organisms.
3. Classify pesticides on the basis of mode of action.
4. What are the benefits of pesticides?
5. Write down the effects of pesticides on health, environment and economy.
6. How pesticide toxicity can be controlled?
7. What are the sources of heavy metal poisoning?
8. Write down Mechanism of heavy metal toxicity of any 3 metals.
9. What is the biochemical mechanism of heavy metal toxicity?

10. What are the effects of heavy metal on human health?
11. Write down the remediation method of heavy metal toxicity.
12. What is ionizing radiation? How it is measured?
13. What are the sources of radiation pollution?
14. What is ionizing radiation?
15. Describe different types of radiation toxicity.
16. What are the effects of radiation pollution?
17. What are the effects of ionizing radiation on DNA?
18. Describe the health hazards caused by food additives.
19. Describe different types of food additives with examples.
20. How radiation pollution can be controlled?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-VI

Plant Allelochemicals: Types and its role in insect-plant interaction. Plant secondary metabolites in insect response

Objective: In this unit you will learn about plant allelochemicals. Its types and its role in insect -plant interaction. We will also learn about role of plant secondary metabolites in insect response.

Introduction:

Allelopathy refers to the chemical interaction between organisms, where one species releases biochemicals (allelochemicals) that influence the germination, growth, or survival of another. These compounds—secondary metabolites like flavonoids, phenolics, terpenes—are released through leaching, volatilization, exudation, or decomposition and may inhibit or stimulate target organisms.

Allelopathic effects differ from resource competition by their direct biochemical action. Kairomones, allomones, and synomones are types of allelochemicals that respectively benefit the receiver, the emitter, or both. Allelopathy plays key roles in plant defense, species distribution, and ecological succession, and contributes to the success of invasive species like *Garlic mustard* and *Ailanthus*.

Historically recognized since Theophrastus (300 BC), the term was formalized by Molisch in 1937. By the late 20th century, studies clarified allelopathic mechanisms, distinguishing them from mere competition. Modern research (e.g., Liu & Lovett, Nilsson) has demonstrated measurable allelopathic suppression in crops and forests.

Applications include using allelochemicals as natural herbicides (e.g., mesotrione), improving sustainable agriculture, and enhancing weed control (e.g., in rice and sorghum). Studies show crop-to-weed allelopathy, allelopathic traits in forest trees (e.g., *Juglans nigra*, *Eucalyptus*), and suppression of mutualistic fungi in invaded ecosystems.

In sum, allelopathy is a significant ecological and agronomic phenomenon with promising applications in sustainable weed management, forest ecology, and crop improvement.

Effect of some allelochemicals on insects:

a. Phenolics (allomones): non-nitrogen compounds, hydroxyl group attached to the benzene rings; they affect nutritional quality of plants; the major groups are

phenylpropanoids, flavonoids, quinones. They have deleterious effects on larval growth of the insects. Compounds harmful to one insect may have little effect on another. For example, proanthocyanins or condensed tanninsare feeding inhibitors, however anthocyanins promote pollinator attraction, rotenone, an isoflavanoid has insecticidal properties. Protein inhibitors in plants are found in seeds, tubers and foliage and inhibitory activity of protein inhibitors is specific to digestive proteinases.

b. Terpenoids: monoterpenes act as attractants/repellents, diterpenes exhibit considerable biological activity in relation to the action of toxins and hormones produced by plants. These chemicals are non toxic to the plant itself but on being consumed by insects are activated into lethal cytotoxins. Terpene induces cytochrome P-450 in insect to higher activity. Such activity may influence the hormone balance or pheromone products in the insect so that regulation of reproductive processes by these allelochemicals is implicated. Oligosaccharides are also reported to regulate not only the activation of defense mechanism but also regulate the various aspects of plant product and morphogenesis.If the plant allelochemicals stimulate mating, dependence of the female on the plant is greater so that the adjustment of fecundity to the carrying capacity of the environment is better. For example, some phytophagous insects do not mate without eating the pollen of particular hosts, thus flower stimulates vitellogenesis and induces the female to a state for oviposition.

Plant secondary metabolites and interaction with insects:

Plants and insects have co-evolved over 350 million years, leading to a dynamic arms race where plants have developed both **direct** and **indirect defense strategies** against herbivory. These include physical barriers (e.g., trichomes, spines, thickened tissues) and biochemical defenses (e.g., terpenoids, alkaloids, phenols, proteinase inhibitors) that deter, intoxicate, or impair insect development and reproduction.

Direct defenses hinder herbivore feeding, digestion, and survival via structural traits (sclerophylly, pubescence, spinescence) and toxic secondary metabolites. **Trichomes**, both glandular and non-glandular, serve as key morphological defenses, physically obstructing insects and secreting deterrent or toxic compounds such as flavonoids and alkaloids.

Indirect defenses include the emission of herbivore-induced volatiles (HIPVs) and secretion of extra-floral nectar that attract predators and parasitoids of herbivores, thereby reducing pest pressure.

Induced defenses, activated upon insect attack, lead to dynamic changes in gene expression and biochemical pathways (e.g., activation of enzymes like POD, PPO, PAL), enhancing plant resistance. Though metabolically costly, these defenses confer adaptive flexibility (phenotypic plasticity), reducing the risk of herbivore adaptation.

Understanding plant-insect chemical ecology and defensive gene expression has significant implications for breeding pest-resistant crops, potentially reducing reliance on chemical pesticides and enhancing sustainable agricultural practices.

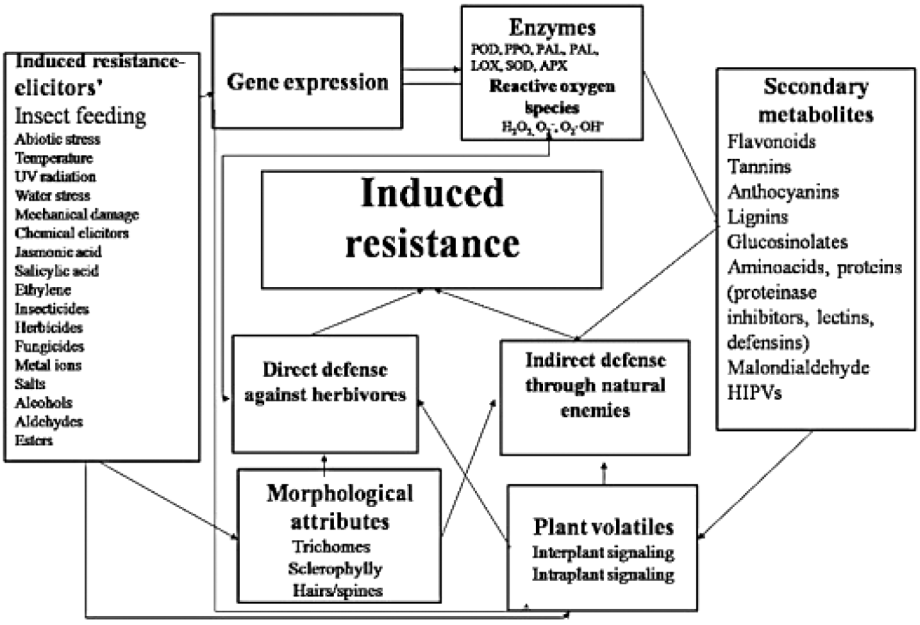


Figure 1. Mechanism of induced resistance in plants POD, peroxidase; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; TAL, tyrosine alanine ammonia lyase; LOX, lipoxygenase; SOD, superoxide dismutase; APX, ascorbate peroxidase; HIPVs, herbivore induced plant volatiles.

Secondary metabolites and plant defense:

Plants synthesize secondary metabolites that, while not essential for primary growth, play a critical role in defense by deterring herbivores and pathogens. These compounds can be constitutively present (phytoanticipins) or induced upon attack (phytoalexins). Activation often involves β-glucosidases, as seen with glucosinolates and benzoxazinoids in Poaceae, which convert into toxic aglycones upon tissue damage.

Key classes include:

- **Phenolics**: These interfere with insect digestion and development via enzyme inhibition, ROS scavenging, and lignification. Polyphenol oxidases (PPOs) and peroxidases (PODs) catalyze the formation of quinones, which reduce palatability and exhibit direct toxicity.
- **Flavonoids**: Multifunctional compounds acting as antifeedants, antioxidants, and signaling modulators. They alter insect behavior, inhibit growth, and protect against oxidative stress.

- **Tannins**: Polyphenols that bind proteins, reduce nutrient absorption, and form complexes with digestive enzymes, thereby impairing herbivore physiology. Condensed tannins (proanthocyanidins) show strong deterrent effects and are inducible by herbivory and light stress.

Defense-Related Proteins and Enzymes:

Plants also produce a suite of proteins that modulate insect nutrition and physiology:

- **Proteinase Inhibitors (PIs)**: These bind to insect digestive enzymes, causing amino acid deficiency and growth retardation. However, insects may evolve PI-insensitive proteases, necessitating combinatorial defense strategies.
- **Lectins**: Carbohydrate-binding proteins that survive digestion and disrupt insect gut function by binding epithelial glycoproteins, leading to systemic toxicity. Transgenic plants expressing lectins show resistance to various insect orders.
- **Oxidative Enzymes**: Including PODs, PPOs, and lipoxygenases (LOXs), these catalyze the formation of toxic quinones and reactive oxygen species (ROS), damaging insect tissues and reducing nutrient availability. LOX also initiates jasmonic acid (JA) signaling, essential for activating both direct (e.g., PIs) and indirect (e.g., VOCs) defenses.

Integration and Applications:

These defenses—structural, biochemical, and inducible—are regulated through complex signaling networks (i.e., JA: Jasmonic Acid, SA: Salicylic Acid and ET: Ethylene pathways) and can vary by species, tissue, and type of herbivore. Advances in metabolomics and transcriptomics have enhanced our understanding of these responses, enabling genetic manipulation for improved crop resistance while minimizing reliance on synthetic pesticides.

Indirect Plant Defenses and Herbivore-Induced Volatiles (HIPVs)::

Plants employ indirect defenses by attracting the natural enemies of herbivores through the release of chemical signals. These defenses can be constitutive or induced in response to herbivore attack, mechanical injury, or herbivore-associated elicitors. Key strategies include the production of herbivore-induced plant volatiles (HIPVs) and secretion of extra floral nectar (EFN), which enhance the recruitment of parasitoids and predators, reducing herbivore populations.

HIPVs are volatile organic compounds (VOCs), such as terpenes, green leaf volatiles (GLVs), methyl salicylate (MeSA), ethylene, and homoterpenes, released from aerial or

root tissues. Their composition and quantity depend on plant and herbivore species, developmental stage, and mode of feeding. HIPVs serve multiple roles: attracting natural enemies, repelling herbivores, acting as oviposition deterrents, or priming neighboring or undamaged plant parts for defense.

GLVs, derived via the hydroperoxide lyase (HPL) branch of the oxylipin pathway, include C6-aldehydes and their alcohol or ester derivatives (e.g., (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexen-1-yl acetate). These volatiles play a significant role in tritrophic interactions by attracting predators like ladybird beetles, lacewings, and parasitic wasps. Notably, compounds like MeSA are central to indirect defense but may also attract certain herbivores, highlighting ecological trade-offs.

HIPVs also mediate intraplant and interplant communication, often through priming—a mechanism where a sub-lethal signal preconditions plants to respond more robustly upon actual attack. Volatile blends from plants like maize, lima bean, or Arabidopsis are tailored to specific herbivore threats, and their emission is often enhanced by prior exposure to GLVs.

Genetically modified plants overexpressing terpene synthases (TPS), such as nerolidol synthase or TPS10, have demonstrated increased attractiveness to natural enemies. Similarly, root-emitted HIPVs (e.g., (E)-β-caryophyllene, 1,8-cineole) defend against soil-dwelling pests and attract entomopathogenic nematodes. However, some root volatiles may inhibit growth of nearby plants due to allelopathic effects on cell division.

Elicitors in Insect Oral Secretions:

Herbivore-derived elicitors in oral secretions (OS), such as fatty acid–amino acid conjugates (FACs), inceptins, caeliferins, and β-glucosidases, initiate complex defense signaling in plants. FACs like volicitin activate jasmonic acid (JA) and MAPK pathways, enhancing volatile emissions and the synthesis of defense proteins. These elicitors trigger transcriptomic, proteomic, and metabolomic reprogramming, increasing resistance through production of defensive secondary metabolites, proteinase inhibitors, and structural reinforcements.

FACs in lepidopteran OS stimulate early signaling cascades, including calcium influx and the activation of MAPKs such as SIPK and WIPK, which in turn induce production of JA, JA-Ile, salicylic acid (SA), and ethylene (ET). Inceptins (ATP synthase fragments) and caeliferins (sulfated fatty acids) further amplify oxylipin and JA pathway activity.

Role of phytohormones in induced resistance in plants:

Role of Phytohormones in Induced Resistance in Plants

Plant defense against herbivores involves a complex signaling network of phytohormones, primarily **jasmonic acid (JA)**, **salicylic acid (SA)**, and **ethylene (ET)**. These hormones coordinate diverse biochemical pathways, regulating gene expression and activating systemic and localized defense responses.

Jasmonic Acid (JA): JA, synthesized via the octadecanoid pathway from linolenic acid, is central to defense against chewing insects. Herbivory induces its accumulation, initiating direct defenses (e.g., proteinase inhibitors, alkaloids, trichomes, VOCs, EFN secretion) and indirect defenses (e.g., attraction of natural enemies). JA biosynthesis involves the conversion of linolenic acid to 12-OPDA and subsequently to JA via OPDA reductase. JA or its active form JA-Ile binds to the SCFCOI1-JAZ complex, leading to degradation of JAZ repressors and activation of JA-responsive genes. JA also influences calcium-dependent protein kinases (CDPKs), modulating redox status and intracellular Ca^{2+} levels. EFN, induced by JA, supports indirect resistance by nourishing insect predators.

Salicylic Acid (SA): SA, a benzoic acid derivative, is crucial in defense against piercing-sucking insects and systemic acquired resistance (SAR). SA-mediated responses are regulated by the NPR1 protein, which, upon redox-triggered activation, modulates transcription through partner factors. SA enhances ROS generation, especially H, O,, which damages insect digestive systems. SA also stimulates VOC emission to attract natural predators (e.g., in tomato and lima bean). However, SA and JA exhibit antagonistic crosstalk, where activation of one can suppress the other. **Methyl salicylate (MeSA)**, a volatile SA derivative, acts as a systemic signal and attracts arthropod predators under field conditions.

Together, JA and SA orchestrate a sophisticated defense network tailored to insect feeding behaviour, enabling plants to optimize both direct and ecological defense strategies.

Ethylene:

Ethylene is an important phytohormone, which plays an active role in plant defense against many insects. Ethylene signaling pathway plays an important role in induced plant defense against herbivores and pathogens both directly and indirectly, however, there are limited reports on its role in indirect defense through the emission of HIPVs. ET signaling pathway works either synergistically or antagonistically, with JA in expression of plant defense responses against pathogens and herbivorous insects. It has been reported that ET and JA work together in tomato in PIs expression. Infestation by *A. alni*induced the emission of ethylene and release of various valatiles in *Alnus glutinosa*L. leaves in

addition to mono-, sesqui and homoterpenes. ETprecursor, 1-amino-cyclopropane-1-carboxylic acid has been reported to enhance the volatile emission from the JA treated detached leaves. Ethylene further induced the emission of volatiles induced by volicitin, JA or (Z)-3-hexen-ol in maize.

Role of Calcium Ions (Ca^{2+}) in Plant Defense:

Calcium ions (Ca^{2+}) serve as early secondary messengers in herbivore-induced plant defense, modulating signal transduction through changes in cytosolic Ca^{2+} levels. Herbivory induces Ca^{2+} influxes and membrane depolarization, triggering activation of calcium sensors such as calmodulin, calmodulin-binding proteins, and CDPKs. CDPKs, possessing both kinase and calmodulin-like domains, mediate downstream responses including MAPK activation and transcriptional regulation. In species like tobacco and Arabidopsis, specific CDPKs regulate defense gene expression. Elevated Ca^{2+} signaling is detected both locally and systemically upon insect feeding, indicating a systemic calcium-based defense response.

Role of Reactive Oxygen Species (ROS) in Plant Defense:

ROS, including superoxide (O, {), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are rapidly produced during herbivore attack, a phenomenon termed "oxidative burst." ROS act as both direct toxins and signaling molecules, triggering defense gene expression and structural reinforcement of cell walls. H, O,, being stable and diffusible, plays a central role in defense signaling, often generated by NADPH oxidase and converted from superoxide via SOD. ROS accumulation not only damages insect digestive systems but also mediates cross-linking of cell wall proteins via peroxidase and activates MAPKs and other transcriptional networks. H, O, has been observed to accumulate rapidly in response to herbivore damage in various crops such as barley, wheat, and oats.

Gene Expression and Molecular Basis of Plant Defense:

Herbivory induces large-scale transcriptional reprogramming in plants, influencing thousands of genes. Technologies such as DNA microarrays, RNA-seq, and eQTL mapping enable detailed analysis of plant responses. These responses are herbivore- and genotype-specific. For example, M. persicae induces more gene responses than P. rapae. While lepidopteran insects affect genes involved in detoxification and signal transduction, aphids modulate genes linked to oxidative stress and cell wall restructuring. Cross-species and cultivar differences are common, reflecting diversity in defense mechanisms. Integration of genomic, transcriptomic, and proteomic tools is essential for advancing understanding of inducible plant defenses against insect herbivores.

Transgenerational induced resistance to herbivores:

Biotic and abiotic stresses can induce resistance not only in the maternal plant but also in its progeny, enhancing seed vigor and seedling defense—an effect termed *transgenerational immunity*. Evidence from species like *Raphanus raphanistrum* and *Arabidopsis thaliana* suggests that herbivory or environmental stress (e.g., cold, heat, flood) leads to increased resistance in offspring through mechanisms such as genome methylation and elevated homologous recombination. Low-to-moderate maternal herbivore damage has been linked to enhanced progeny fitness. However, the underlying genetic and epigenetic mechanisms—including siRNA signaling and DNA methylation inheritance—remain inadequately explored. Further research is essential to utilize maternal ecological cues for effective pest management and to deepen our understanding of heritable plant defense strategies.

Probable Questions:

1. What is allelochemicals?
2. Define allelopathy.
3. Discuss about effect of allelochemicals on insects.
4. What are the applications of allelopathy?
5. What are direct responses of plants to herbivores.
6. How secondary metabolites help plants in defence?
7. Describe the role of flavonoids in plant defence.
8. Describe the role tannins in plant defence
9. Describe the role enzymes in plant defence.
10. Describe the role polyphenol oxidases in plant defence.
11. Describe the role Lipoxygenases in plant defence.
12. Describe the role peroxidises in plant defence.
13. Describe the role Proteinase inhibitors in plant defence.
14. Describe the role plant lectins in plant defence.
15. Describe role of phytohormones in induced resistance in plants.
16. Describe the role plant jasmonic acid in plant defence.
17. Describe the role of ROS in plant defence.

18. Describe the role of ethylene in plant defence.
19. Describe the role of salicylic acid in plant defence.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen.
5. Mechanisms of Plant Defense against Insect Herbivores. War *et al.* 2012. Plant Signaling& Behavior 7:10, 1306-1320

UNIT-VII

Group Characteristics and function of pesticides: Organochlorines, Organophosphates insecticides, Carbamates, Pyrethroids, other plant origin bio-insecticides, neonicotinoids and nitrogenous insecticides; fumigants; IGRs, attractants, repellents and anti-feedants

Objective: In this unit we will discuss different types of pesticides. Their action, structure, advantages, disadvantages will be discussed.

Pesticides: Definition, Types, and Historical Context:

Pesticides are chemical or biological agents (e.g., carbamates, bacteria, viruses, fungi) designed to kill, incapacitate, or repel pests—including insects, weeds, microbes, nematodes, rodents, birds, and others that harm crops, property, or transmit diseases. Common pesticide classes include herbicides, insecticides, fungicides, nematicides, rodenticides, and antimicrobials, with herbicides accounting for ~80% of global usage. Some biocontrol agents (e.g., *Alternaria* against *Salvinia*) also serve as pesticides. Historically, pest control dates back to 2000 BC (e.g., sulfur by Sumerians, plant toxins in Rigveda). Toxic metals like arsenic and lead were common by the 15th century. Natural compounds like nicotine sulfate, pyrethrum, and rotenone gained prominence in the 17th–19th centuries. The discovery of DDT by Paul Müller revolutionized pest control in the 20th century, but due to environmental and health concerns, DDT and related organochlorines were replaced by organophosphates, carbamates, and later, pyrethrins and modern herbicides like glyphosate.*The FAO defines a pesticide as any substance (or mixture) intended to prevent or control pests affecting crops, animals, stored goods, or food production systems.*

Types of Pesticides

Pesticides are also referred to by the type of pest they control. Pesticides can be either biodegradable pesticides, that break down into harmless compounds by bacteria and other living organisms, or persistent/Non-Biodegradable pesticides, which can take months or years to break down.

Classification of pesticides are according to the types of pests they kill

Grouped by the pest types they kill;

- Insecticides – Insects
- Herbicide – Plants
- Rodenticides – Rodents (rats & mice)
- Bactericides – Bacteria
- Fungicides – Fungicide
- Larvicides – Larvae

In modern agriculture a large number of pesticides and insecticides are used.

They persist in the environment for a long time and accumulate in certain vital tissues of organisms and other items, which are used by humans.

The important categories of pesticides and their toxicity are mentioned below:

1. Organ chlorine insecticides:

These are chlorinated ethane derivations, such as DDT and Methoxychlor; cyclodienes such as endrin, aldrin, dieldrin, chlordane, heptachlor, and mirex; and hexachlorocyclohexanes (HCH) such as Lindane. Of these, methoxychlor is less toxic than DDT; endrin is extremely toxic; Lindane is also highly toxic but less cumulative; the remaining are less toxic.

They stimulate the nervous system and induce irritability, disturbed equilibrium, paresthesia, tremor, and convulsions. Some of these chemicals, such as aldrin, dieldrin and lindane affect neurotransmitter activity. DDT may exert its toxic effects in the nervous system by adversely affecting ion transport across the axon membrane (Doherty, 1979; Narahashi, 1980). Some organ chlorine insecticides including DDT are hepato toxic.

2. Organophosphorus insecticide:

These are esters of phosphoric acid (Dichlorvos) and theophosphoric acid (Parathion). Other pesticides in this group are Diazinon, Dimethoate, Malathion, Mevinphos and Dipterex (Trichlorfon). Their toxic effects vary over a wide range. They act by inhibiting acetylcholinesterase (AchE). As a result, the accumulated acetylcholine (Ach) induces tremor, incoordination and convulsion. The accumulation of Ach at the neuromuscular synapse will lead to contraction of the muscles, loss of reflexes and paralysis. Several organophosphorus compounds may cause delayed neurotoxicity.

3. Carbamate Insecticides:

Insecticides of this class include Carbaryl (Sevin), Aldicarb (Temik), Carbofliran Methomyl and Propoxur (Baygon). These are esters of N. methylcarbamic acid. They also act by inhibiting AchE. However, inhibition of AchE by a carbamate is readily reversible.

4. Herbicides:

Several types of herbicides are used. Herbicides like 2, 4-D (2, 4-dichlorophenoxy-acetic acid). Paraquat and Diquat have been widely used. Some herbicides retard the growth of weeds by inhibiting photosynthesis, respiration, cell division or protein or lipid synthesis. Their toxicities in animals are relatively low. Paraquat and Diquat exert their toxicity via the formation of free radicals. Paraquat causes lung damage after inhalation and also after ingestion. It also causes hemorrhage and fibrosis.

5. Rodenticides:

A number of rodenticides including Warfarin, Thioureas, Sodium fluoroacetate and Sodium fluoroacetamide have been used. Warfarin is an antimetabolite to vitamin K, thus acts as an anticoagulant. Thioureas main toxicity is pulmonary edema and pleural effusion. They are highly toxic to rats but moderately toxic to humans. Sodium fluoroacetate exerts its toxic effect through blockage of the citric acid cycle.

6. Fungicides:

Methyl and ethyl mercury are very effective fungicides. However, permanent neurologic damage and deaths have been reported after their use. Fungicides which have been widely used in agriculture are Dicarboximides but they are reported to have carcinogenic effects.

Depending on how biodegradable they are

Biodegradable Pesticides

Biodegradable pesticides are those that can be broken down into harmless compounds by microbes and other living organisms within less period of time.

Non-Biodegradable Pesticides

Few pesticides are known as non-biodegradable, also called persistent pesticides. The most long-lived pesticide materials include aldrin, parathion, DDT, chlordane, and endrin, they take a long period of time to break down. These pesticides can survive in the soil for over 15 years or more.Another way of thinking about pesticides is considering the chemical pesticides extracted from a common source or some production method.

Chemical pesticides:

Organophosphates: Many organophosphates are insecticides that impact on the nervous system by compromising the enzyme that regulates the neurotransmitter.

Carbamate: Carbamate pesticides affect the nervous system by compromising

the enzyme that regulates the neurotransmitter similar to the organophosphates, but carbamate enzyme effects are usually reversible.

Organochlorine Insecticides: This type was common in the early years when pesticides came into the market. Many countries have banned organochlorine insecticides from their markets because of their impacts and persistence on health and the environmental factors (e.g., DDT, chlordane and toxaphene).

Pyrethroid: There are synthetic variants of pyrethrin, a naturally occurring pesticide present in chrysanthemums (Flower). Their development is such a way they can maximize their environmental resilience.

Sulfonylurea herbicides: The commercial production of sulfonylureas herbicides was for weed control like flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, pyrithiobac-sodium, cyclosulfamuron, bispyribac-sodium, terbacil, sulfometuron-methyl Sulfosulfuron.

Biopesticides

The biopesticides are a type of pesticides obtained from natural resources such as animals, plants, bacteria, and certain minerals.

Uses

- Pesticides are useful in controlling organisms that are toxic or harmful to their environment.
- Herbicides are useful in controlling algae and weeds.
- They are useful in grocery stores and food storage facilities to control rats and insects infesting on food.
- They are in use to kill mosquitoes that can spread life-threatening diseases such as the West Nile virus, yellow fever, and malaria.
- Also, they are useful in the agricultural sector to prevent or kill insects and other organisms that feed on crops.

Types of Pesticides:

These are grouped according to the types of pests which they kill:

Grouped by Types of Pests They Kill

- Insecticides – insects
- Herbicides – plants
- Rodenticides – rodents (rats & mice)
- Bactericides – bacteria
- Fungicides – fungi
- Larvicides – larvae

Based on how biodegradable they are:

Pesticides can also be considered as:

- **Biodegradable:** The biodegradable kind is those which can be broken down by microbes and other living beings into harmless compounds.
- **Persistent:** While the persistent ones are those which may take months or years to break down.

Another way to classify these is to consider those that are chemical forms or are derived from a common source or production method.

Chemically-related pesticides:

- **Organophosphate:**

  Most organophosphates are insecticides, they affect the nervous system by disrupting the enzyme that regulates a neurotransmitter.

- **Carbamate:**

  Similar to the organophosphorus pesticides, the carbamate pesticides also affect the nervous system by disrupting an enzyme that regulates the neurotransmitter. However, the enzyme effects are usually reversible.

- **Organochlorine insecticides:**

  They were commonly used earlier, but now many countries have been removed Organochlorine insecticides from their market due to their health and environmental effects and their persistence (e.g., DDT, chlordane, and toxaphene).

- **Pyrethroid:**

  These are a synthetic version of pyrethrin, a naturally occurring pesticide, found in chrysanthemums (Flower). They were developed in such a way as to maximise their stability in the environment.

- **Sulfonylurea herbicides:**

  The sulfonylureas herbicides have been commercialized for weed control such as pyrithiobac-sodium, cyclosulfamuron, bispyribac-sodium, terbacil, sulfometuron-methyl Sulfosulfuron, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron.

Examples of pesticides

Examples of pesticides are fungicides, herbicides, and insecticides. Examples of specific synthetic chemical pesticides are glyphosate, Acephate, Deet, Propoxur, Metaldehyde, Boric Acid, Diazinon, Dursban, DDT, Malathion, etc.

Benefits of Pesticides

The major advantage of pesticides is that they can save farmers. By protecting crops from insects and other pests. However, below are some other primary benefits of it.

- Controlling pests and plant disease vectors.
- Controlling human/livestock disease vectors and nuisance organisms.
- Controlling organisms that harm other human activities and structures.

Effects of Pesticides

- The toxic chemicals in these are designed to deliberately released into the environment. Though each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Instead, they enter the air, water, sediments, and even end up in our food.
- Pesticides have been linked with human health hazards, from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm.
- The use of these also decreases the general biodiversity in the soil. If there are no chemicals in the soil there is higher soil quality, and this allows for higher water retention, which is necessary for plants to grow.

Bio-pesticides Types: Bio-Herbicides and Bio-Insecticides:

- Bio-pesticides are those biological agents that are used for control of weeds, insects and pathogens.
- The micro-organisms used as bio-pesticides are viruses, bacteria, protozoa, fungi and mites. Some of the bio-pesticides are being used on a commercial scale.

- Most important example is the soil bacterium, Bacillus thuringiensis (Bt). Spores of this bacterium possess the insecticidal Cry protein.
- Therefore, spores of this bacterium kill larvae of certain insects. The commercial preparations of *B. thuringiensis* contain a mixture of spores, Cry protein and an inert carrier.
- This bacterium was the first bio-pesticide to be used on a commercial scale in the world, and is the first bio-pesticide being produced on a commercial scale in India.

Bio-pesticides are of two types: bio-herbicides and bio-insecticides:

(i) Bio-herbicides: Herbicides inhibit unwanted plant growth, but chemical weedicides pose ecological risks. Genetic engineering has enabled herbicide-resistant crops like transgenic tomato and tobacco, reducing reliance on chemicals. Some crops (e.g., barley, rye, sorghum, sunflower) naturally suppress weeds through allelopathy and can be used in crop rotation for weed control. Biological control involves deploying weed-specific natural enemies. For example, *Cactoblastis cactorum* controls *Opuntia*, and *Chrysolina* beetles reduce *Hypericum perforatum*. Bioherbicides—organisms that eliminate weeds without harming crops—offer sustainable alternatives. The first, a mycoherbicide (*Phytophthora palmivora*), was used in 1981 to suppress milkweed in citrus groves. Other examples include *Cercospora rodmanii* and *Alternaria eichhorniae* for water hyacinth, and *Puccinia chondrilla* for skeleton weed. Commercial fungal biocontrol products like **Devine** and **Collego** contain durable spores effective against specific weeds.

(ii) Bio-insecticides:

Bio-insecticides are those biological agents that are used to control harmful insects. They include the following.

(a) Predators:

Destructive insects or plant pests can be brought under control through introduction of their natural predators. The predators should be specific and unable to harm the useful insects. Introduction of ladybugs (Lady Bird Beetles) and Praying Mantis has been successful in combating scale insects or aphids which feed on plant sap.

(b) Parasites and Pathogens:

This is alternate biological control of plant pests through the search of their natural parasites and pathogens. They include viruses, bacteria, fungi and insect parasitoids. Parasitoids are organisms that live as parasites for some time (as early or larval stage) and free living at other times, e.g., Trichogramma. Nucleopolyhedrovirus (NPV) are species specific.

For example, Baculovirus heliothis (a virus) can control Cotton bollworm (HeliothisZea). Similarly, Bacillus thuringenesis (a bacterium) is effective against the cabbage looper (*Trichoplausia ni*) and *Entomophthora ignobilis* (a fungus) the green peach aphid of Potato (Myzuspersicae). In U.S.S.R. the fungus *Beauveria bassiana* has been successfully employed in controlling Potato beetle and Codling moth.

(c) Natural Insecticides:

These are eco-friendly compounds derived from plants or microbes that control insect pests without harming non-target organisms.

- **Azadirachtin** (from *Azadirachta indica* or neem): A potent antifeedant deterring pests like Japanese beetles by disrupting feeding behavior.
- **Rotenone** (from *Derris elliptica* and *Lonchocarpus nicou*): An effective insecticide safe for warm-blooded animals, historically used in Asia.
- **Red Squill** (*Urginea maritima*): Produces rodenticidal compounds with minimal impact on non-target animals.
- **Nicotine** (from *Nicotiana tabaccum*): A toxic alkaloid used in the form of nicotine sulfate for pest control.
- **Pyrethrum** (from *Chrysanthemum* sp.): Contains pyrethrins and cinerins; widely used in mosquito coils, aerosols, and fly sprays.
- **Thurioside** (produced by *Bacillus thuringiensis* or Bt): A microbial toxin lethal to insects by disrupting gut integrity; widely adopted in biopesticide formulations.
- **Bt Crops**: Genetically modified plants (e.g., Bt cotton, Bt tomato) containing *cry* genes from Bt, which confer inherent resistance to specific insect larvae.

Anti-feedant:

Antifeedants are compounds that interfere with insect feeding behavior, often by acting on specific gustatory receptors.

- These substances may either deter feeding initiation (feeding deterrents) or suppress ongoing feeding (feeding suppressants).
- They can disrupt normal feeding cues, such as sugars or amino acids, by blocking stimulant receptors—similar to how **DEET** repels blood-feeding arthropods.
- **Azadirachtin** acts by lowering the sensitivity of sugar-sensing neurons in herbivores, leading to rejection of treated plant tissue.
- True antifeedants act through specific neural mechanisms, while general toxicants affecting all sensory cells are not classified as true antifeedants.
- Chemically, most antifeedants are **alkaloids**, **terpenes**, or **flavonoids**, and are typically bitter-tasting secondary metabolites.

Insecticidal and antiparasitic activity:

An antifeedant effect is a main strategy related to the use of quinoline and quinazoline alkaloids against insects. Dictamnine (**25**) was deterrent against three insect pests, including *Sitophilus zeamays, Trilobiumcastaneum*, and *Spodoptera litura*, for plant protection. Evolitrine (**103**) (Figure below) exhibited antifeedant activity against fourth instar larvae of the tobacco caterpillar *S. litura*, and acetylcupreine and 3,4-dihydroxyquinoline-2-carboxylic acid (**104**) (Figure below) presented powerful feeding deterrent effects toward the potato beetle *Leptinotarsa decemlineata* and the fish species *Blennius sphynx,* respectively. In addition, tryptanthrin (**31**) showed antifeedant activity against larvae of the house longhorn beetle *Hylotrupesbajulus* and the termite *Reticulitermissantonensis*.At levels of 0.05 and 0.1%, vasicine (**2**) (Fig. 2), vasicinone (**105**), and vasicinol (**106**) (Figure below) showed feeding deterrence against two beetle species *Aulacophorafoveicollis* and *Epilachna vijintioctopunctata*.

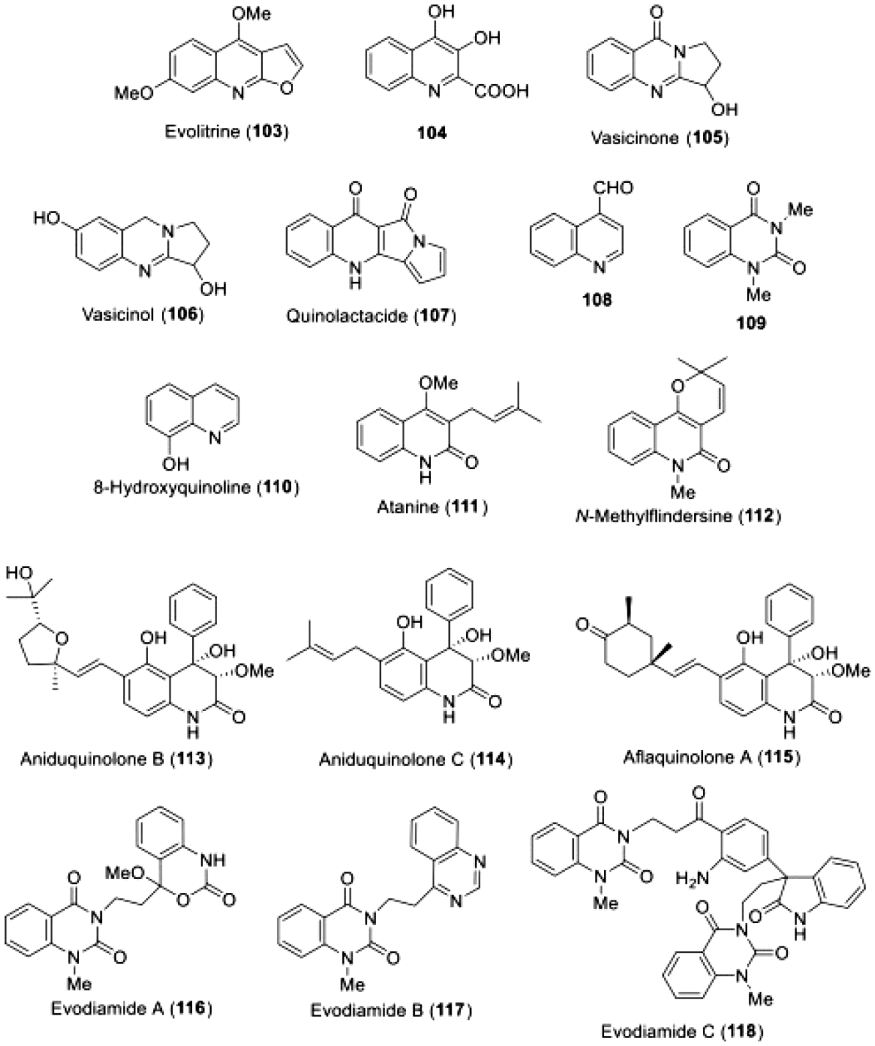


Fig. Chemical structures of some anti-feedant compounds

Furthermore, quinolactacide (**107**)) from the fermentation broth of *Penicillium citrinum* Thom F 1539 showed potent insecticidal activity against the green peach aphid *Myzuspersicae*. Quinoline-4-carbaldehyde (**108**) from *Rutachalepensis* exhibited insecticidal activity against the rice weevil *Sitophilus oryzae* with LD_{50} values of 0.084 mg/cm^2 using the fumigant method and 0.065 mg/cm^2 using the contact method. Changing the position of the aldehyde group on the quinoline skeleton affected the insecticidal activity.Another plant protection strategy is the use of insecticidal sex pheromones. Wojtasek and Leal found that 1,3-dimethylquinazoline-2,4-dione (**109**) has agricultural potential as a sex pheromone of the chafer beetle *Phylloperthadiversa* and is highly specific to males of this species.

Quinoline and quinazoline alkaloids also exhibit potent activity against human and animal, as well as plant, parasites. With an IC_{50} value of 0.213 μM, 8-hydroxyquinoline (**110**) was effective against the growth of *Toxoplasma*, the causative parasite of human toxoplasmosis. Febrifugine (**72**) from *Hydrangea macrophylla* showed anticoccidial activity against *Eimeria* parasites in chickens. Atanine (**111**) from *Evodia rutaecarpa* showed antiparasitic and anthelmintic activity against larvae of the blood fluke *Schistosoma mansoni* and the soil nematode *Caenorhabditis elegans*.

Luo et al. found that *N*-methylflindersine (**112**) from *Micromelumfalcatum* is highly toxic toward brine shrimp (*Artemia salina*) with an LD_{50} value of 1.39 μg/mL. Two 4-phenyl-3,4-dihydroquinolones aniduquinolones B and C (**113** and **114**), as well as aflaquinolone A (**115**) from the endophytic fungus *Aspergillus nidulans* exhibited LD_{50} values of 7.1, 4.5 and 5.5 μM, respectively, against brine shrimp. Three new quinazoline alkaloids, evodiamides A, B, and C (**116–118**), were isolated from *E. rutaecarpa* in 2018; however, they did not show significant pesticidal or antibacterial activity.

Fumigants

Fumigants are toxic gases or gas-releasing agents used to control pests, especially rodents and insects, through inhalation. They are advantageous as they require no behavioral response from the target pest. Fumigants are commonly applied in two ways:

- **Structural Fumigation**: Used in buildings, storage units, or vehicles; requires certified professionals and regulatory compliance.
- **Burrow Fumigation**: Targets burrowing pests (e.g., rats, ground squirrels, gophers) by sealing fumigants within burrows.

Two main types include:

- **Aluminum Phosphide**: A restricted-use pesticide that releases lethal phosphine gas upon reacting with moisture.

- **Gas Cartridges**: Readily available, these produce toxic smoke upon ignition and are used by sealing the smoke inside pest dens.

Soil type, moisture, and sealing efficiency affect fumigant success. Caution is essential due to fire hazards and proximity to buildings.

Other Fumigants and Applications:

Common fumigants include **hydrogen cyanide, methyl bromide, phosphine, chloropicrin**, and **ethylene dibromide**. These may be:

- **Inorganic**: e.g., aluminum phosphide, hydrogen cyanide
- **Organic**: e.g., methyl bromide, dibromochloropropane
- In agriculture, fumigants sterilize soil before planting and preserve stored crops by eliminating pests, fungi, nematodes, and bacteria. Due to their high toxicity and vapor pressure, most are regulated and require protective equipment.

Attractants and Repellents

- **Attractants** are stimuli (primarily chemical but also visual, auditory, thermal) that lure pests toward food or reproductive sites (e.g., pheromone traps, light sources).
- **Repellents** deter pests by inhibiting attraction or feeding. These do not necessarily drive pests away but rather block behavioral cues (e.g., DEET masks host-detection in mosquitoes).

The term “repellent” can be misleading; not all substances marketed as repellents effectively prevent contact or biting. Repellents may act on gustatory, olfactory, or other sensory systems, and their effect depends on concentration, mode of application, and pest species.

Conclusion

Fumigants are potent, broad-spectrum pest control agents but require careful application due to environmental and human safety risks. Attractants and repellents provide behaviour-based pest management alternatives, especially in integrated pest control strategies.

Attractants and Repellents: Mechanisms and Applications:

Attractants

Insect attractants are derived from natural cues such as food, oviposition sites, and sex pheromones. For instance, **lysine**, a blood component, attracts mosquitoes. Protein

hydrolysates are also known to be broadly attractive to Diptera and are widely used in agricultural entomology. Moisture may enhance attraction to oviposition and feeding sites. However, attractants are underutilized in public health vector control.

Repellents

Early repellents, like **citronella and camphor oils**, function through volatile odors. Later synthetic compounds, such as **dimethyl phthalate**, challenged the belief that only high-volatility substances are effective. Although vapors do play a role, repellency is not solely olfactory. Common properties of repellents include **mucosal irritation**, **plasticizing behavior**, and sometimes **toxicity** to insects.

Repellents may act by:

- Blocking **olfactory receptors** or **feeding cues**
- **Inhibiting neural responses** related to feeding and reproduction
- Creating **irritation** or interfering with **receptor activity**

Their mechanisms remain partially understood, with theories including **infrared absorption** and **non-specific receptor inactivation**.

Applications and Future Directions:

Though underexplored, **attractants** have potential in vector control, trap enhancement, and as spray additives to counteract repellent effects of formulations. Repellents are widely used on **skin and clothing** and may also be applied in **space** or **airflow systems** where dermal application is not feasible.

Improving repellents depends on understanding their **site- and stage-specific actions**—from preventing approach (olfactory), deterring landing (chemoreception), to blocking feeding (gustatory). These insights could enable tailored formulations targeting each stage of insect-host interaction.Orally active repellents remain largely ineffective in mosquitoes, though promising for other pests like fleas. Behavioral resistance—such as altered host preference—can arise with long-term repellent use, highlighting the importance of integrated pest management strategies.

Conclusion

Both attractants and repellents represent critical tools in pest control, with potential to reduce reliance on conventional insecticides and help combat resistance development. Their rational application depends on deeper understanding of insect sensory biology and chemical interactions.

Probable Questions:

1. Classify pesticides according to the types of pests they kill.
2. Differentiate biodegradable and non biodegradable pesticides.
3. What is Biopesticides? What are the uses of it?
4. What are the effects of pesticides?
5. What is anti feedant? How it is used?
6. What is fumigant? How it is used?
7. Discuss Attractants and Repellents pesticides.
8. Discuss different types of fumigants.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-VIII

Safer pesticides: Next generation molecules to be used as pesticides for plant protection and their chemistry

Objective: In this unit we will discuss about next generation pesticides which have less impact on environmentand also on human health.

Introduction and Characteristics of Modern Pesticides:

Pesticides continue to be widely used across the globe. In 2013, EU pesticide sales totaled nearly 360,000 tonnes, with Spain, France, Italy, Germany, and Poland accounting for over 70% of the total. However, the discovery and commercialization of new pesticides have significantly slowed due to complex, expensive, and time-consuming approval procedures. On average, only 1 in 70,000 synthesized or isolated compounds reaches market readiness, with development costs averaging €90 million and a time frame of about nine years.

Modern or new-generation pesticides aim to replace older, more toxic compounds to reduce environmental and human health impacts. Nevertheless, recent studies reveal that these newer pesticides are still frequently detected in surface waters, especially in intensive agricultural zones. For example, California stream studies found over 80% of samples contained multiple pesticide residues, with newer diamide insecticides being most prevalent and toxic, particularly to invertebrates.

The high development costs and regulatory hurdles have resulted in continued reliance on older pesticide groups such as macrocyclic lactones, chloronicotinyls, tetranordtriterpenoids, and others.

Pesticide Categories and Their Properties

- **Macrocyclic Lactones (e.g., Avermectins)**: Derived from *Streptomyces avermitilis*, these compounds, such as abamectin and ivermectin, are widely used antiparasitics. They are lipophilic, low in water solubility, degrade via photolysis and oxidation, and exhibit UV-sensitive isomerization.
- **Chloronicotinyls**: These systemic insecticides (e.g., imidacloprid, acetamiprid) act on cholinergic receptors and are effective at low doses. They degrade into 6-chloronicotinic acid in soil, with minimal persistence beyond three months.
- **Azadirachtin**: A natural limonoid from neem seeds, known for its eco-friendly insect antifeedant and growth-inhibiting effects. It is polar, UV-sensitive, degrades under heat and basic conditions, and consists of isomers, mainly type A.

- **Bipyridyliums (e.g., Diquat, Paraquat)**: Nonselective contact herbicides with high water solubility, stable in acidic media, and used for defoliation and desiccation. Their herbicidal activity arises from stable resonant structures.
- **Dinitroanilines**: Pre-emergence herbicides targeting grasses and broadleaf weeds. They inhibit microtubule assembly and are strongly adsorbed to soil colloids. Their degradation is faster under anaerobic soil conditions.
- **Chloroacetamides (e.g., Acetochlor, Alachlor)**: Widely used herbicides and fungicides in cereal and vegetable crops. They inhibit protein synthesis and RNA synthesis in susceptible organisms and are moderately water soluble.
- **Oximes**: Synthetic compounds used to manage glyphosate-resistant weeds. Examples include clethodim and sethoxydim, which inhibit fatty acid synthesis. These degrade quickly in light and acidic aqueous solutions.
- **Triazoles**: Used as herbicides, fungicides, and growth regulators. They are often chiral, with enantiomer-specific activities and toxicities. For instance, R-diniconazole is more fungicidal, while S-uniconazole promotes plant growth.
- **Pyridine-Based Molecules**: These include herbicides like pyridate, norflurazon, and fluridone. They vary in application (e.g., contact vs. soil-applied) and stability. Pyridate is stable in neutral media but degrades in strong acids or bases.

Toxicology:

Toxicological Aspects of Select Pesticide Classes

Pesticide residues are regulated based on their toxicity and potential human exposure. Among them, macrocyclic lactones act as neurotoxins by modulating ã-aminobutyric acid (GABA) pathways, with selective activity due to the mammalian blood–brain barrier. Despite their low LD50, poor dermal absorption limits their toxicity through skin exposure. Avermectin B1a enhances GABA binding and is metabolized into hydroxymethyl or desmethyl derivatives, excreted mostly unchanged. However, compounds like abamectin are teratogenic, and low doses can induce neurological symptoms in mammals.

1. Chloronicotinyl insecticides (e.g., imidacloprid) act on postsynaptic nicotinic acetylcholine receptors. Though they show low toxicity to vertebrates, metabolites like olefin and nitrosimine are more toxic to insects and persistent in soil, depending on environmental factors. They are used systemically as contact and stomach poisons.
2. Azadirachtin, a biopesticide from neem, is ecofriendly and non-toxic to mammals. It interferes with insect development by disrupting molting, feeding, and reproduction.

3. PQ (paraquat) primarily targets the lungs, generating superoxide radicals through redox cycling that damage membranes and deplete NADPH. DQ (diquat) shows nephrotoxicity and causes cataracts but lacks pulmonary toxicity. PQ is also teratogenic, disrupting collagen synthesis.
4. Growth regulators like chlormequat (CQ) and mepiquat (MQ) limit shoot elongation but can disrupt reproduction and inhibit cholinesterase activity, leading to acetylcholine accumulation. MQ is considered slightly hazardous with established maximum residue limits.
5. Substituted anilines, including alachlor and acetochlor, can induce methemoglobinemia and are considered likely carcinogens. Alachlor induces tumors via non-genotoxic pathways; acetochlor acts by suppressing protein synthesis in weed seedlings.
6. Triazole fungicides are widely applied and are known endocrine disruptors. Lipophilic and persistent, they bioaccumulate and affect steroid metabolism, disrupting hormonal balance. Compounds like myclobutanil and triadimefon induce hepatic toxicity and reproductive effects in rodents.
7. Amitrole was reclassified by IARC due to insufficient evidence of human carcinogenicity despite its ability to induce thyroid tumors in rodents by disrupting thyroid peroxidase.
8. Oximes like clethodim inhibit acetyl CoA carboxylase and are used for selective postemergence weed control. Though generally effective, some like sethoxydim may cause hepatic and bone marrow damage.
9. Pyridine-based pesticides such as fluoridone and chloridazon have shown cytotoxic effects and interfere with nerve membrane function. Their usage has declined due to safety concerns.

Probable Questions:

1. What are the needs of introduction of new generation safer pesticides?
2. What are the characteristics of safer pesticides?
3. What are the toxicological evaluations of safer new generation pesticides?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis

UNIT-IX

Metabolism of Pesticides: Phase I and Phase II reactions and metabolism of pesticides

Objective: In this unit we will discuss about how pesticides are metabolized. We will discuss Phase I and Phase II reactions and metabolism of different pesticides.

1. Introduction and Historical Context Human activities have introduced over one billion pounds of toxins annually into the environment. Of approximately 6 million synthesized chemicals, 60,000–95,000 are commercially used, including pesticides. Initially derived from natural compounds (e.g., sulfur, arsenic), pesticide use expanded rapidly post-WWII with chemicals like DDT and 2,4-D due to their effectiveness and affordability. Despite their benefits, excessive and indiscriminate application has led to environmental and health issues, resistance in pests, and contamination of non-target ecosystems.

2. Classification and Usage Trends Pesticides include herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides, and plant growth regulators. They protect crops, reduce losses (e.g., 78% in fruits without pesticides), and enhance productivity. Classification based on chemical structure aids in correlating toxicity, persistence, and degradation. Global usage exceeds 4 million tons annually, with herbicides being the dominant class. Asia, Europe, China, the US, and Brazil are major consumers. Pesticides are most widely applied in fruit, vegetable, and maize cultivation.

3. Environmental and Health Impacts Pesticides degrade, volatilize, or leach into water and soil, rarely reaching their intended targets. Persistent residues bioaccumulate, disrupt food chains, and contribute to pest resistance. Environmental effects include:

- Decline of phytoplankton and zooplankton
- Neurotoxicity, carcinogenicity, and reproductive harm in wildlife
- Pollinator decline and alteration of nutrient cycles
- Water body contamination via leaching and soil adsorption

Public health impacts arise from acute occupational exposure and chronic ingestion via contaminated air, water, or food.

4. Obsolete Pesticides and Contamination Many countries hold large stocks of obsolete or banned pesticides, often stored or disposed improperly, posing risks to ecosystems and communities. Globally, over 500,000 tons of such chemicals remain, especially in Africa, Asia, and Latin America. In Mexico alone, over 26,000 liters and 147,000 kg of obsolete pesticides have been recorded, with extensive soil contamination and thousands of empty pesticide containers contributing to pollution.

5. Microbial Biodegradation of Pesticides Certain microorganisms, especially soil-dwelling bacteria and fungi, can degrade pesticides. Biodegradation is influenced by microbial physiology, environmental parameters (moisture, temperature, nutrient presence), and pesticide structure. Key genera include:

- **Bacteria**: *Pseudomonas*, *Rhodococcus*, known for degrading compounds like aroclor 1242 and triazines
- **Fungi**: *Aspergillus*, *Absidia*, *Fusarium*, *Botrytis*, capable of metabolizing metribuzin, linuron, and metroburon

Mechanisms involve hydrolysis of chemical bonds (e.g., P-O, P-S, P-F) commonly present in organophosphates. Transformation products (e.g., nitrite, ammonia) suggest efficient degradation pathways.

6. Advancements in Microbial Tools and Omics Approaches Modern bioremediation integrates molecular biology and omics tools (genomics, proteomics, transcriptomics, metabolomics) to characterize degradation pathways and identify microbial consortia. These tools allow prediction and enhancement of biodegradative potential and environmental restoration strategies.

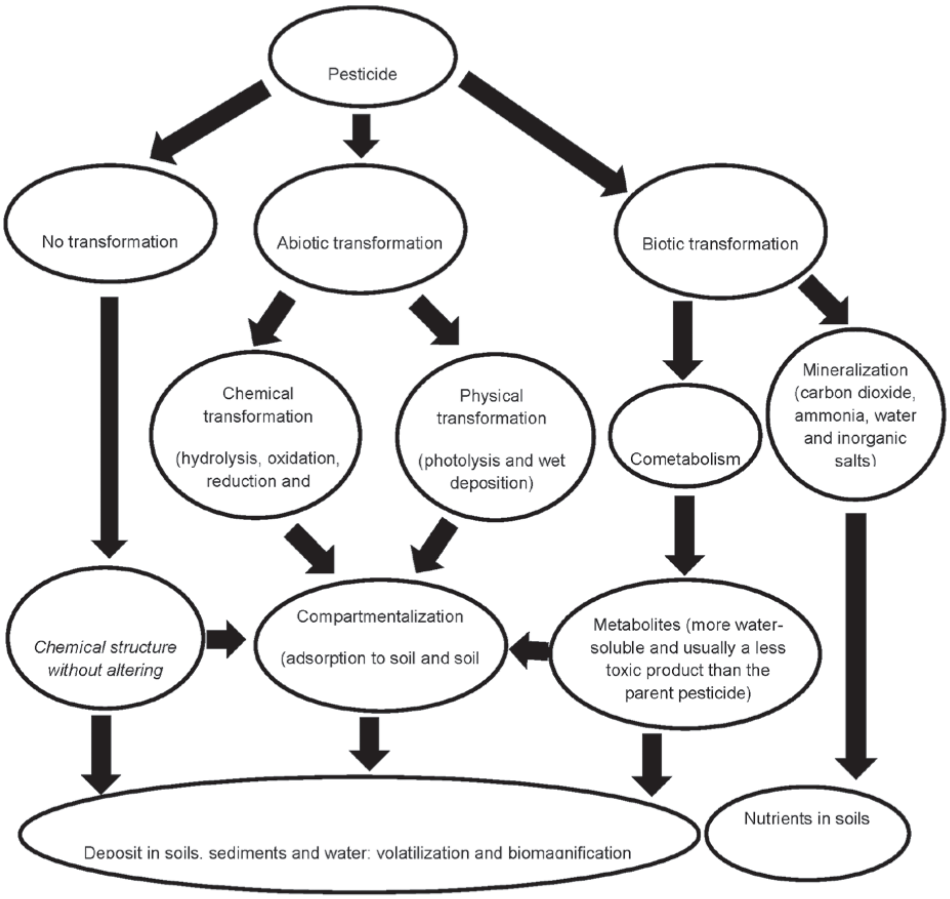


Figure 1. Fate of pesticides in the environment.

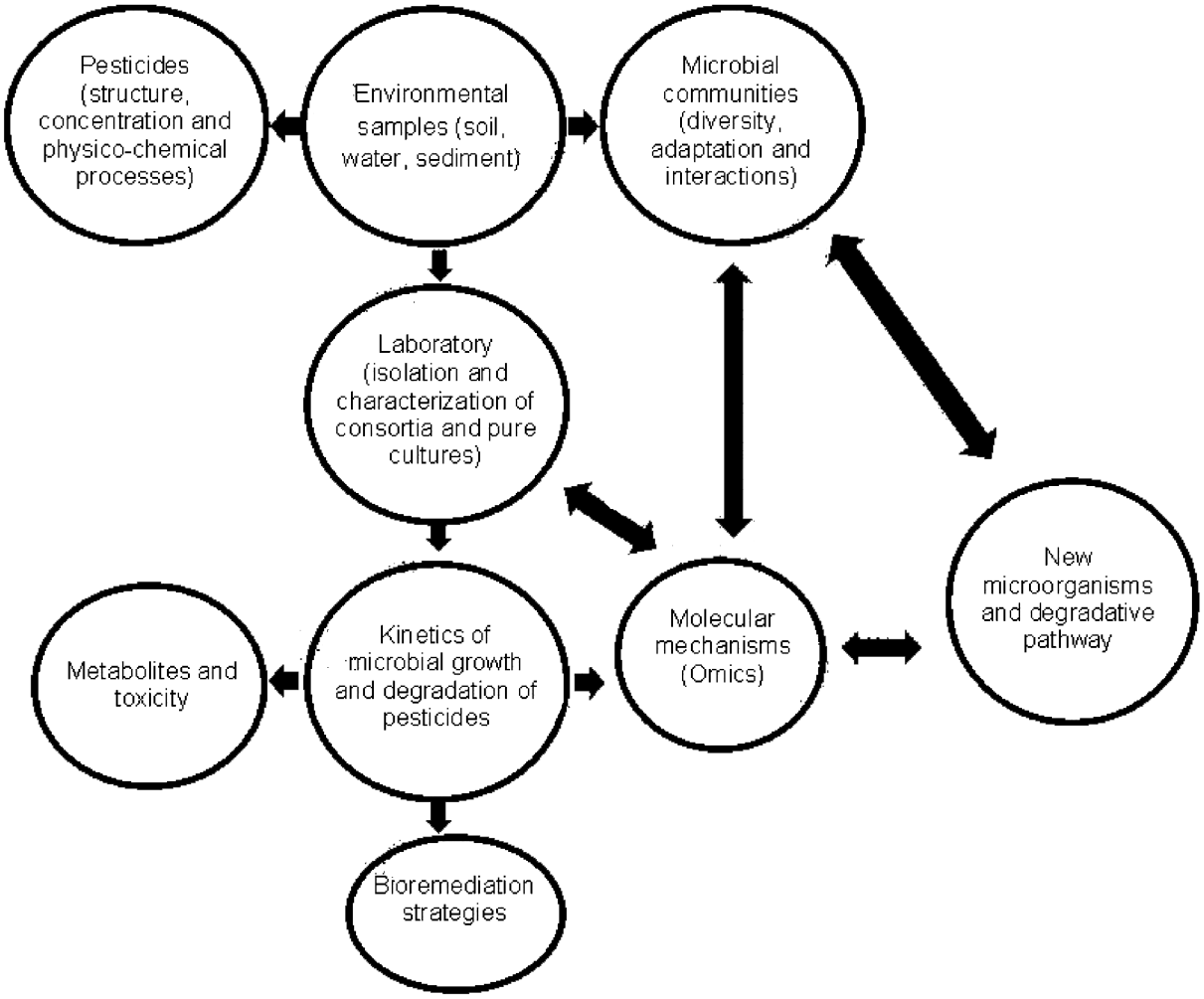


Figure 2. Representation of the relationships between pesticides, microbial communities, and the discovery of new biodegradation processes, Omics = high throughput-based characterization of biomolecules characteristic of bioprocesses; DNA, genomics; mRNA, transcriptomics; protein, proteomics; metabolites, metabolomics.

Biodegradation Mechanisms: A Microbial Approach to Pesticide Detoxification:

Biodegradation refers to the microbial breakdown of organic pollutants into simpler, often inorganic, non-toxic compounds. It is one of the most efficient, eco-friendly, and cost-effective strategies for removing environmental contaminants, particularly pesticides. This process is driven by microbial metabolism, which may be energy-generating, detoxifying, or a result of co-metabolism.Microorganisms—due to their vast diversity, abundance, and biochemical adaptability—play a central role in biodegradation. Their ability to

operate under aerobic and anaerobic conditions, including extreme environments, makes them valuable tools in pollutant removal. In nature, biodegradation is not performed by isolated species but through a cooperative microbial community that facilitates metabolic interactions—commonly referred to as **metabolic cooperation**.

Among microbial taxa, **bacteria, fungi, and actinomycetes** are predominant in pesticide degradation:

- **Fungi** generally initiate transformation by structurally modifying xenobiotics via extracellular enzymes, reducing toxicity and rendering compounds more accessible to bacterial degradation.
- **White-rot fungi**, in particular, secrete broad-spectrum ligninolytic enzymes like **laccases, manganese peroxidases, and lignin peroxidases**, which are highly effective in degrading complex organic pollutants.
- **Bacteria** employ diverse intracellular enzymatic systems to metabolize pesticides further.

Three major enzyme families dominate pesticide degradation:

1. **Hydrolases** (including **esterases**): Break down ester or amide bonds.
2. **Mixed Function Oxidases (MFOs)**: Catalyze oxidation-reduction reactions.
3. **Glutathione S-transferases (GSTs)**: Detoxify electrophilic compounds via conjugation with glutathione.

Biodegradation typically occurs in **three metabolic phases**:

- **Phase I**: Functionalization—modifies the parent pesticide via **oxidation, reduction, or hydrolysis**, making it more polar and water-soluble.
- **Phase II**: Conjugation—attaches the pesticide or its metabolite to **sugars or amino acids**, further enhancing solubility and reducing toxicity.
- **Phase III**: Sequestration—produces stable, non-toxic **secondary conjugates**.

These transformations are mediated by a wide array of enzymes, including **oxidases, oxygenases, dehalogenases, reductases**, and others capable of altering side chains, cleaving aromatic rings, or replacing functional groups. The choice of enzymatic route depends on both the chemical nature of the pesticide and the bioavailability of the compound in the environment.

Recent research highlights the importance of:

- **Omics technologies** (genomics, proteomics, metabolomics) in identifying key microbial degraders and understanding enzymatic pathways.

- **Transferases, hydrolases, isomerases, and ligases** in central metabolic pathways that facilitate integration of pesticide intermediates into core metabolism.

In summary, effective biodegradation of pesticides is contingent upon the metabolic potential of microbial communities, availability of catalytic enzymes, and environmental conditions that support microbial activity. Advancing this field holds promise for sustainable bioremediation and environmental restoration

1. **Hydrolases:** Hydrolases are key enzymes that catalyze the hydrolysis of pesticide bonds (e.g., esters, amides, thioesters, and halides) without requiring redox cofactors, making them ideal for bioremediation. They degrade pesticides like carbofuran, producing various less toxic metabolites.
2. **Phosphotriesterases (PTEs):** PTEs, among the most studied, hydrolyze organophosphate (OP) pesticides, mitigating their toxicity by preventing acetylcholinesterase inhibition. Encoded by the opd gene (e.g., in *Pseudomonas diminuta* and *Flavobacterium*), these enzymes target phosphoester bonds (P–O, P–F, etc.). Other related enzymes include organophosphorus hydrolase (OPH), methyl-parathion hydrolase (MPH), and HOCA. PTEs utilize a zinc-activated water molecule for nucleophilic attack on phosphorus, leading to detoxification.
3. **Esterases:** Esterases catalyze the hydrolysis of ester-containing pesticides (e.g., organophosphates, carbamates, pyrethroids). They convert esters into alcohols and acids and are classified under EC 3.1.1.1. Carboxylesterases (A- and B-type) differ in their active site residues (Cys or Ser) and their interaction with OPs. A-type hydrolyze via SH groups, while B-type form irreversible P=O bonds with Ser, inhibiting activity. These enzymes act as both detoxifying agents and protective sinks against pesticide toxicity.
4. **Oxidoreductases:** These enzymes mediate electron transfer between molecules and include oxidases and dehydrogenases. A notable case is the degradation of endosulfan, a persistent organochlorine pesticide, which undergoes oxidation or hydrolysis by microbial enzymes. Microbial strains (e.g., *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*) degrade endosulfan to metabolites like endosulfan diol and sulfate. While the diol is less toxic, the sulfate metabolite is persistent and ecotoxic. Enzymes like monooxygenases (e.g., Ese, Esd) in *Arthrobacter* use reduced flavin for oxidation, particularly under sulfur-limited conditions. Hydrolysis can also occur abiotically under alkaline pH.
5. **Mixed Function Oxidases (MFOs):** MFOs (EC 1.14.14.1), especially cytochrome P450s, incorporate one oxygen atom into substrates and reduce the other to water, requiring NADPH and O_2. The P450 system includes over 200 isozymes with broad substrate specificity, ideal for degrading stable pesticides. P450s, with

an iron-containing heme group, oxidize recalcitrant bonds (e.g., C-H) and are active in detoxification processes across organisms. However, their dependence on cofactors limits use outside living systems. In insects, MFOs regulate detoxification, growth, and reproduction, metabolizing diverse pesticide classes.

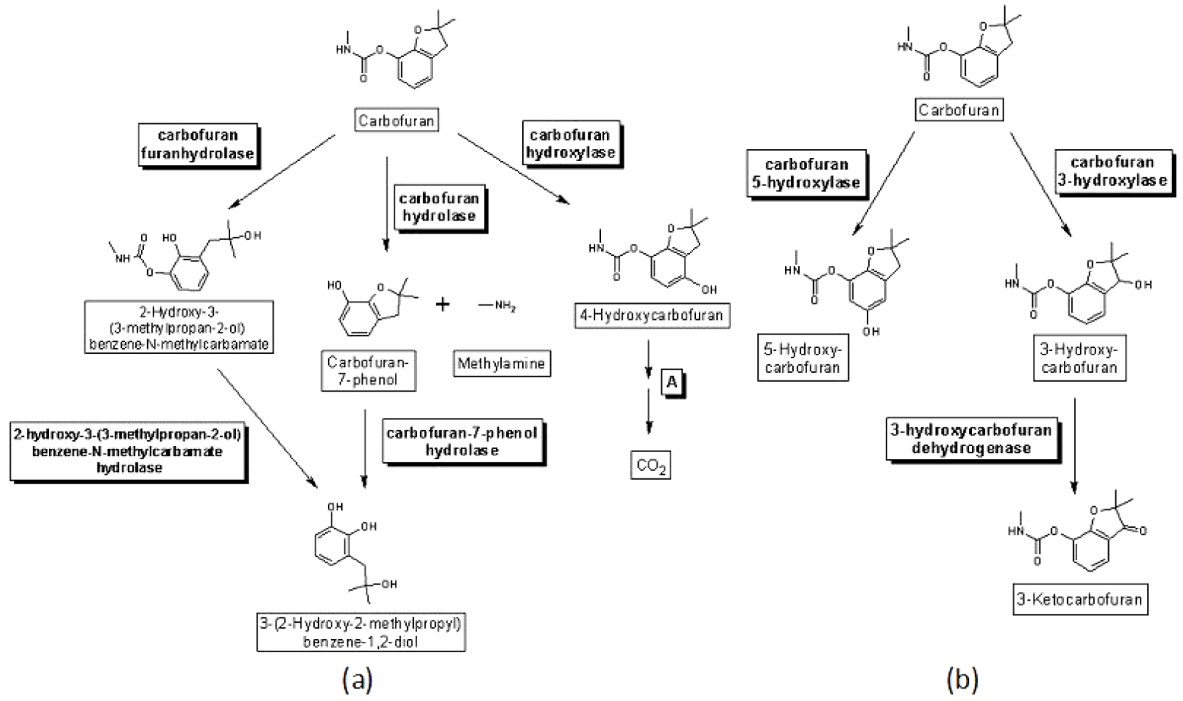
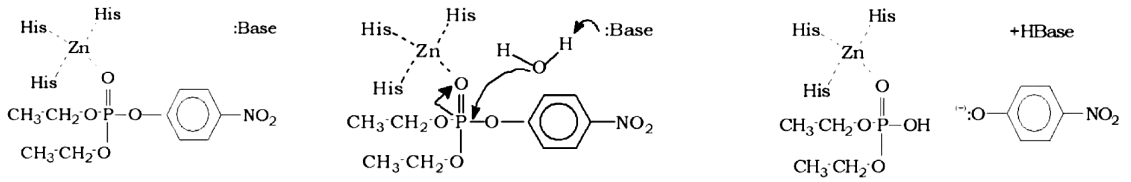


Figure 3. Degradation pathway of carbofuran. In a) several bacteria are involved in the hydrolysis of metabolites and b) fungal degradation of carbofuran may occur via hydroxylation at the three position and oxidation to 3-ketocarbofuran



1) Zinc helps positioning the enzyme with the substrate

2) The base residue deprotonates the water activating it for a nucleophiles attack to the central atom of phosphorous

3) A strong negative charge in the oxygen atom from the ester link leads to the rupture and loss of the p-nitrophenol

Figure 4. Proposed mechanism for PTE activity. Zinc's active site functions in phosphate polarization, making phosphor more susceptible to the attack. 1) A base subtracts a proton from a water molecule with the subsequent attack of the hydroxyl to the central phosphorous. 2) The intermediary complex originates the products 3) p-nitrophenol and diethyl thiophosphate [6].

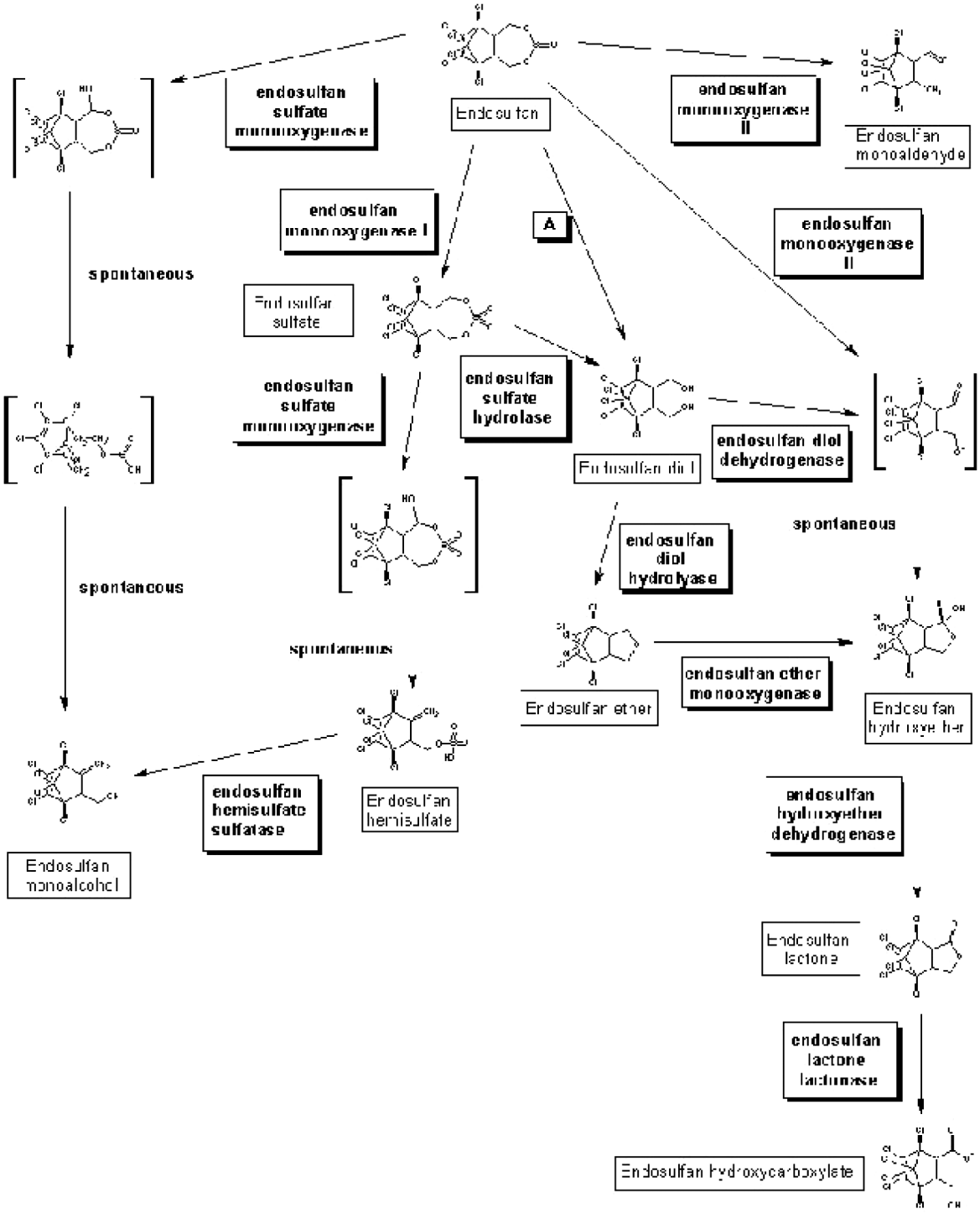


Figure 5. Degradation pathway of endosulfan

6. Glutathione S-Transferase (GST)

The GSTs (EC 2.5.1.18) are a group of enzymes that catalyze the conjugation

of hydrophobic components with the tripeptide glutathione (Figure 6). In this reaction, the thiol group of glutathione reacts with an electrophilic place in the target compound to form a conjugate which can be metabolized or excreted, and they are involved in many cellular physiological activities, such as detoxification of endogenous and xenobiotic compounds, intracellular transport, biosynthesis of hormones and protection against oxidative stress.

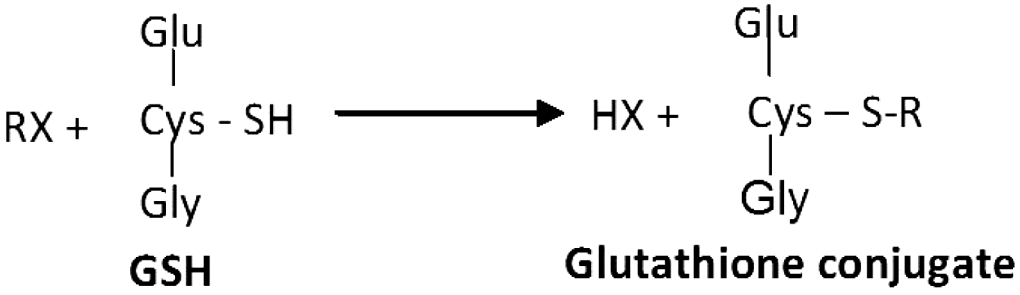


Figure 6 Representation of the conjugation reaction catalyzed by glutathione S-transferase (GST).

Genetics and Genomic Strategies for Pesticide Biodegradation

Microbial degradation of pesticides is governed by specific catabolic genes, often located on chromosomes, plasmids, or transposons. The advancement of metagenomics and genome sequencing has expanded access to both culturable and nonculturable microbes, enabling the identification of novel genes and regulatory elements involved in pesticide breakdown. Mobile genetic elements, especially plasmids and transposons, have been found to encode crucial degradation enzymes, such as organophosphorus hydrolase (OPH; *opd* gene) and methyl-parathion hydrolase (MPH; *mpd* gene). These genes are often conserved and have been detected across diverse bacterial strains.

Genetic Engineering and Detoxification

Recombinant DNA technology has accelerated microbial adaptation to pollutants through gene amplification, pathway optimization, and insertion of heterologous genes. Genetically engineered strains, including *E. coli*, *P. pseudoalcaligenes*, and *Yarrowialipolytica*, have been developed to express degradation enzymes. Directed evolution has further improved enzyme efficiency, such as OPH variants exhibiting significantly enhanced hydrolysis rates for chlorpyrifos and methyl parathion.

Metagenomics and Functional Genomics

Metagenomics accesses unculturable microbial diversity by analyzing environmental DNA, revealing previously unknown degradation genes. Functional genomics, including transcriptomics and proteomics, elucidates gene functions and regulatory networks involved in biodegradation. These technologies, combined with metabolic engineering, enable rational design of microbial pathways for efficient pesticide remediation.

Cell Immobilization Techniques

Cell immobilization enhances pesticide degradation by maintaining high cell density, improving resistance to environmental stress, and enabling reuse. Methods include physical entrapment in polymeric gels or adhesion via biofilm formation. Supports such as alginate, agarose, cellulose, and volcanic rock (e.g., tezontle) facilitate immobilization. Biofilms form structured microbial communities with enhanced resilience and metabolic activity, regulated via quorum sensing.

Natural carriers like loofa sponge (*Luffa cylindrica*) offer high porosity, mechanical strength, and biocompatibility for microbial immobilization. These matrices enable efficient degradation of xenobiotics, including organophosphates, and are reusable and environmentally friendly.

Conclusion

Understanding the genetic and enzymatic basis of pesticide degradation is crucial for designing effective bioremediation strategies. Techniques like genetic engineering, functional genomics, and cell immobilization offer robust tools for in situ remediation, reducing environmental and health risks from pesticide contamination.

Probable Questions:

1. Briefly discuss how microorganisms help in biodegradation of pesticides.
2. What is the role of hydrolases in degradation of pesticides?
3. What is the role of Phosphotriesterases in degradation of pesticides?
4. What is the role of Esterases in degradation of pesticides?
5. What is the role of Oxidoreductases in degradation of pesticides?
6. What is the role of Mixed Function Oxidases in degradation of pesticides?
7. What is the role of Glutathione S-Transferase in degradation of pesticides?
8. How genetic engineering helps in degradation of pesticides?

9. What is metagenomics? How it is related to metabolism of pesticides?
10. What is functional genomics? How it is related to metabolism of pesticides?
11. Discuss case cell immobilization.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-X

Toxicological symptoms of Organochlorines, Organophosphorus, Carbamates, Pyrethroids, plant origin insecticides and other bioinsecticides

Objective: In this unit we will discuss about toxicological symptoms of various pesticides such as Organochlorines, Organophosphorus, Carbamates, Pyrethroids, plant origin insecticides and other bioinsecticides

Introduction:

Pesticide applicators should understand the hazards and risks associated with the pesticides they use. Pesticides vary greatly in toxicity. Toxicity depends on the chemical and physical properties of a substance and may be defined as the quality of being poisonous or harmful to animals or plants. Pesticides have many different modes of action, but in general cause biochemical changes which interfere with normal cell functions.

The toxicity of any compound is related to the dose. A highly toxic substance causes severe symptoms of poisoning with small doses. A substance with a low toxicity generally requires large doses to produce mild symptoms. Even common substances like coffee or salt become poisons if large amounts are consumed.

Toxicity can be either *acute* or *chronic*.

- **Acute toxicity** is the ability of a substance to cause harmful effects which develop rapidly following exposure, i.e. a few hours or a day.
- **Chronic toxicity** is the ability of a substance to cause adverse health effects resulting from long-term exposure to a substance.

There is a great range in the toxicity of pesticides to humans. The relative hazard of a pesticide is dependent upon the toxicity of the pesticide, the dose and the length of time exposed. The hazard in using a pesticide is related to the likelihood of exposure to harmful amounts of the pesticide. The toxicity of pesticides can't be changed but the risk of exposure can be reduced with the use of proper personal protective equipment (PPE), proper handling and application procedures.

Pesticide Toxicity: Some pesticides are dangerous after one large dose (acute toxicity). Others can be dangerous after small, repeated doses (chronic toxicity).

Measuring Acute Toxicity (LD_{50} And LC_{50} Values): Acute toxicity of a pesticide refers to the effects from a single dose or repeated exposure over a short time (e.g. one day),

such as an accident during mixing or applying pesticides. Acute toxicity is measured by LD_{50} and LC_{50} values. The LD_{50} value is the amount of pesticide (lethal dose) which kills 50% of the test animals. These treatments are through the skin (dermal) or through the mouth (oral). These values are given in milligrams per kilogram of body weight of the animal (mg/kg body wt.). A pesticide with a lower LD_{50} is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the test animals. The LC_{50} value is a measure of the toxicity of a pesticide when test animals breathe air mixed with pesticide dust, vapours or spray mist. The LC_{50} is the concentration of pesticide which is lethal to 50% of a population of test animals and is usually determined for a specific exposure period (e.g. inhalation for 4 hours). The length of exposure is important because shorter exposure periods generally require higher pesticide concentrations to produce toxic effects. LC_{50} values for pesticides in air are expressed as the ratio of pesticide to air, in parts per million (ppm) or parts per billion (ppb). LC_{50} values are also determined for fish and aquatic organisms based on the concentration of pesticide in water.

Important characteristics to note about LD50 and LC50 values:

- they are based on a single dose (LD_{50}) or short exposure (LC_{50});
- they do not indicate cumulative effects of small doses;
- they are an indicator of the amount of chemical required to kill or severely injure animals, and do not indicate the amount of chemical causing less severe toxic effects.

Chronic Toxicity

Chronic toxicity refers to the effects of long-term or repeated lower level exposures to a toxic substance, such as when a pesticide applicator is frequently wetted with spray during unsafe spray practices. The effects of chronic exposure do not appear immediately after first exposure and may take years to produce symptoms. Pesticides which have a tendency to accumulate, or which break down slowly in body tissues, usually represent the greatest chronic exposure hazard. Someone who is frequently exposed to low doses of such pesticides may develop symptoms of poisoning long after the first exposure. Chronic exposure may include chronic oral, chronic dermal or chronic inhalation poisoning. Very few pesticides now in use are known to cause chronic effects, if used according to label directions. However, a few pesticides are suspected or known to cause chronic illness in test animals or humans when exposure levels are high. The registration of some pesticides has been cancelled because the suspected or identified chronic effects represented a significant health hazard.

Exposure

There are three ways in which pesticides can enter the human body:

1. through the skin or eyes (dermal),
2. through the mouth (oral) and
3. through the lungs (respiratory or inhalation).

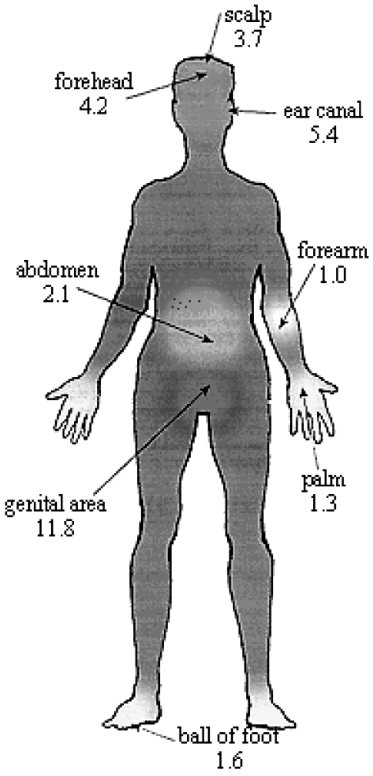
Dermal Exposure

In typical work situations, skin absorption is the most common route of pesticide poisoning. Absorption will continue as long as the pesticide remains in contact with the skin. The rate of absorption is different for each part of the body (see diagram). The head (especially the scalp and ear canal) and the genital areas are particularly vulnerable. Absorption may occur as a result of a splash, spill or drift when mixing, loading or applying a pesticide. Applicators may also be exposed to residues on application equipment, protective clothing or treated surfaces after pesticide application. Following exposure, residues can also be transferred from one part of the body to another. A cut or skin abrasion can greatly increase pesticide absorption.

The dermal toxicity of a pesticide depends on the pesticide formulation, the area of the body contaminated and the duration of the exposure. In general, liquids are more easily absorbed through the skin than wettable powders or granules. The hazard from skin absorption increases when workers are mixing pesticides because they are handling concentrated pesticides that contain a high percentage of active ingredient.

Protect yourself from dermal exposure. Follow these guidelines:

1. Wear protective clothing and equipment when using pesticides or repairing contaminated equipment.
2. Spray during periods when there is little or no wind.
3. Do not re-enter a sprayed field without protective clothing until the re-entry time has elapsed.
4. If your clothes become contaminated, change immediately. Wash affected areas of the skin.
5. Change clothes as part of the clean-up after pesticide use at the end of the day.
6. Wash and shower after using pesticides.
7. Wear clean clothes at the start of each day during pesticide application.



Relative absorption rates, as compared to the forearm (1.0)

Eye Exposure

The tissues of the eyes are particularly absorbent. Enough pesticide can be absorbed through the eyes to result in serious or fatal poisoning. In addition, some pesticides may cause chemical injury to the eye itself. Eye protection is needed when measuring or mixing concentrated or highly toxic pesticides. Protective face shields or goggles should be worn whenever there is a chance that pesticide sprays or dusts may come in contact with the eyes.

Protect yourself from eye exposure. Follow these guidelines:

1. Always wear eye protection when you measure or mix pesticides.
2. Always wear eye protection when pesticide sprays or dusts may contact your eyes.
3. Do Not wipe your eyes with contaminated gloves or hands.
4. Be prepared to respond to accidental eye exposure quickly

Oral Exposure

Pesticides taken through the mouth result in the most severe poisoning, compared to other types of exposure. Pesticides can be ingested by accident, through carelessness, or intentionally. The most frequent cases of accidental oral exposure are those in which pesticides have been stored in an unlabelled bottle or food container. There are many cases where people, especially children, have been poisoned by drinking pesticides from a soft drink bottle. People have also been poisoned by drinking water stored in contaminated containers. Workers handling pesticides or application equipment can also consume excessive levels of pesticides if they do not wash their hands before eating or smoking.

Protect yourself from oral exposure. Follow these guidelines:

1. Always store pesticides in their original labeled containers.
2. Never put pesticides in an unlabelled bottle or food container.
3. Never use your mouth to clear a spray hose or nozzle, or to begin siphoning a pesticide.
4. Always wash after handling pesticides and before eating, drinking, smoking, or using the toilet.
5. Never leave pesticides unattended.
6. Avoid splashes or dusts when mixing pesticides.
7. Label your pesticide measuring containers.

Respiratory Exposure

Inhalation of pesticides can lead to serious health effects due to rapid absorption through lung tissues. Fine particles, aerosols, and vapours pose the highest risks, especially during mixing of concentrated powders or use of high-pressure, ultra-low volume (ULV), or fogging equipment. These methods generate fine droplets that remain airborne and may travel significant distances via air currents.

Vapour hazards increase with temperature and are especially high in enclosed or poorly ventilated spaces, such as greenhouses or storage areas. Fumigants, designed to act through vapours, pose the greatest risk, but even non-fumigant formulations may emit harmful vapours. Some pesticides produce strong odours or cause irritation, acting as warning signs, while others remain undetectable, increasing the risk of inadvertent exposure.

Precautionary Guidelines

- **Always wear a properly fitted respirator**:
  - If required on the label;
  - When handling pesticides in confined or poorly ventilated areas;
  - If inhalation of vapours, sprays, or dusts is possible.
- **Avoid early re-entry** into treated areas; follow label-specified re-entry intervals.
- **Ensure proper ventilation** in greenhouses or enclosed areas post-application.
- **Do not apply pesticides in temperatures above 30°C**, as heat enhances vapour release.

Key Concept

The **toxicity of pesticides** depends on exposure route (dermal, oral, or respiratory), **concentration**, and **duration**. Prolonged exposure significantly elevates health risks, necessitating stringent respiratory protection and environmental control during pesticide application.

PesticideToxicity

Pesticide toxicity to people can be measured severalways, although it is not easy, since humans cannot be used as test subjects. Because of this, other animals, such as rats, are used. If a pesticide is poisonous to rats, however, it is not necessarily poisonous to dogs, cows, wildlife, or people.

Toxicity studies are only guidelines: they are used to estimate how poisonous one pesticide is compared with another. Some pesticides are dangerous in one large dose or exposure, which is known as acute toxicity. Others can be dangerous after small, repeated doses, called chronic toxicity.

Measuring toxicity: The LD_{50} (lethal dose, 50 percent) describes the dose of pesticide that will kill half of a group of test animals (rats, mice, or rabbits) from a single exposure or dose by a dermal, oral, or inhalation route. The LD_{50} is the dose per unit of body weight, such as milligrams per kilogram (mg/kg). A pesticide with a lower LD_{50} is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the testanimals. For example, a pesticide with an LD_{50} of 10mg/kg is much more toxic than a pesticide with an LD_{50} of 1,000 mg/kg. The toxicity of fumigant pesticides is described in terms of the concentration of the pesticide in the air, LC_{50} (lethal concentration, 50 percent). Researchers use a similar system to test the potential effects of pesticides on aquatic organisms in water.

Acute toxicity of a pesticide refers to the effects from a single exposure or repeated exposures over a short time, such as an accident when mixing or applying pesticides. Various signs and symptoms are associated with acute poisonings. A pesticide with a high acute toxicity can be deadly even if asmall amount is absorbed. Acute toxicity can be measured in terms of oral, dermal, or inhalation.

Chronic toxicity refers to the effects of long-term or repeated low-level exposures to a toxic substance.The effects of chronic exposure do not appear immediately after the first exposure: years may pass before signs and symptoms develop. Possible effects of long-term exposure to some pesticides include:

- cancer, either alone or in combination with other chemicals;
- genetic changes;
- birth defects in offspring following exposure of the pregnant female;
- tumors, not necessarily cancerous;
- liver damage;
- reproductive disorders;
- nerve damage;
- interfering with the endocrine system (hormones and glands that regulate many body functions); and
- sensitivity or allergic reactions such as irritation of the skin and/or respiratory tract.

Signal Words

Nearly all pesticides are toxic at some dose. They differ only in the degree of toxicity. All pesticides are potentially dangerous to people who have had excessive exposure. The label of a pesticide product will have one of three signal words that clearly indicates the degree of toxicity associated with that product (*Table I*). The signal word indicates the degree of risk to a user, not the effectiveness of the product in controlling the target pest. The signal word “Caution” is not required to appear on the label of a relatively nontoxic pesticide, but is required for slightly toxic pesticides.

Recognizing Signs and Symptoms of Poisoning

Anyone who may be exposed to pesticides or is working with someone who may be exposed should be aware of thesigns and symptoms of pesticide poisoning. Signs, such as vomiting, sweating, and pinpoint pupils, can be observed by others. Symptoms are any changes in normal condition that can be described by the victim of poisoning, including nausea, headache, weakness, dizziness, and others. Knowing these signs and

symptoms will allow for prompt treatment and help prevent serious injury. People who are frequently involved with pesticides should become familiar with the following important steps.

1. Recognize the signs and symptoms of pesticide poisoning for those pesticides commonly used, or to which people may be exposed. Often, pesticide poisoning resembles flu symptoms.
2. If you suspect poisoning due to a pesticide, get immediate help from a local hospital, physician, or the nearest Poison Control Center.
3. Identify the pesticide to which the victim was exposed, giving the chemical name and Environmental Protection Agency (EPA) registration number found on the label, if possible. Provide this information to medical professionals.
4. Have a copy of the pesticide label available when medical attention begins. The label provides useful information to those assisting a victim of pesticide poisoning. The Safety Data Sheet (SDS) has helpful information as well; supplying the SDS to medical professionals is required when the Worker Protection Standard applies.
5. Know emergency measures you can undertake until help arrives or the victim can be taken to the hospital. Both first aid and medical treatment procedures are listed on the product label.

Recognizing Common Pesticide Poisonings

Pesticides from the same chemical group usually affect the body similarly, though the severity depends on their concentration, formulation, toxicity, and exposure route (inhalation, skin, or ingestion). Recognizing the type of pesticide and its associated symptoms is crucial for early detection and treatment.

High-risk pesticide groups with known modes of action and symptoms are categorized together. Even unregistered or outdated pesticides can still pose risks if stored.

The **Agricultural Health Study (AHS),** involving 90,000 applicators and spouses from Iowa and North Carolina. The AHS states that the study "began in 1993 with the goal of answering important questions about how agricultural, lifestyle and genetic factors affect the health of farming populations. The study is a collaborative effort involving investigators from the National Cancer Institute, the National Institute of Environmental Health Sciences, the Environmental Protection Agency, and the National Institute for Occupational Safety and Health." The AHS relies mainly on participant memory to determine dose-related exposures. Also, keep in mind that an association does not automatically mean there is a cause-and-effect relationship. An association shows that more research is needed.

Some general findings of the AHS are listed below.

- Farmers have lower rates of many diseases compared with the rest of the population, perhaps because they are less likely to smoke and are more physically active.
- Farmers have a higher risk for developing some cancers, including prostate cancer.
- Gloves matter. Use of chemical-resistant gloves can reduce pesticide exposure 50 to 80 percent.
- Accidental high pesticide exposure events may affect health later in life.

Insecticides

Insecticides have many different modes of action. Some act on the insect's nervous system. Others slow the production of energy that an insect needs to survive. Another type slows or stops production of chitin, a major component of an insect exoskeleton, so the insect can't molt. Insect growth regulators, another type, also may prevent an insect from molting or keep it from maturing and reproducing. Some insecticides disrupt the water balance in an insect, causing rapid water loss and eventual death. Modes of action involving the nervous system and energy production may affect not only insects, but other animals as well. Insecticides such as insect growth regulators typically are specific to insects. The following is a list of insecticides grouped by their chemical makeup.

Insecticide Poisoning: Organophosphates, Carbamates, Organochlorines, and Pyrethroids

Organophosphates and Carbamates: These insecticides inhibit acetyl cholinesterase, an enzyme vital for nervous system function, leading to uncontrolled nerve impulses. Symptoms appear quickly—within minutes to hours—and include headache, dizziness, nausea, blurred vision, muscle twitching, and slowed heartbeat. Severe cases may result in unconsciousness, seizures, and incontinence. Risks are elevated for individuals with respiratory or neurological issues, and alcohol may exacerbate symptoms. Antidotes are available, making early diagnosis and treatment critical.

Organochlorines: Though largely banned, organochlorines like DDT and lindane persist in the environment. They disrupt nerve function and may cause nausea, dizziness, twitching, seizures, and unconsciousness. Chronic exposure has been linked to cancer, birth defects, and endocrine disorders. These chemicals can still be found in old storage and industrial waste. No antidotes exist—treatment involves immediate decontamination and supportive care. Vomiting should not be induced in unconscious individuals.

Pyrethroids: Synthetic analogs of natural pyrethrins, pyrethroids target insect nerves without inhibiting cholinesterase. They are widely used and pose low risk via inhalation or skin contact. Human poisonings are rare but may cause skin irritation, tingling, allergic reactions, or mild neurological effects like tremors and vomiting if ingested in large amounts. Pyrethroids are generally excreted efficiently by the kidneys.

Biological Insecticides

Insecticides produced from plant materials or bacteria are called biological insecticides.

Azadirachtin, derived from the Neem tree, is an insect growth regulator that interferes with the insect molting process. For humans, exposure to azadirachtin causes slight skin and gastrointestinal irritation. Stimulation and depression of the central nervous system also have been reported.

Eugenol is derived from clove oil and is used as both an insect attractant and insecticide. In humans, exposure to skin or eyes can cause irritation and burns. Ingestion of extremely large doses may result in liver problems and coma.

Pyrethrum and pyrethrins. Pyrethrum is found in the flowers of *Chrysanthemum cinerariaefolium*. Crude pyrethrum is a dermal and respiratory allergen for people. Skin irritation and asthma have occurred following exposures. Refined pyrethrins are less allergenic, but appear to retain some irritant and/or sensitizing properties.

In cases of human exposure to commercial pyrethrum products, realize that other toxicants may be present and listed on the label. Synergists may be added to insecticide products to enhance the killing power of the active ingredi ent. Synergists such as piperonyl butoxide, discussed later, have low toxic potential in humans, but organophosphates or carbamates included in the product may have significant toxicity. Pyrethrins themselves do not inhibit the cholinesterase enzyme.

Rotenone is a naturally occurring substance found in several tropical plants. Until 2011, it was formulated as dusts, powders, and sprays for use in gardens and on food crops. The AHS showed a relationship between exposure to rotenone and the incidence of Parkinson's disease. More research is needed to reach any conclusions on the specifics of that relationship. Rotenone manufacturers have voluntarily stopped producing the pesticide for all uses except to manage undesirable fish species. Rotenone is now a restricted use pesticide.

Antibiotics include abamectin, *Bacillus thuringiensis* (Bt), spinosad, and streptomycin. These compounds are prac tically nontoxic to humans. In studies involving deliberate ingestion by human subjects, slight inflammation of the gut occurred. Antibiotic insecticides in the form of emulsifiable concentrates may cause slight to moderate eye

irritation and mild skin irritation due to the solvent carriers. Antibiotic pesticides are different from antibiotics taken by people to cure bacterial infections.

Inorganic Insecticides

Boric acid and borates. Boric acid, derived from borax and usually combined with an anti-caking agent, is commonly used to kill cockroaches. It can be harmful to humans if accidentally ingested, especially by children. Avoid inhaling the dust during application. The label may indicate that respiratory protection is required. Inhaled borax dust irritates the respiratory tract and causes shortness of breath. Borax dust is moderately irritating to skin. Infants have developed a red skin rash that most often affects palms, soles of the feet, buttocks, and scrotum in severe poisonings. The skin developed a "boiled lobster appearance" followed by extensive skin peeling.

Diatomaceous earth (DE) is mined from the fossilized silica shell remains of diatoms, which are microscopic sea animals. Labels may refer to this ingredient as silicon dioxide, or silicon dioxide from diatomaceous earth. DE is used commercially to control crawling insects, such as cockroaches, ants, and insects that infest grain. It is virtually nontoxic to humans. Avoid inhaling diatomaceous earth, however, as it can irritate eyes and lungs.

Silica gel is a nonabrasive, chemically inert substance used as a dehydrating agent because the small particles absorb moisture and oils. Avoid inhaling the dust. Some grades of diatomaceous earth contain small amounts of crystalline silica, known to cause a respiratory disease called silicosis, and cancer. The cancer risk depends on the duration and level of exposure. Pesticide-quality diatomaceous earth and silica gel are amorphous (non-crystalline), and do not cause silicosis or cancer.

Sulfur is moderately irritating to skin and has been associated with skin inflammation. Dust is irritating to the eyes and respiratory tract. If swallowed, it acts like a strong laxative.

Other Insecticides

Neonicotinoids were introduced in the 1990s. Chemically similar to nicotine, they have a lower toxicity to humans than do organophosphates and carbamates. Imidacloprid and thiamethoxam are used to control termites, turf insects, and some crop insects. Neonicotinoids are being studied for their risk to honeybees and other pollinators. Farm workers reported skin or eye irritation, dizziness, breathlessness, confusion, or vomiting after they were exposed to pesticides containing imidacloprid. Similar symptoms, along with increased heart and breathing rates, also were noted after a victim ingested a product containing imidacloprid; the victim suffered severe cardiac toxicity and death 12 hours after oral exposure.

Pyrazoles: Fipronil is a moderately toxic pyrazole that may cause mild irritation to the eyes and skin. It is used to control termites (Termidor®, Taurus™), cockroaches (Combat®, Maxforce®), certain insect pests of corn, and fleas and ticks of cats and dogs (Frontline®, Effipro®, PetArmor™). Lab animals exhibited reduced feeding, reduced urination, increased excitability, and seizures following a toxic oral dose. After ingesting fipronil, humans have reported sweating, nausea, vomiting, headaches, abdominal pain, dizziness, agitation, and weakness. Direct, short-term contact with skin can result in slight skin irritation. Inhalation or dermal contact while spraying fipronil for five hours may have caused head-ache, nausea, dizziness, and weakness. Symptoms developed two hours after spraying and then disappeared. The National Pesticide Information Center reports that signs and symp- toms from a brief exposure to fipronil generally improve and clear up without treatment.

Pyrroles: Chlorfenapyr (Phantom®, Pylon®) is the only active ingredient in this group. It is formulated to control ants, cockroaches, termites, and some insect and mite pests on fruits and vegetables. It is slightly toxic if swallowed or contacts skin, and can moderately irritate eyes and skin.

Tetronic acids: Spiromesifen is the sole active ingredient in this group. It is used to control mites and whiteflies on some vegetable crops (Oberon®) and ornamental trees (Forbid™, Judo™, Oberon®). No indication of eye irritation has been reported.

Tetramic acids: Spirotetramat (Kontos®, Movento®) is a systemic insecticide that controls a number of major sucking insects and mites that are pests of trees, vegetables, potatoes, and other plants. Some products with tetramic acids may cause moderate eye irritation. Prolonged or repeated skin contact may cause allergic reactions in some individuals.

Insect Growth Regulators:

Insect growth regulators (IGR) act on insects in different ways. Those that mimic juvenile hormones keep insects in immature stages and prevent insect reproduction. Chitin synthesis inhibitors prevent insects from molting and growing into adults. In general, IGRs are very low in toxicity and cause mild skin irritation with limited exposure. No human poisonings or adverse reactions in exposed workers have been reported.

Mosquito Repellents: Toxicological Overview and Safe Use

1. DEET (Diethyltoluamide):

Originally developed by the U.S. Army in 1946 and widely available since 1957, DEET is one of the most effective synthetic mosquito repellents. It is generally well tolerated

when applied to intact human skin, though prolonged exposure may lead to irritation, redness, rashes, or exacerbation of preexisting dermatological conditions. Ocular exposure results in significant irritation. If ingested, DEET may induce gastrointestinal symptoms like nausea and vomiting.

Severe skin reactions, including blistering and ulceration, have occurred particularly under hot, humid conditions when DEET was not washed off before sleeping. Rare neurotoxic effects (e.g., seizures) have been linked to inappropriate use, including ingestion or misuse contrary to product labeling.

Precaution in Children:

- Use only low-concentration formulations.
- Avoid applying on hands, face, or near mucous membranes.
- Not recommended for infants under 2 months (as per AAP).
- Monitor for behavioral changes post-application.

2. Picaridin:

A synthetic derivative structurally similar to a compound in black pepper, picaridin has been in use in the U.S. since 2005. It is considered safe for topical use, with minimal reports of skin or eye irritation. Animal studies at high doses suggest possible kidney toxicity, but inhalation toxicity is negligible. Current evidence does not indicate greater sensitivity in children.

3. Oil of Citronella:

Registered in 1948, citronella is a plant-based repellent commonly used in candles, sprays, and lotions. While its efficacy is variable, it poses minimal risk when used as directed. The main concern is potential skin irritation, warranting precautionary labeling. The EPA has deemed citronella products safe for all populations, including children, provided label instructions are followed.

Fumigants

Fumigants deliver the active ingredient to the target site in the form of a gas. Fumigants can completely fill a space, and many have tremendous penetrating power. They can be used to treat objects such as furniture, structures, grain, and soil for insects and other pests. Fumigants are among the most hazardous pesticide products to use, due to danger of inhalation.

Various fumigants produce differing physiological effects. Headache, dizziness, nausea, and vomiting are common early signs and symptoms of excessive exposure. Prompt medical treatment is critical with fumigant poisoning. After donning appropriate PPE, immediately move a victim of fumigant inhalation to fresh air. Keep the individual quiet

in a semi-reclining position even if initial signs and symptoms are mild. If breathing has stopped, give mouth-to-mouth or mouth-to-nose resuscitation. If the victim has no pulse, immediately give cardiopulmonary resuscitation (CPR) using chest compression. Some fumigant products, along with signs and symptoms of poisoning, are listed below.

Chloropicrin causes severe irritation of the upper respiratory tract, eyes, and mucous membranes. Symptoms of exposure include burning eyes, tearing, coughing, difficulty breathing, headaches, nausea, and vomiting. Chloropicrin may be a stand-alone fumigant or may be combined with other fumigants to increase their potency. Chloropicrin can cause eye irritation and tearing in concentrations as low as 0.15 ppm. Some fumigant formulations include small amounts as a warning agent to clear people from an area.

Sulfuryl fluoride (Vikane®) poisoning symptoms include depression, slowed walking pattern, slurred speech, nausea, vomiting, stomach pain, stupor, itching, numbness, twitching, and seizures. Inhalation of high concentrations may irritate the respiratory tract and may be fatal due to respiratory failure. Sulfuryl fluoride almost always is applied with chloropicrin, so the first signs of poisoning are often associated with severe irritation of the eyes and mucous membranes. Skin contact with gaseous sulfuryl fluoride normally poses no hazard, but contact with liquid sulfuryl fluoride can cause pain and frost- bite due to cold temperatures from rapid evaporation.

Phosphine fumigants, such as aluminum and magnesium phosphide (Phostoxin®, PhosFume®, Fumitoxin®, and FumiCel®) affect cell function in the liver and lungs. Mild exposure is signaled by a sensation of cold, chest pains, diarrhea, and vomiting. Exposures that are somewhat more serious will be evidenced by cough, tightness in the chest, difficulty breathing, weakness, thirst, and anxiety. Signs and symptoms of severe exposure include stomach pain, loss of coordination, blue skin color, pain in limbs, enlarged pupils, choking, fluid in the lungs, and stupor. Severe poisonings can lead to seizures, coma, and death.

Methyl bromide (Metabron, Meth-O-Gas®) affects the central nervous system, lungs, heart, and liver. People poisoned by methyl bromide experience the common signs and symptoms of fumigant poisoning along with abdominal pain, weakness, slurred speech, mental confusion, muscle twitching, and convulsions similar to epileptic seizures. Methyl bromide is corrosive to eyes; damage may have a delayed on- set after exposure. Some liquid fumigants cause skin injuries such as redness or blisters that rupture, leaving raw skin or deep ulcers.

Acrolein (Magnacide H®) is an extremely irritating gas used as an aquatic herbicide. Inhaling vapors causes irritation in the upper respiratory tract, which may lead to a buildup of fluids in and narrowing of the air passages. Acrolein is corrosive to the eyes. If ingested, it attacks the stomach lining, resulting in open sores and cell death. Contact with skin may cause blistering.

Dazomet (Basamid® G) is a granular soil fumigant. It is used to sterilize soil to eliminate weeds, nematodes, and soilborne diseases. Dazomet is highly toxic if swallowed and can be fatal. Frequent or prolonged exposure to skin can result in irritation or more serious skin problems for some individuals. Exposure to the eyes can cause irreversible eye damage. Inhalation can cause a variety of acute and chronic lung conditions, including local irritation, inflammation, fluid buildup, and lung disease.

Metam sodium (Vapam®) is a soil fumigant used to kill fungi, bacteria, weed seeds, nematodes, and insects. When combined with water, it produces a gas that is very irritating to respiratory mucous membranes, eyes, and lungs. Inhalation can cause severe respiratory distress, including coughing blood and frothy sputum. It can only be used outdoors, and precautions must be taken to avoid inhaling the gas.

Dichloropropene (Telone®) is very irritating to skin, eyes, and the respiratory tract. Inhalation may cause spasms of the bronchi, where air passes into lungs. Although limited data for humans exist, animals have experienced liver, kidney, and cardiac damage. Most dichloropropene products contain chloropicrin; severe irritation of the eyes and mucous membranes is an early sign of exposure. Apparently, risk for oral toxicity is low for humans unless large quantities of dichloro- propene are ingested.

Rodenticides

Rodenticides, intended to kill mammals, pose significant human health risks due to shared physiological targets. They are classified into:

First-generation anticoagulants – Require multiple ingestions; act by inhibiting blood clotting, causing death by internal bleeding over 5–7 days.

Second-generation anticoagulants – More potent; a single dose can be lethal. They persist longer in tissues, increasing risk of secondary poisoning in predators and scavengers consuming contaminated carcasses.

Non-anticoagulants – Act through neurotoxic or organ-specific mechanisms and were developed to control anticoagulant-resistant rodent populations.

Because rodents dwell near human habitats, accidental or indirect exposures are common. Proper handling and ecological consideration are essential to prevent unintended harm.

First-generation Anticoagulants

Coumarins are anticoagulants: they slow blood's ability to clot, and disrupt capillary and liver function. Examples include warfarin (Kaput® Mole Gel Bait and Mouse Blocks). The main signs and symptoms are nosebleeds, bleeding gums, blood in the urine, tar-

colored feces, and large irregular blue-black to greenish-brown spots on the skin. Vitamin K is an antidote.

Indandiones include chlorophacinone (Rozol®) and diphacinone (Ditrac®, d-CON® IX and XI, Kaput Pocket Gopher Bait and Prairie Dog Bait, Ramik®). Main signs and symptoms are similar to coumarin compounds, but some indandiones cause nerve, heart, and blood system damage in laboratory rats, leading to death before hemorrhage occurs. None of these signs and symptoms have been reported in human poisonings. Vitamin K is an antidote.

Second-generation Anticoagulants

Coumarins also may be second-generation anticoagulants, developed with increased toxicity. Examples include brodifacoum (Jaguar®, Talon®, WeatherBlok®), and bromadiolone (Contrac®, Maki®). The main signs and symptoms are nosebleeds, bleeding gums, blood in the urine, tar-colored feces, and large irregular blue-black to greenish-brown spots on the skin. Vitamin K is an antidote.

Non-anticoagulants

Benzenamines: Bromethalin (Tomcat® Mouse Killer), the only chemical in this class of rodenticide, acts on the central nervous system. Possible signs and symptoms of exposure to this compound include skin and eye irritation, headache, confusion, muscle twitching, convulsive seizures, and difficulty breathing. Bromethalin poisoning in dogs usually results in paralysis or convulsions, and sometimes, abdominal swelling or bloating.

Cholecalciferols. (Terad 3 Blox®, d-CON XVI and XVII). This rodenticide is an activated form of vitamin D, and affects the liver and kidneys. It causes elevated levels of calcium in the blood; rodents die due to problems such as blockages in the circulatory system. For humans, signs and symptoms include fatigue, headache, weakness, and nausea. This rodenticide has poisoned dogs and cats. A high dosage may cause death in humans. Labels caution against direct contact with skin; gloves are required when handling bait or retrieving carcasses.

Strychnine is not easily absorbed through the skin nor does it accumulate in the human body. When ingested, however, it acts on the central nervous system within 10 to 30 minutes. Convulsions also can occur. Treatment of strychnine poisoning is geared toward eliminating outside stimuli. If strychnine poisoning occurs, place the victim in a warm, dark room to reduce outside stimuli that trigger convulsions. Consequently, in the case of strychnine poisoning, bring medical help to the victim rather than transporting the victim to a medical center, because movement will trigger the convulsions.

Zinc phosphide causes severe irritation if ingested. It reacts with water and stomach juices to release phosphine gas, which enters the bloodstream and affects lungs, liver, kidneys, heart, and central nervous system. Zinc phosphide can be absorbed through skin, and inhaled from fumes. With repeated exposure, it accumulates in the body to dangerous levels. Signs and symptoms of mild zinc phosphide poisoning include diarrhea and stomach pains. In more severe cases, nausea, vomiting, chest tightness, coldness, loss of conscious- ness, coma, and death can occur from fluid buildup in lungs, and liver damage. No antidote for zinc phosphide poisoning exists. It is a slow-acting material, which allows time to get the victim medical assistance.

Herbicides

Herbicides kill weeds by affecting metabolic processes in plants. Therefore, risk to humans and other mammals is relatively low. Some herbicides, however, can pose a risk of poisoning if not handled according to label directions. Regardless of their chemical structure, the vast majority of herbicides often affect the human body in a similar way. In general, they can irritate the skin, eyes, and respiratory tract. Always read and follow label recommendations carefully to avoid any of these health risks. Herbicides that present the greatest potential health risks are covered in the next four sections.

Chlorophenoxy Herbicides

2,4-D and **MCPA** are examples of chlorophenoxy her bicides. These compounds are moderately irritating to skin and mucous membranes. Inhalation may cause a burning sensation in the nose, sinuses, and chest, which may result in coughing. Prolonged inhalation sometimes causes dizziness.

Stomach irritation usually leads to vomiting soon after ingestion. Victims may experience chest and abdominal pain and diarrhea. Headache, mental confusion, and bizarre behavior are early signs and symptoms of severe poisoning, which may progress to unconsciousness.

Arsenical Herbicides

Ansar®, Montar®, MSMA, and cacodylic acid are examples of arsenical herbicides. Arsenical herbicides (e.g., MSMA, cacodylic acid) are highly toxic and can cause acute poisoning within an hour of ingestion, often identifiable by garlic-scented breath and feces. Key symptoms include severe gastrointestinal distress (burning pain, vomiting, bloody diarrhea), neurological effects (headache, dizziness, seizures, coma), liver dysfunction (jaundice), hematological suppression, and death due to circulatory collapse within 1–3 days.Chronic exposure, especially through dermal contact, poses greater risk than acute

ingestion. Long-term effects include cancer, corneal thickening, hyperkeratosis, edema, nail discoloration or loss, alopecia, and mucosal pigmentation.

Other Herbicides

Endothall (Aquathol®) is commonly used as an aquat- ic herbicide or algaecide. It is irritating to skin, eyes, and mucous membranes. In one case, a man died after ingesting endothall. In this case, bleeding and swelling were noted in the gut and the lungs.

Sodium chlorate (Drexel®, Defol®) is used as a defoliant, nonselective herbicide, and soil sterilant. It is irritating to skin, eyes, and stomach. Even though sodium chlorate is poorly absorbed in the digestive tract, ingesting a large dose will cause severe poisoning. Irritation to the gut causes nau- sea, vomiting, and abdominal pain. Bluish skin sometimes is the only visible sign of poisoning. Dark brown blood and urine can indicate sodium chlorate poisoning.

Fungicides and Associated Human Health Risks

Fungicides are widely used in agriculture and domestic settings, but generally pose **low systemic toxicity** due to limited absorption and low mammalian toxicity. However, **skin irritation and allergic sensitization** can occur. Notably, the Agricultural Health Study (AHS) identified a potential link between fungicide exposure—especially to **benomyl, captan, chlorothalonil, maneb, and metalaxyl**—and **retinal degeneration**, particularly among orchard workers using high-exposure methods like **hand sprayers or foggers**.

Application Methods and Inhalation Hazards

Methods such as **Total Release Foggers (TRFs)** increase exposure risks. Improper use—like failing to vacate premises—has led to **respiratory, neurological, and dermal symptoms,** particularly in vulnerable groups (e.g., elderly, asthmatics). Despite their availability, **TRFs are not generally recommended** due to limited efficacy and significant risk.

First Response in Pesticide Poisoning

Prompt medical action is essential. Suspected pesticide poisoning should trigger **immediate contact with Poison Control Centers**. Key emergency steps include:

- Removing the victim from exposure.
- Flushing affected skin or eyes with water.
- Administering oxygen if needed.

Inducing vomiting (e.g., with syrup of ipecac) may be advised unless contraindicated—such as when the label warns against it, the patient has seizures, is unconscious, or ingested petroleum-based compounds.

Understanding **product labels**, exposure pathways, and proper emergency protocols is critical to minimizing health risks and improving outcomes in pesticide-related incidents.

Probable Questions:

1. Briefly discuss chronic toxicity.
2. What are the guidelines for avoiding dermal exposure to toxic chemicals?
3. What are the guidelines for avoiding oral exposure to toxic chemicals?
4. What are the guidelines for avoiding respiratory exposure to toxic chemicals?
5. What is pesticide toxicity?
6. How toxicity is measured?
7. Discuss Signs and Symptoms of Poisoning.
8. How Organophosphate and Carbamate Insecticides work?
9. How PyrethroidInsecticides works?
10. What is biological pesticides?
11. Discuss the mechanism of mosquito repellants.
12. How rodenticides are used for pest control?
13. How Fumigants are used in pest control?
14. How Herbicides are used?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-XI

Classification of hormones; general principles, nature of hormone receptors (cell surface receptors and intracellular receptors)

Objective: In this unit we will discuss about classification oof hormones, hormone receptors types and mode of action.

Definition of Hormones:

Hormones are chemical messengers (may be of proteins, lipids or amines), secreted from special cells of endocrine glands and maintain the physiological activities very specifically on target cells through circulation and disintegrated after action.

Characteristics of Hormones:

The hormones possess the following specific properties:

1. They are chemical entities produced by special cells of endocrine glands.
2. They are transported to the target cells/ tissue/organ via circulation.
3. Their actions are species specific...
4. They are active in very minute quantities.
5. They are mostly water soluble.
6. They are low in molecular weight.
7. They are destroyed after their actions.
8. Chemically they are heterogeneous substances.
9. They cannot be stored for a long time; usually they are synthesized and secreted during the time of requirement.
10. They usually activate target cells by forming hormone receptor complex.

Mechanism of Hormone Action:

1. Enhancement of enzyme synthesis:

The steroid hormones and the thyroid hormones enter the cell and combine with the specific receptor protein to form 'receptor protein-hormone complex'. This complex

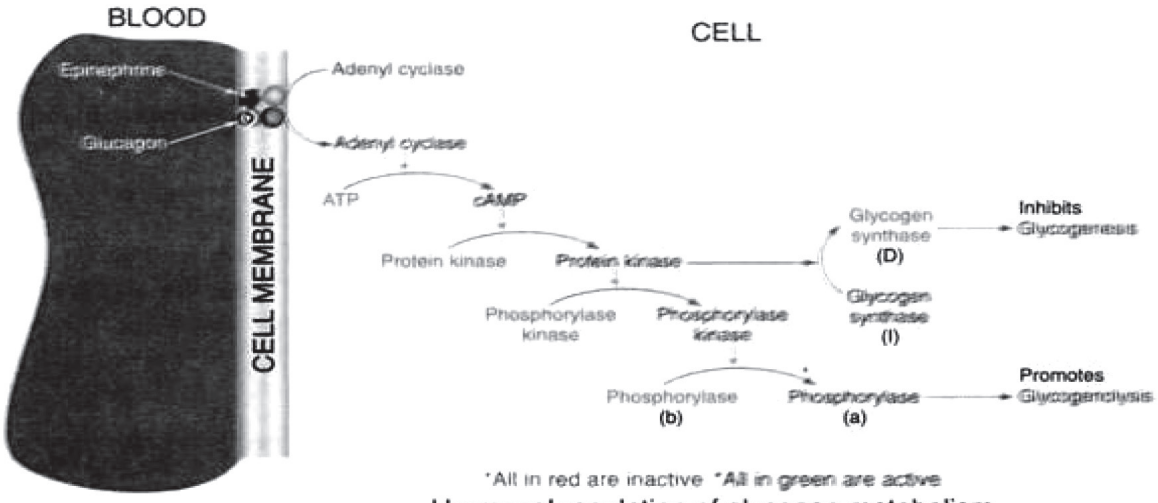
will then bind to a specific site on DNA and initiate or enhance the synthesis of mRNA which in turn synthesizes the protein i.e. enzymes. Therefore the cell reactions speed up.

2. Change in cell permeability:

Hormones like insulin binds to a specific receptor on the cell membrane which results in alteration of the permeability of the cell to certain substances like glucose, amino acids and ions. The entry of these substances will bring a change in cell reactions.

3. Action through a second messenger (cAMP):

Hormones like epinephrine, glucagon bind to a regulatory site on the cell membrane. On the inner side of this regulatory site, an enzyme known as adenyl cyclase is present that converts ATP to cAMP which then activates certain protein kinases that in turn will phosphorylate certain enzymes. Some enzymes on phosphorylation become active whereas some other enzymes become inactive. Certain reactions are therefore stimulated while others are inhibited.



Hormonal regulation of glycogen metabolism

There are two mechanism by which hormones exerts its effect:

Mechanism - 1. Mode of Protein Hormone Action through Extracellular Receptors:

(i) Formation of Hormone Receptor Complex:

Every hormone has its own receptor. The number of receptors for each hormone varies. Insulin receptors for most cells is less than 100 but for some liver cells their number may be more than 1,00,000. The molecules of amino acid derivatives, peptides

or polypeptide protein hormones bind to specific receptor molecules located on the plasma membrane to form the hormone receptor complex.

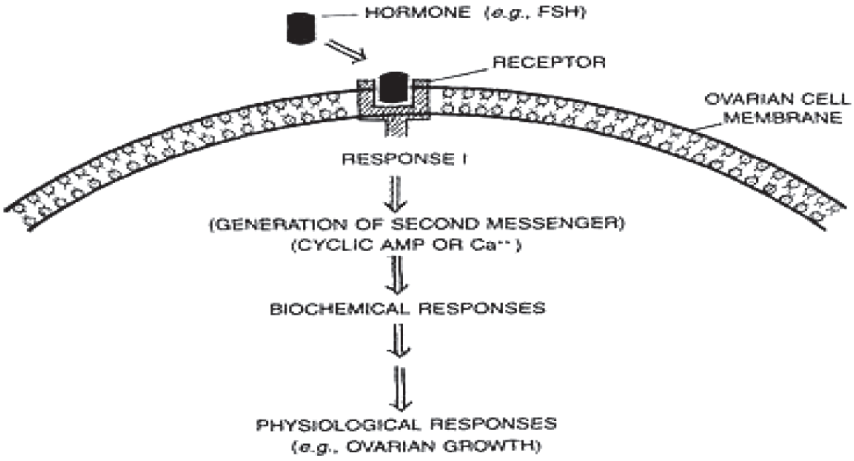


Fig. 22.18. Diagrammatic representation of mechanism of protein hormone action.

(ii) Formation of Secondary Messengers—the Mediators:

The hormone-receptor complex does not directly stimulates adenyl cyclase present in the cell membrane. It is done through a transducer G protein. Alfred Gilmans has shown that the G protein is a peripheral membrane protein consisting of α, β and γ subunits (Fig 22.19).It interconverts between a GDP form and GTP form. In muscle or liver cells, the hormones such as adrenaline bind receptor to form the hormone-receptor complex in the plasma membrane.

The hormone- receptor complex induces the release of GDP from the G protein. The α-subunit bearing GTP separates from the combined β and γ subunits. The β and γ subunits do not separate from each other. The activated β and γ subunits of G protein activate adenyl cyclase. The activated adenyl cyclase catalyses the formation of cyclic adenosine monophosphate (cAMP) from ATP.

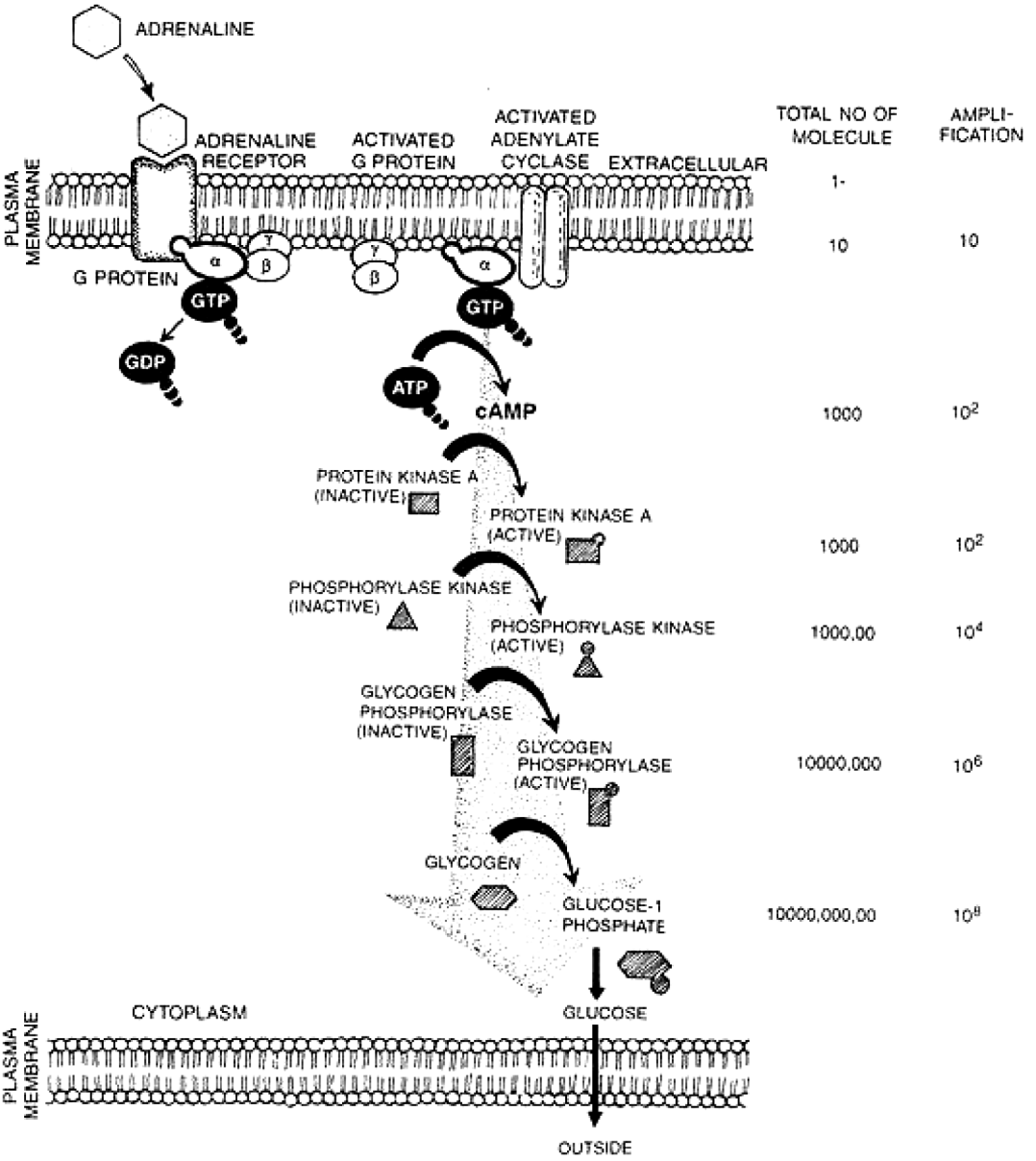


Fig. 22.19. Mode of hormone action through the extracellular receptor and amplification.

The hormone is called the first messenger and cAMP is termed the second messenger.

$$ATP \xrightarrow[\text{adenyl cyclase} + Mg^{++}]{\text{Activated}} cAMP + PPi$$

The hormones which interact with membrane-bound receptors normally do not enter the taget cell, but generate second messengers (e.g., cAMP).

Besides, cAMP, certain other intracellular second messengers are cyclic guanosine monophosphate (cGMP), diacyl-glycerol (DAG), inositol triphosphate (IP_3) and Ca^{++} responsible for amplification of signal. Earl W. Sutherland Jr (1915-1974) discovered

cAMP in 1965. He got Nobel prize in physiology of medicine in 1971 for his discovery, "Role of cAMP in hormone action".

(iii) Amplification of Signal:

Single activated molecule of adenyl cyclase can generate about 100 cAMP molecules. Four molecules of cAMP now bind to inactive protein-kinase complex to activate protein-kinase A enzyme. Further steps as shown in involve cascade effect. In cascade effect, every activated molecule in turn activates many molecules of inactive enzyme of next category in the target cell. This process is repeated a number of times.

n the cytoplasm a molecule of protein kinase A activates several molecules of phosphorylase kinase. This enzyme changes inactive form of glycogen phosphorylase into active one. Glycogen phosphorylase converts glycogen into glucose-1 phosphate. The latter changes to glucose. As a result single molecule of adrenaline hormone may lead to the release of 100 million glucose molecules within 1 to 2 minutes. This increases the blood glucose level.

(iv) Antagonistic Effect:

The effect of hormones which act against each other are called antagonistic effects. Many body cells use more than one second messenger. In heart cells cAMP acts as a second messenger that increases muscle cell contraction in response to adrenaline, while cGMP acts as another second messenger which decreases muscle contraction in response to acetylcholine.

Thus the sympathetic and parasympathetic nervous systems achieve antagonize effect on heart beat. Another example of antagonistic effect is of insulin and glucagon. Insulin lowers blood sugar level and glucagon raises blood sugar level.

(v) Synergistic Effect:

When two or more hormones complement each other's actions and they are needed for full expression of the hormone effects are called synergistic effects. For example, the production and ejection of milk by mammary glands require the synergistic effects of oestrogens, progesterone, prolactin and oxytocin hormones.

Hormones that Bind to Cell Membrane Receptor mediate their actions through many second messengers, some of which are discussed below:

A. cAMP as the Second Messenger:

i. For the formation of cAMP from ATP needs: Receptor, GS protein, Adenylate cyclase.

ii. cAMP (Cyclic adenosine 32 -52 monophosphate) is formed from ATP by the action of the enzyme adenylate cyclase and converted to physiologically inactivated 52 - AMP by the action of enzyme phosphodiesterase.

iii. Hormone receptor complex combines with G_s or G_i; (s = stimulatory, i = inhibitory) type of GTP dependent trimeric nucleotide regulatory complex of the cell membrane.

iv. Both G_s or G_1 are made up of 3 subunits. G_s contains $\alpha_s\beta\gamma$ and G_i contains $\alpha_i\beta\gamma$.

v. The α subunit (either G_s or G_i) is bound to GDP. When binding of hormone to R_s or R_i results in a receptor-mediated activation of G, then GDP is exchanged for GTP and the a subunit separates from the combined β and γ subunits.

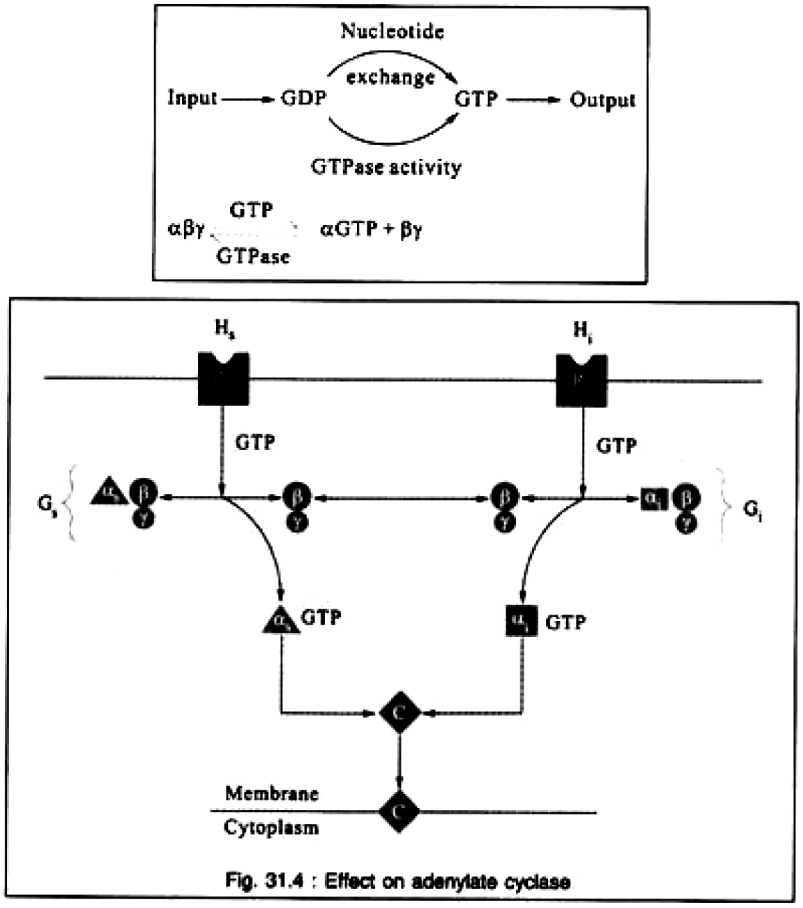


Fig. 31.4 : Effect on adenylate cyclase

vi. This GTP-α_s activates effectors (adenylate cyclase). The intrinsic GTPase activity of the α-subunit then converts GTP and GDP and leads to re-association of the α with $\beta\gamma$ subunit.

vii. On the other hand, α_i-GTP inhibits adenylate cyclase by binding with it. This lowers the intracellular concentration of cAMP. Hormones that stimulate adenylate cyclase: ACTH, ADH, FSH, Glucagon. Hormones that inhibit adenylate cyclase: Acetylcholine, Angiotensin II.

viii. cAMP binds to a protein kinase that is a hetero tetrameric molecule consisting of 2 regulatory subunits (R) and 2 catalytic subunits (C). cAMP binding results in the following reaction.

$4cAMP + R_2C_2 \rightarrow R_2 (4cAMP) + 2C$

ix. The R_2C_2 complex has no enzymatic activity but the binding of cAMP by R dissociates R from C, thereby activating protein kinase. This activated protein kinase catalyzes the transfer of the y phosphate of ATP (Mg^{++}) to a serine or threonine residue in a variety of proteins. Thus they regulate the conformational changes of phosphoprotein and physiologic effect occurs.

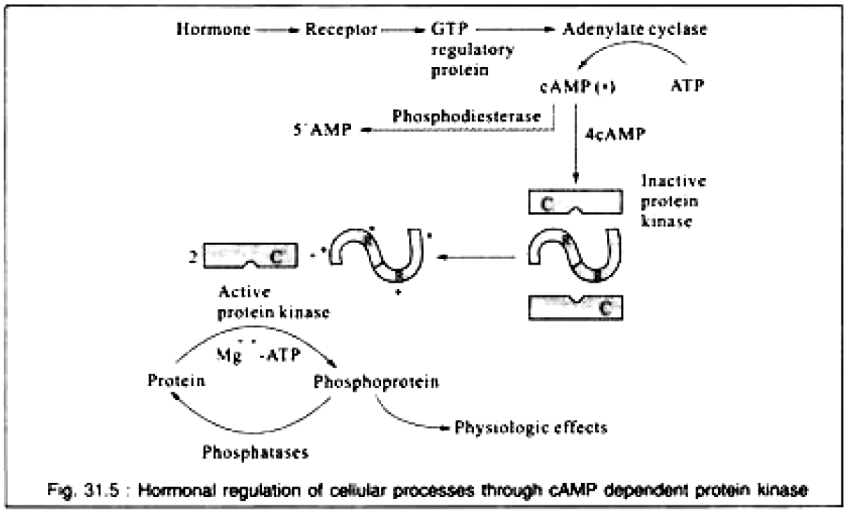


Fig. 31.5 : Hormonal regulation of cellular processes through cAMP dependent protein kinase

B. Role of cGMP in Hormone Action:

i. Hormones such as insulin and growth hormone, affect the guanylate cyclase cGMP system. This will increase the intracellular concentration of cGMP and activate cGMP dependent protein kinases.

ii. The active cGMP protein kinase would in turn bring about phosphorylation of specific cellular proteins to change their activities, leading to relaxation of smooth muscles, vasodilatation and other effects.

iii. The idea of cGMP as second messenger has not been accepted as yet. It is likely that Ca^{++} may act as second messenger to activate guanylate cyclase and thereby increasing the concentration of cGMP inside the cell.

iv. It appears that cGMP has its unique place in hormone action. The atriopeptins, a family of peptides, produced in cardiac atrial tissues cause natriuretic, diuresis, vasodilatation and inhibition of aldosterone secretion.

v. These peptides (e.g., atrial natriuretic factor) bind to and activate the membrane bound form of guanylate cyclase. This results in an increase of cGMP.

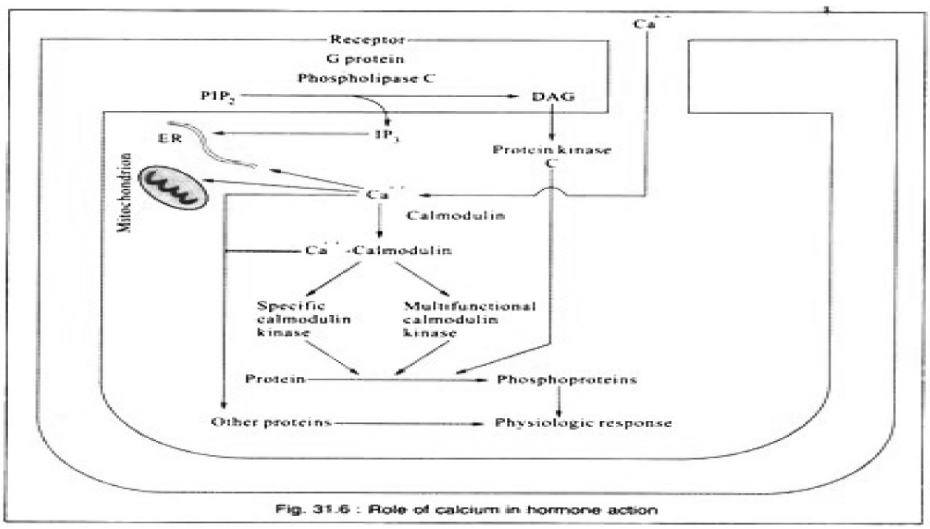


Fig. 31.6 : Role of calcium in hormone action

C. Role of Calcium in Hormone Action:

i. It is suggested that ionized calcium of the cytosol is the important signal for hormone action than cAMP.

ii. The extracellular calcium (Ca^{++}) concentration is about 5 mmol/L, the intracellular concentration of this free ion is much lower 0.1-10 µmol/L.

iii. The hormones that bind cell membrane receptor enhance membrane permeability to Ca^{++} and thereby increase Ca^{++} influx. This is probably accomplished by an Na^{+}/ Ca^{++} exchange mechanism that has a high capacity but a low affinity for Ca^{++}. There is a $Ca^{2+}/2H^{+}$-ATPase dependent pump that extrudes Ca^{2+} in exchange for H^{+}. This has a high affinity for Ca^{2+} but a low capacity.

iv. Cell surface receptors such as those for acetylcholine, ADH, when occupied by their respective ligands, potent activators of phospholipase c.

v. Receptor binding and activation of phospholipase c are coupled by a unique G protein.

vi. Phospholipase c catalyses the hydrolysis of phosphatidyl inositol 4, 5-bisphosphate to inositol triphosphate and 1, 2 diacylglcerol.

vii. The diacylglycerol is itself capable of activating protein kinase c, the activity of which also depends upon free ionic calcium.

viii. Inositol triphosphate is an effective releaser of calcium from intracellular storage sites such as endoplasmic reticulum, and mitochondria.

ix. Thus, the hydrolysis of PIP_2 leads to activation of protein kinase c and promotes an increase of cytoplasmic calcium ion.

x. The calcium dependent regulatory protein is now referred to as calmodulin. Calmodulin has $4Ca^{++}$ binding sites. Ca^{++}– calmodulin complex can activate specific kinases. These then modify the conformational changes of phosphoprotein and alters physiologic responses.

xi. The activated protein kinase c can phosphorylate specific substrates and alter physiologic processes.

Mechanism - 2. Mode of Steroid Hormone Action through Intracellular Receptors:

Steroid hormones are lipid-soluble and easily pass through the cell membrane of a target cell into the cytoplasm. In the cytoplasm they bind to specific intracellular receptors (proteins) to form a hormone receptor complex that enters the nucleus.

In the nucleus, hormones which interact with intracellular receptors (e.g., steroid hormones, iodothyromines, etc.) mostly regulate gene expression or chromosome function by the interaction of hormone-receptor complex with the genome. Biochemical actions result in physiological and developmental effects (tissue growth and differentiation, etc.). In-fact the hormone receptor complex binds to a specific regulatory site on the chromosome and activates certain genes (DNA).

The activated gene transcribes mRNA which directs the synthesis of proteins and usually enzymes in the cytoplasm. The enzymes promote the metabolic reactions in the cell. The actions of lipid soluble hormones are slower and last longer than the action of water- soluble hormones.

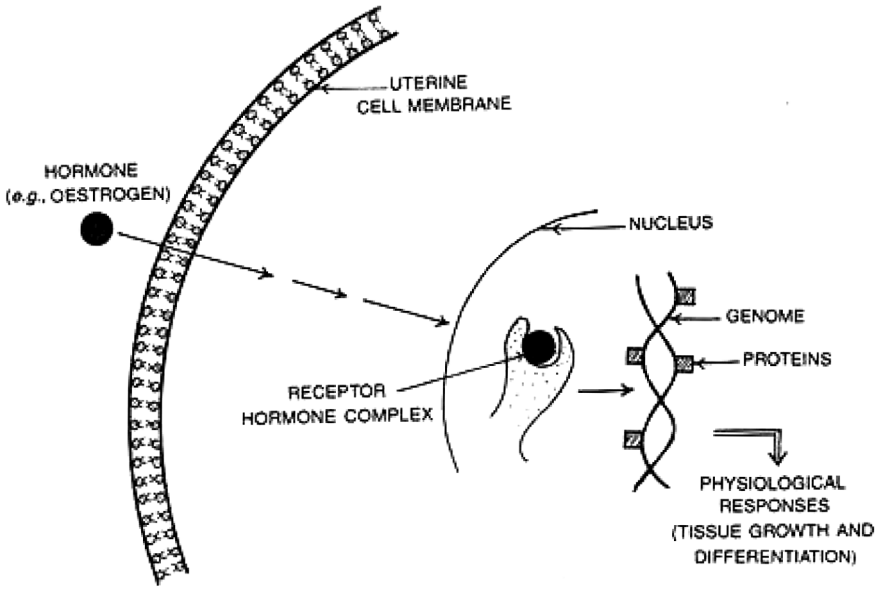


Fig. 22.20. Diagrammatic representation of the mechanism of Steroid hormone.

Role of Hormones as Messengers and Regulators (Role of Hormones in Homeostasis):

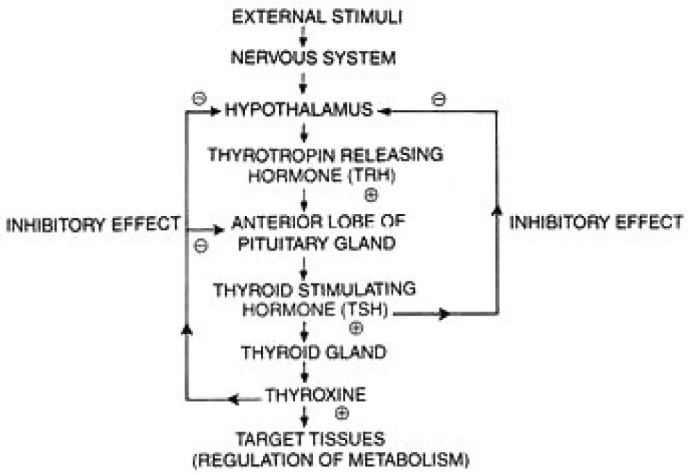


Fig. 22.21. Feed back control involving the hypothalamus, anterior lobe of pituitary gland, thyroid gland and target tissues.

Hormones as Messengers [Hypothalamus-hypophysial (pituitary) Axis]:

Hypothalamus is a part of the fore brain. Its hypothalamic nuclei— masses of grey matter containing neurons, are located in the white matter in the floor of the third ventricle of the brain. The neurons (neurosecretory cells) of hypothalamic nuclei secrete some hormones called neurohormones (releasing factors) into the blood.

The neurohormones are carried to the anterior lobe of the pituitary gland (= hypophysis) by a pair of hypophysial portal veins. In the pituitary gland (hypophysis) the neurohormones stimulate it to release various hormones. Hence the neurohormones are also called "releasing factors".

Hormones as Regulators (Feed Back Control):

Homeostasis means keeping the internal environment of the body constant. Hormones help in maintaining internal environment of the body. When the secretion of hormones is under the control of factors or other hormones it is called feedback control. The regulation of secretion of thyroxine from the thyroid gland is an example of such feedback control mechanism.

Degradation and excretion of hormones:

All the hormones are degraded and excreted. Peptide hormones are degraded in the liver and/or kidney. The catecholamine's, steroids and the thyroid hormones are inactivated directly by enzymatic modification in the blood and/or in the liver.

Feed back control is of two types:

(i) Negative Feed Back Control:

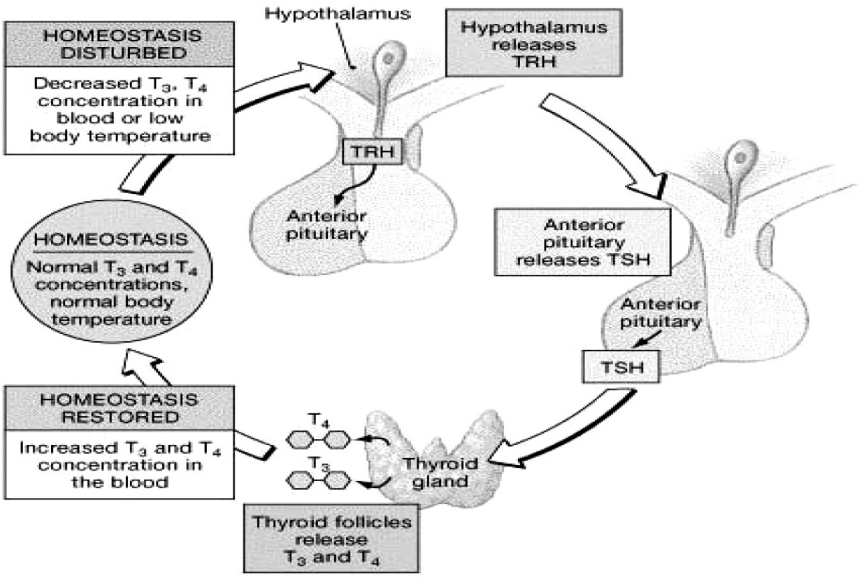
The receptors (sensory cells) present on the body of vertebrates constantly monitors the reference point of internal environment. Any changes in the internal environment can activates the receptor cells, which relay messages to the control centre (Brain or spinal cord). The control centre determines the deviation and activates the effectors. Effectors are generally muscles or glands. The effectors respond to the stimulus and corrects the reference point either by increasing or decreasing the activities. As soon as the system is corrected, the control centre and effectors are turned off by the mechanism called Negative feed-back.

In negative feed-back mechanism, changes occurring in the system automatically activates the corrective mechanism, which reverse the changes and bringback the system to the normal. The principle of thermostat is analog to the Negative feed-back mechanism. In thermostat, when the temperature exceeds the normal ranges, the receptor detects the changes and signals the control center of thermostat to turn off the heating plate, allowing the thermostat to cool down. When the thermostat cool down below the set point, it turn ON the heating plates, so the temperature starts rise again.

The mechanism of Negative feed-back in biological system can be illustrated with the example given below.

Negative feed-back mechanism of thyroid gland

Lower concentration of thyroxine hormone in blood alters the cellular activities ie. Decrease in basic metabolic rates or temperature. Decreases in BMR stimulates neurosecretory cells of hypothalamus to secrete thyrotropin releasing hormone (TRH). The releasing of TRH causes anterior pituitary gland to secrete thyroid stimulating hormone (TSH). This TSH then stimulates the thyroid gland to release thyroxine. Thyroxin causes an increase in the metabolic activity, generating ATP energy and heat and eventually restore homeostasis. Both the raised body temperature and higher thyroxine levels in the body feed-back to inhibit the releasing of TRH and TSH.



(ii) Positive Feed Back Control:

Positive feedback mechanism causes destabilizing effects in the body, so does not results in homeostasis. It is mainly responsible for amplification of the changes caused by the stimulus. Positive feedback is relatively less common than negative feedback, since it leads to unstable condition and extreme state. Most positive feedback mechanisms are harmful and in some cases resulting in death. For example, if a person breathes air that has very high carbon dioxide content. The amount of oxygen in blood decreases while the concentration of carbon-dioxide in blood increases. This is sensed by carbon dioxide receptors, which cause the breathing rate to increase. So the person breathes faster, taking in more carbon dioxide, which stimulates the receptors even more, so they breathe faster and faster which ultimately results in death.

In some cases, the positivefeed-back is very useful, such as during blood clotting, fever, child birth, breast feeding etc. Positive feedback also plays a role in the contractions of the uterus during child birth. The contraction of uterine wall is caused by oxytocin hormone. In this case, stretching of the uterus by the fetus stimulates oxytocin release which results in contraction of uterus, and contraction causes further stretching and release of oxytocin; the cycle continues until the fetus is expelled from the uterus.

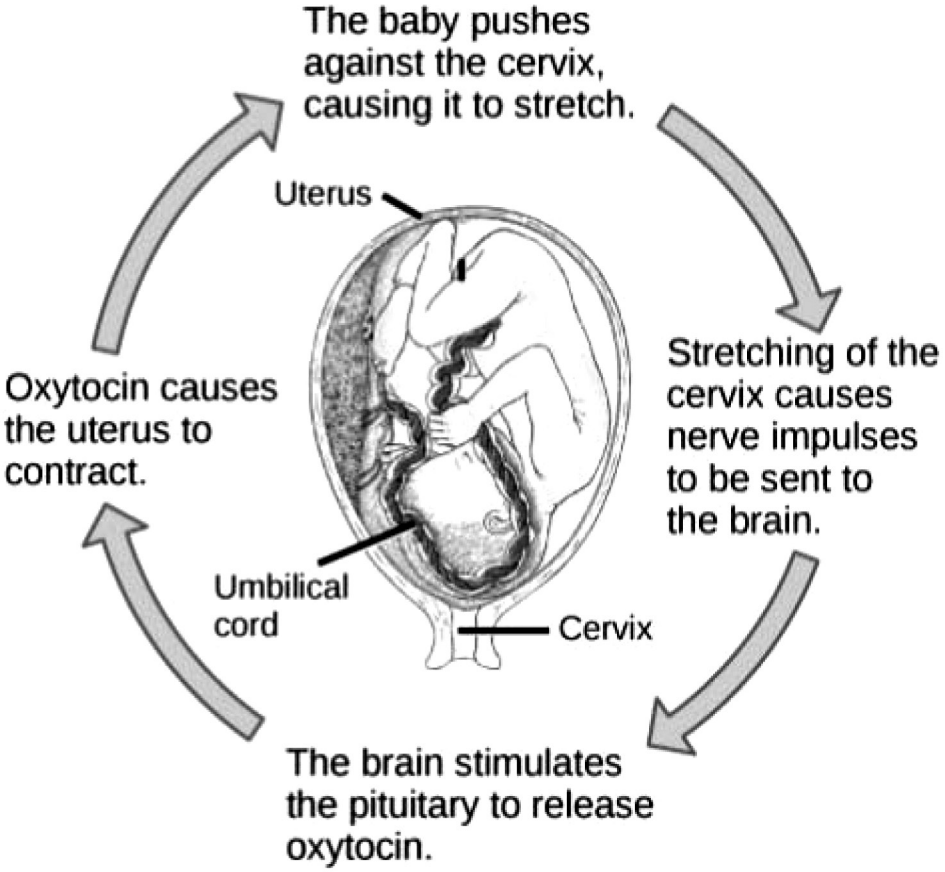


Figure: Regulation of oxytocin hormone; an example of positive feedback mechanism

Hormone Receptors:

Meaning of Hormone Receptors:

A hormone receptor is a receptor protein on the surface of a cell or in its interior that binds to a specific hormone. The hormone causes many changes that take place in the cell. Binding of hormones to hormone receptors often trigger the start of a biophysical signal that can lead to further signal transduction pathways, or trigger the activation or inhibition of genes.

Types of Hormone Receptors

Peptide Hormone Receptors:

Are often trans membrane proteins. They are also called G-protein- coupled receptors, sensory receptors or ionotropic receptors. These receptors generally function via intracellular second messengers, including cyclic AMP (cAMP), inositol 1, 4, 5-triphosphate (IP_3) and the calcium (Ca^{2+})—calmodulin system.

Steroid Hormone Receptors and Related Receptors:

Are generally soluble proteins that function through gene activation. Their response elements are DNA sequences (promoters) that are bound by the complex of the steroid bound to its receptor. The receptors themselves are zinc-finger proteins. These receptors include those for glucocorticoids, estrogens, androgens, thyroid hormone (T3), calcitriol (the active form of vitamin D), and the retinoids (vitamin A).

Receptors for Peptide Hormones:

With the exception of the thyroid hormone receptor, the receptors for amino acid derived and peptide hormones are located in the plasma membrane. Receptor structure is varied. Some receptors consist of a single polypeptide chain with a domain on either side of the membrane, connected by a membrane-spanning domain. Some receptors are comprised of a single polypeptide chain that is passed back and forth in serpentine fashion across the membrane, giving multiple intracellular, trans membrane, and extracellular domains. Other receptors are composed of multiple polypeptides. Ex. The insulin receptor is a disulfide linked tetramer with the β-subunits spanning the membrane and the α-subunits located on the exterior surface.

Subsequent to hormone binding, a signal is transduced to the interior of the cell, where second messengers and phosphorylated proteins generate appropriate metabolic responses. The main second messengers are cAMP, Ca^{2+}, inositol triphosphate (IP3), and diacylglycerol (DAG).

Proteins are phosphorylated on serine and threonine by cAMP-dependent protein kinase (PKA) and DAG-activated protein kinase C (PKC). Additionally a series of membrane-associated and intracellular tyrosine kinases phosphorylate specific tyrosine residues on target enzymes and other regulatory proteins. The hormone-binding signal of most, but not all, plasma membrane receptors is transduced to the interior of cells by the binding of receptor-ligand complexes to a series of membrane-localized GDP/GTP binding proteins known as G-proteins. The classic interactions between receptors, G-protein transducer, and membrane-localized adenylate cyclase are illustrated using the pancreatic hormone glucagon as an example.

When G-proteins bind to receptors, GTP exchanges with GDP bound to the *a*-subunit of the G-protein. The G_a-GTP complex binds adenylate cyclase, activating the enzyme. The activation of adenylate cyclase leads to cAMP production in the cytosol and to the activation of PKA, followed by regulatory phosphorylation of numerous enzymes. Stimulatory G-proteins are designated Gs, inhibitory G-proteins are designated Gi. A second class of peptide hormones induces the transduction of 2 second messengers, DAG and IP3. Hormone binding is followed by interaction with a stimulatory G-protein

which is followed in turn by G-protein activation of membrane-localized phospholipase C-γ, (PLC-γ). PLC-γ hydrolyzes phosphatidylinositol bisphosphate to produce 2 messengers viz. IP_3, which is soluble in the cytosol, and DAG, which remains in the membrane phase.

Cytosolic IP_3 binds to sites on the endoplasmic reticulum, opening Ca^{2+} channels and allowing stored Ca^{2+} to flood the cytosol. There it activates numerous enzymes, many by activating their calmodulin or calmodulin-like subunits. DAG has 2 roles-it binds and activates PKC, and it opens Ca^{2+} channels in the plasma membrane, reinforcing the effect of IP_3. Like PKA, PKC phosphorylates serine and threonine residues of many proteins, thus modulating their catalytic activity.

Insulin Receptor:

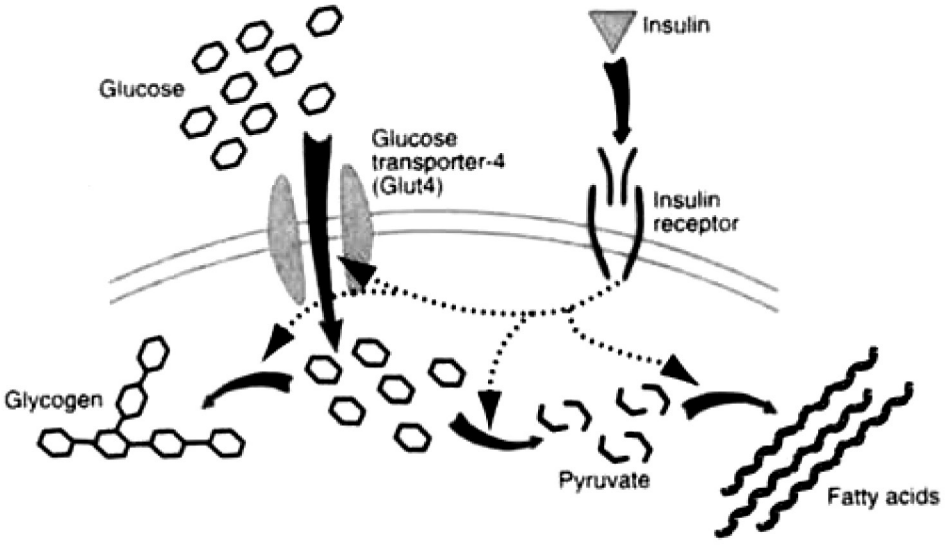
Is a trans membrane receptor that is activated by insulin. It belongs to the large class of tyrosine kinase receptors. Two alpha subunits and two beta subunits make up the insulin receptor. The beta subunits pass through the cellular membrane and are linked by disulfide bonds. The alpha and beta subunits are encoded by a single gene (INSR). The insulin receptor has been designated as CD_{220} (cluster of differentiation 220).

Function of insulin receptor-effect of insulin on glucose uptake and metabolism:

Insulin binds to its receptor which in turn starts many protein activation cascades.

These include—

i. Translocation of Glut-4 transporter to the plasma membrane and influx of glucose
ii. Glycogen synthesis
iii. Glycolysis and fatty acid synthesis



Insulin receptors (a family of tyrosine kinase receptors), mediate their activity by causing the addition of a phosphate group to particular tyrosine's on certain proteins within a cell. The 'substrate' proteins which are phosphorylated by the insulin receptor include a protein called 'IRS-1' for 'Insulin Receptor Substrate-1'.

IRS-1 binding and phosphorylation eventually leads to an increase in the high affinity glucose transporter (Glut4) molecules on the outer membrane of insulin-responsive tissues, including muscle cells and adipose tissue, and therefore to an increase in the uptake of glucose from blood into these tissues. Briefly, the glucose transporter ($Glut_4$) is transported from cellular vesicles to the cell surface, where it then can mediate the transport of glucose into the cell. Glycogen synthesis is also stimulated by the insulin receptor via IRS-1.

Pathology of insulin receptors:

The main activity of activation of the insulin receptor is inducing glucose uptake. For this reason 'insulin insensitivity', or a decrease in insulin receptor signalling, leads to diabetes mellitus type 2 – the cells are unable to take up glucose, and the result is hyperglycemia (an increase in circulating glucose), and all the sequelae which result from diabetes. Patients with insulin resistance may display acanthosis nigricans.A few patients with homozygous mutations in the INSR gene have been described, which causes Donohue syndrome or Leprechauns. This autosomal recessive disorder results in a totally non-functional insulin receptor. These patients have low set, often protuberant ears, flared nostrils, thickened lips, and severe growth retardation.

In most cases, the outlook for these patients is extremely poor with death occurring within the first year of life. Other mutations of the same gene cause the less severe Rabson-Mendenhall syndrome, in which patients have characteristically abnormal teeth, hypertrophic gingiva (gums) and enlargement of the pineal gland. Both diseases present with fluctuations of the glucose level—after a meal the glucose is initially very high, and then falls rapidly to abnormally low levels.

Degradation of insulin and its receptors:

Once an insulin molecule has docked onto the receptor and effected its action, it may be released back into the extracellular environment or it may be degraded by the cell. Degradation normally involves endocytosis of the insulin-receptor complex followed by the action of insulin degrading enzyme. Most insulin molecules are degraded by liver cells. It has been estimated that a typical insulin molecule is finally degraded about 71 minutes after its initial release into circulation.

It is a 62 kDa peptide that is activated by glucagon and is a member of the G- protein

coupled family of receptors, coupled to Gs. Stimulation of the receptor results in activation of adenylate cyclase and increased levels of intracellular cAMP. Glucagon receptors are mainly expressed in liver and in kidney with lesser amounts found in heart, adipose tissue, spleen, thymus, adrenal glands, pancreas, cerebral cortex, and G.I. tract.

Steroid Hormone Receptors:

Are proteins that have a binding site for a particular steroid molecule. Their response elements are DNA sequences that are bound by the complex of the steroid bound to its receptor. The response element is part of the promoter of a gene. Binding by the receptor activates or represses, as the case may be, the gene controlled by that promoter. It is through this mechanism that steroid hormones turn genes on (or off).

The DNA sequence of the glucocorticoid (a protein homodimer) response element is:

5′ -AGAACAnnnTGTTCT-3′

3′ TCTT GTnnnACAAGA-5′

where n represents any nucleotide (a palindromic sequence)

The glucocorticoid receptor, like all steroid hormone receptors, is a zinc-finger transcription factor; there are four zinc atoms each attached to four cysteine's.

For a steroid hormone to turn gene transcription on, its receptor must:

(i) Bind to the hormone
(ii) Bind to a second copy of itself to form a homodimer
(iii) Be in the nucleus, moving from the cytosol if necessary
(iv) Bind to its response element
(v) Activate other transcription factors to start transcription

Each of these functions depends upon a particular region of the protein (Ex. The zinc fingers for binding DNA). Mutations in any one region may upset the function of that region without necessarily interfering with other functions of the receptor.

Nuclear Receptor Superfamily:

The zinc-finger proteins that serve as receptors for glucocorticoids and progesterone are members of a large family of similar proteins that serve as receptors for a variety of small, hydrophobic molecules. These include other steroid hormones like the mineralocorticoid-aldosterone, oestrogens, the thyroid hormone (T_3), calcitriol (the active

form of vitamin D), vitamin A (retinol) and its relatives-retinal/retinoic acid, bile acids and fatty acids. These bind members of the superfamily called Peroxisome Proliferator Activated Receptors (PPARs). They got their name from their initial discovery as the receptors for drugs that increase the number and size of peroxisomes in cells.

In every case, the receptors consists of at least three functional modules or domains from N-terminal to C-terminal, these are:

i. A domain needed for the receptor to activate the promoters of the genes being controlled

ii. The zinc-finger domain needed for DNA binding (to the response element)

iii. The domain responsible for binding the particular hormone as well as the second unit of the dimer.

Receptors for Thyroid Hormones:

Are members of a large family of nuclear receptors that include those of the steroid hormones. They function as hormone-activated transcription factors and thereby act by modulating gene expression.

Thyroid hormone receptors bind DNA in absence of hormone:

Usually leading to transcriptional repression. Hormone binding is associated with a conformational change in the receptor that causes it to function as a transcriptional activator.

Mammalian thyroid hormone receptors are encoded by two genes, designated alpha and beta. Further, the primary transcript for each gene can be alternatively spliced, generating different alpha and beta receptor isoforms. Currently, four different thyroid hormone receptors are recognized as-(i) α-1 (ii) α-2 (iii) β-1 and (iv) β-2.

Like other members of the nuclear receptor superfamily, thyroid hormone receptors encapsulate three functional domains:

i. A transactivation domain at the amino terminus that interacts with other transcription factors to form complexes that repress or activate transcription. There is considerable divergence in sequence of the transactivation domains of alpha and beta isoforms and between the two beta isoforms of the receptor.

ii. A DNA-binding domain that binds to sequences of promoter DNA known as hormone response elements.

iii. A ligand-binding and dimerization domain at the carboxy-terminus.

Disorders of thyroid hormone receptors:

A number of humans with a syndrome of thyroid hormone resistance have been identified, and found to have mutations in the receptor beta gene which abolish ligand binding. Clinically, such individuals show a type of hypothyroidism characterized by goiter, elevated serum concentrations of T_3 and thyroxine and normal or elevated serum concentrations of TSH.

More than half of affected children show attention-deficit disorder, which is intriguing considering the role of thyroid hormones in brain development. In most affected families, this disorder is transmitted as a dominant trait, which suggests that the mutant receptors act in a dominant negative manner.

Adrenergic Receptors (or Adrenoceptors):

Are a class of G-protein coupled receptors that are targets of the catecholamine's. Adrenergic receptors specifically bind their endogenous ligands, the catecholamine's adrenaline and noradrenalin (called epinephrine and norepinephrine), and are activated by these.

Many cells possess these receptors, and the binding of an agonist will generally cause a sympathetic response (i.e. the fight-or-flight response) viz. the heart rate will increase and the pupils will dilate, energy will be mobilized, and blood flow diverted from other, non-essential, organs to skeletal muscle. There are several types of adrenergic receptors, but there are two main groups viz. a-adrenergic and P-adrenergic.

α-Adrenergic receptors:

These receptors bind noradrenalin (norepinephrine) and adrenaline (epinephrine). Phenylephrine is a selective agonist of the a-receptor. They exist as α_1-adrenergic receptors and α_2-adrenergic receptors.

β-Adrenergic receptors:

These receptors are linked to Gs proteins, which in turn are linked to adenyl cyclase. Agonist binding thus causes a rise in the intracellular concentration of the second messenger cAMP. Downstream effectors of cAMP include cAMP-dependent protein kinase (PKA), which mediates some of the intracellular events following hormone binding.

Role in circulation:

Epinephrine reacts with both α and β-adrenoreceptors, causing vasoconstricdon and vasodilation, respectively. Although receptors are less sensitive to epinephrine, when activated, they override the vasodilation mediated by β-adrenoreceptors. The result

is that high levels of circulating epinephrine cause vasoconstriction. Lower levels of epinephrine dominates β-adrenoreceptor stimulation, producing an overall vasodilation.

The mechanism of adrenergic receptors:

Adrenaline or noradrenalin is receptor ligands to either α_1, α_2 or β-adrenergic receptors, a, couples to Gq, which results in increased intracellular Ca^{2+} which results in smooth muscle contraction. α_2 on the other hand, couples to Gi, which causes a decrease of cAMP activity, resulting in smooth muscle contraction. α receptors couple to Gs, and increase intracellular cAMP activity, resulting in heart muscle contraction, smooth muscle relaxation and glycogenolysis.

Functions of α-receptors:

α-Receptors have several functions in common. They are:

(i) Vasoconstriction of arteries to heart (coronary artery)
(ii) Vasoconstriction of veins
(iii) Decrease motility of smooth muscle in gastrointestinal tract

Alpha-1 adrenergic receptor:

Alpha-1 -adrenergic receptors are members of the G protein-coupled receptor superfamily. Upon activation, a heterotrimeric G-protein, Gq, activates phospholipase C (PLC), which causes an increase in IP_3 and calcium. This triggers all other effects. Specific actions of the β_1 receptor mainly involve smooth muscle contraction.

It causes vasoconstriction in many blood vessels including those of the skin & gastrointestinal system and to kidney (renal artery) and brain. Other areas of smooth muscle contraction are for instance – ureter, vas deferens, hairs (arrector pili muscles), uterus (when pregnant), urethral sphincter, bronchioles (although minor to the relaxing effect of β_2 receptor on bronchioles). Further effects include glycogenolysis and gluconeogenesis from adipose tissue and liver, as well as secretion from sweat glands and Sodium Na reabsorption from kidney.

Alpha-2 adrenergic receptor:

There are 3 highly homologous subtypes of α_2 receptors viz. α_2A, α_2B, and α_2C. Specific actions of the α_2-receptor include:

i. Inhibition of insulin release in pancreas
ii. Induction of glucagon release from pancreas
iii. Contraction of sphincters of the gastrointestinal tract

Beta-1 adrenergic receptor:

Specific actions of the β_1 receptor include:

i. Increase cardiac output, both by raising heart rate and increasing the volume expelled with each beat (increased ejection fraction)

ii. Renin release from juxtaglomerular cells

iii. Lipolysis in adipose tissue

Beta-2 adrenergic receptor:

Specific actions of the β_2 receptor include:

i. Smooth muscle relaxation, e.g. in bronchi

ii. Relaxes urinary sphincter and pregnant uterus

iii. Relaxes detrusor urinary muscle of bladder wall

iv. Dilates arteries to skeletal muscle

v. Glycogenolysis and gluconeogenesis

vi. Contract sphincters of GI tract

vii. Thickened secretions from salivary glands

viii. Inhibit histamine-release from mast cells

ix. Increase renin secretion from kidney

Comparison of different adrenergic receptors

Receptor type	Agonist potency order	Selected action of agonist	Mechanism	Agonists	Antagonists
α_1:A, B, D	Adrenaline ≥ Noradrenaline >> Isoprenaline	Smooth muscle contraction	Gq: Phospholipase C (PLC) activated, IP3 and Calcium up	Noradrenaline Phenylephrine Methoxamine Cirazoline	(Alpha blockers) Phenoxy-benzamine Phentolamine Prazosin Tamsulosin Terazosin
α_2: A, B, C	Adrenaline ≥ Noradrenaline >> Isoprenaline	Smooth muscle contraction and neurotransmitter inhibition	Gi: Adenylate cyclase inactivated, cAMP down	Clonidine lofexidine Xylazine Tizanine Guanfacine	(Alpha blockers) Metoprolol atenolol
β_1	Isoprenaline > Adrenaline = Noradrenaline	Heart muscle contraction	Gs: Adenylate cyclase activated, cAMP up	Noradrenaline Isoprenaline Dobutamine	(Beta blockers) Metoprolol atenolol

β_2	Isoprenaline > Adrenaline > > Noradrenaline	Smooth muscle relaxation	Gs: Adenylate cyclase activated, cAMP up	Salbutamol Bitolterol Mesylate Formoterol Isoprenaline Levalbuterol Metaproterenol Salmeterol Terbutaline Ritodrine	(Beta blockers) Butoxamine propranolol
β_3	Isoprenaline = Noradrenaline > Adrenaline	Enhance lipolysis	Gs: Adenylate cyclase activated, cAMP up	L-796568	

Probable Questions:

1. Define hormone. What are the main characteristics of a hormone?
2. How hormones exert their effect through extracellular receptors?
3. How steroid hormones exert their effect?
4. What is antagonistic effect and what is synergistic effect of hormone action?
5. State the role of cAMP as hormone second messenger.
6. State the role of cGMP as hormone second messenger.
7. State the role of Calcium ion as hormone second messenger.
8. Discuss about positive feed back of hormone action with suitable example.
9. Discuss about negative feed back of hormone action with suitable example.
10. What is hormone receptor? Describe its importance.
11. How insulin receptor exerts its effect in cell? What happen when there is defect in the receptor?
12. What are the characteristics of steroid receptors?
13. Discuss the role and types of adrenergic receptors.
14. Compare different types of adrenergic receptors.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XII

Biosynthesis, secretion and regulation of hormones: biosynthesis of protein and peptide hormones (Growth Hormone and Insulin) including their post-translational event and release

Objective: In this unit you will learn about biosynthesis release and regulations of different hormones such as growth hormone, insulin, thyroxine and steroid hormones.

Meaning of Growth Hormones:

Growth hormone (GH) also known as somatotropic hormone and is a peptide hormone secreted by acidophils of the anterior pituitary gland. GH is stored in large, dense granules present in acidophil cells. It is a single chain polypeptide with molecular weight of 22,000 having 191 amino acids and two disulphide bridges. As the name indicates, its action is on the growth of the body. It stimulates somatic growth and development and helps to maintain lean body mass and bone mass in adults.

Growth hormone (**GH**) or **somatotropin**, also known as **human growth hormone** (**hGH** or **HGH**) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals. It is thus important in human development. GH also stimulates production of IGF-1 and raises the concentration of glucose and free fatty acids. It is a type of mitogen which is specific only to the receptors on certain types of cells. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

A recombinant form of hGH called somatreopleopin (INN) is used as a prescription drug to treat children's growth disorders and adult growth hormone deficiency. In the United States, it is only available legally from pharmacies by prescription from a licensed health care provider. In recent years in the United States, some health care providers are prescribing growth hormone in the elderly to increase vitality. While legal, the efficacy and safety of this use for HGH has not been tested in a clinical trial. Many of the functions of hGH remain unknown.

In its role as an anabolic agent, HGH has been used by competitors in sports since at least 1982, and has been banned by the IOC and NCAA. Traditional urine analysis does not detect doping with HGH, so the ban was not enforced until the early 2000s, when blood tests that could distinguish between natural and artificial HGH were starting

to be developed. Blood tests conducted by WADA at the 2004 Olympic Games in Athens, Greece targeted primarily HGH. Use of the drug for performance enhancement is not currently approved by the FDA.

GH has been studied for use in raising livestock more efficiently in industrial agriculture and several efforts have been made to obtain governmental approval to use GH in livestock production. These uses have been controversial. In the United States, the only FDA-approved use of GH for livestock is the use of a cow-specific form of GH called bovine somatotropin for increasing milk production in dairy cows. Retailers are permitted to label containers of milk as produced with or without bovine somatotropin.

The mammalian *GH* gene (also called *GH-normal* or *GH-N*) belongs to a gene cluster that includes the genes for prolactin and some placental lactogens, and is primarily expressed in the somatotroph cells of the anterior pituitary gland. GH secretion occurs in a pulsatile fashion owing to the action of two hypothalamic factors, growth hormone releasing hormone (GHRH) which stimulates GH secretion, and somatostatin which inhibits GH secretion. GH secretion is also stimulated by ghrelin, an endogenous GH secretagogue that is primarily secreted by the gastrointestinal tract. In the circulation, GH is bound to the growth hormone binding protein (GHBP) which is a soluble truncated form of the growth hormone receptor (GHR). GHBP is generated either as an alternative splice form of the GHR transcript (in rodents) or by limited proteolysis of the GHR protein (in humans). Thus, GH in the circulation exists as bound and free forms, the predominance of each being dependent on the pulsatile pattern of its secretion.

GH secretion exhibits sexual dimorphism; it is secreted more frequently in females than in males. While this could reflect the differential effects of sex steroids on GH secretion and action, recent data suggest the existence of sex-specific differences in the GH/IGF-1 axis at birth. Moreover, inter-species differences in circulating GH profiles have been observed in mammals. In males, GH secretion occurs nocturnally in humans and in 3-4 hour intervals in rodents; while in females, rats have residual GH levels between periods of GH secretion which are absent in humans and mice.

Nomenclature:

The names *somatotropin* (*STH*) or *somatotropic hormone* refers to the growth hormone produced naturally in animals and extracted from carcasses. Hormone extracted from human cadavers is abbreviated *hGH*. The main growth hormone produced by recombinant DNA technology has the approved generic name (INN) *somatropin* and the brand name *Humatrope*, and is properly abbreviated rhGH in the scientific literature. Since its introduction in 1992 Humatrope has been a banned sports doping agent, and in this context is referred to as HGH.

Structure:

The major isoform of the human growth hormone is a protein of 191 amino acids and a molecular weight of 22,124 daltons. The structure includes four helices necessary for functional interaction with the GH receptor. It appears that, in structure, GH is evolutionarily homologous to prolactin and chorionic somatomammotropin. Despite marked structural similarities between growth hormone from different species, only human and Old World monkey growth hormones have significant effects on the human growth hormone receptor.

Several molecular isoforms of GH exist in the pituitary gland and are released to blood. In particular, a variant of approximately 20 kDa originated by an alternative splicing is present in a rather constant 1:9 ratio, while recently an additional variant of ~ 23-24 kDa has also been reported in post-exercise states at higher proportions. This variant has not been identified, but it has been suggested to coincide with a 22 kDa glycosylated variant of 23 kDa identified in the pituitary gland. Furthermore, these variants circulate partially bound to a protein (growth hormone-binding protein, GHBP), which is the truncated part of the growth hormone receptor, and an acid-labile subunit (ALS).

Regulation of Growth hormone secretion:

Secretion of growth hormone (GH) in the pituitary is regulated by the neurosecretory nuclei of the hypothalamus. These cells release the peptides growth hormone-releasing hormone (GHRH or *somatocrinin*) and growth hormone-inhibiting hormone (GHIH or *somatostatin*) into the hypophyseal portal venous blood surrounding the pituitary. GH release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) and inhibitors (e.g., free fatty acids) of GH secretion.

Somatotropic cells in the anterior pituitary gland then synthesize and secrete GH in a pulsatile manner, in response to these stimuli by the hypothalamus. The largest and most predictable of these GH peaks occurs about an hour after onset of sleep with plasma levels. Otherwise there is wide variation between days and individuals. Nearly fifty percent of GH secretion occurs during the third and fourth NREM sleep stages. Surges of secretion during the day occur at 3- to 5-hour intervals.[3] The plasma concentration of GH during these peaks may range from 5 to even 45 ng/mL. Between the peaks, basal GH levels are low, usually less than 5 ng/mL for most of the day and night. Additional analysis of the pulsatile profile of GH described in all cases less than 1 ng/ml for basal levels while maximum peaks were situated around 10-20 ng/mL.

A number of factors are known to affect GH secretion, such as age, sex, diet, exercise, stress, and other hormones. Young adolescents secrete GH at the rate of about 700 μg/day, while healthy adults secrete GH at the rate of about 400 μg/day. Sleep deprivation generally suppresses GH release, particularly after early adulthood.

Mechanism of Action of Growth Hormones:

i. Receptors for growth hormone are present on the plasma membrane of cells.

ii. Belong to cytokine family of receptors.

iii. Presence of excess of GH down regulates the synthesis of its receptors.

iv. Many hours must elapse after administration of GH before anabolic and growth-promoting actions of the hormones to become evident.

v. Most of the actions of GH require the production of GH induced somatomedin C or insulin-like growth factor (IGF).

vi. The plasma half-life of IGF is much longer than that of GH.

Actions of the hormone can be broadly classified into two types:

a. Indirect growth promoting action

b. Direct anti-insulin action.

1. Indirect growth promoting action (Figs 6.9 and 6.10) is due to the action of growth hormone on liver. When the hormone acts on liver, liver secretes somatomedin C or insulin-like growth factor (IGF- I). This substance acts on skeletal and extraskeletal compartments.

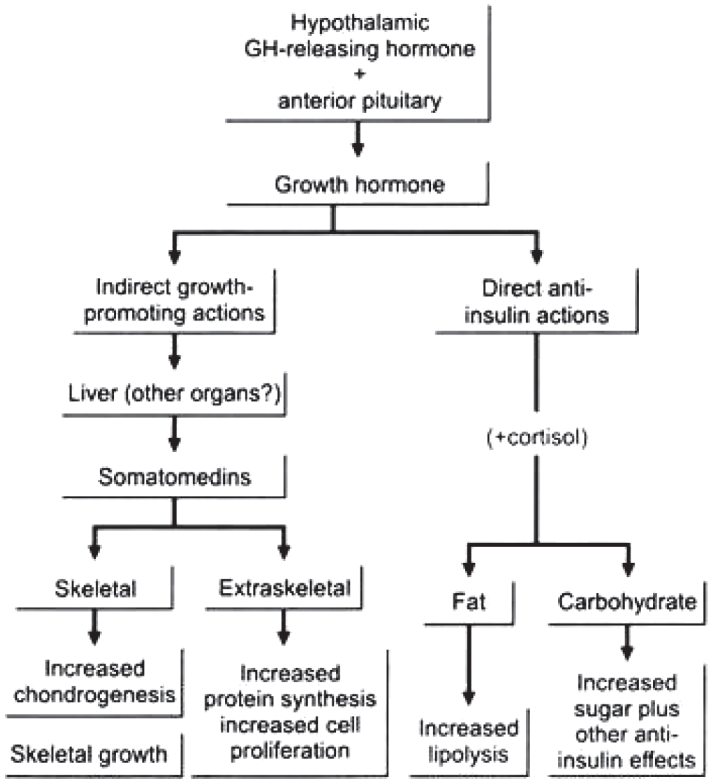


Fig. 6.9: Composite diagram showing actions of GH

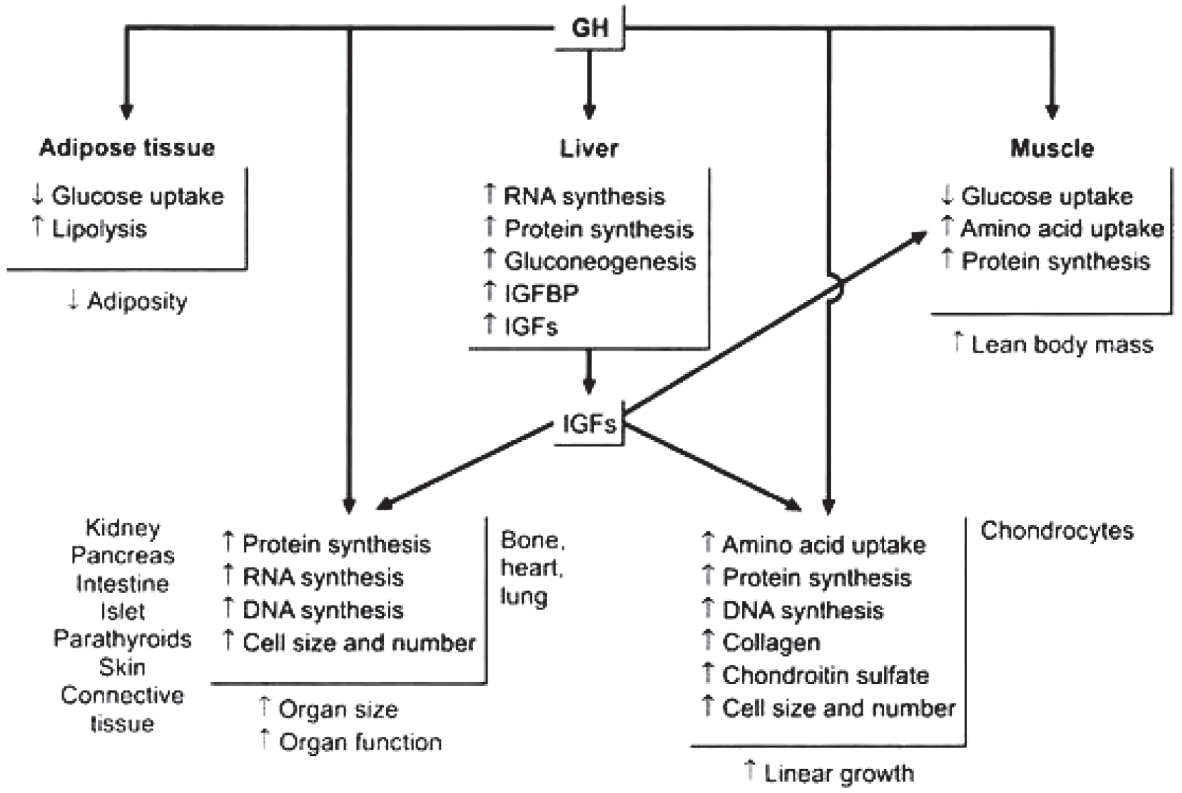


Fig. 6.10: Highlighting various intracellular actions of GH in the body

i. Skeletal compartment:

When somatomedin acts on epiphyseal plate present between the long bones, the epiphyseal plate gets widened. This gives space for the chondrogenesis of the long bones. The long bones grow linearly. Hence, the height of the person increases. The long bones can grow only up to the age of about 18-20 years beyond which the epiphyseal plates get fused with long bones and there can be no more linear growth of body.

ii. Extra-skeletal compartment:

This in general refers to the growth of organ and tissues. The growth is brought about by hyperplasia (stimulating mitotic cell division and hence increase in cell number) and hypertrophy (increase cell size). The various tissues in the body grow. There will be increased protein synthesis because of which it brings about positive nitrogen balance. The proteins synthesized are incorporated for the growth of the organs.

The various parts of the body do not grow in equal proportion at the same time. The growth of the different parts of the body based on chronological age has been shown in Fig. 6.11.

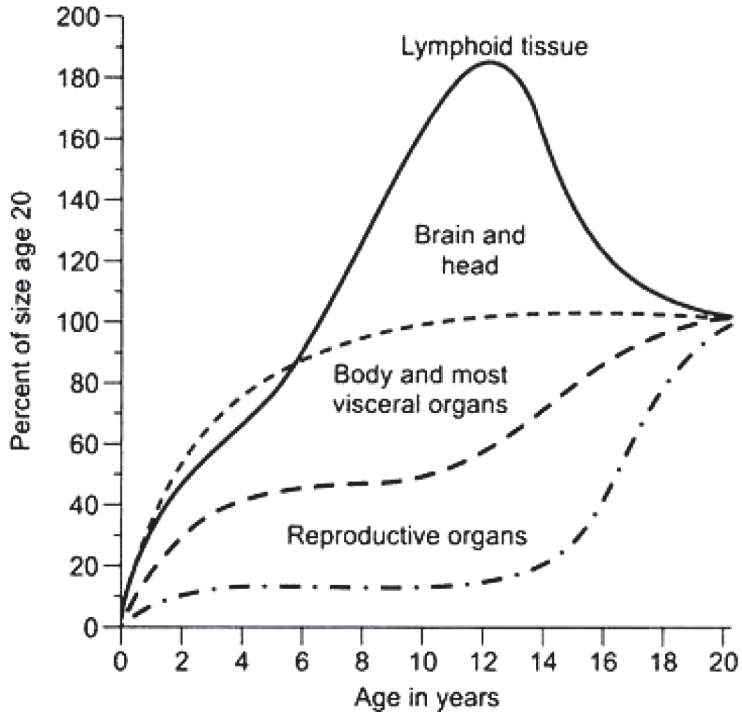


Fig. 6.11: Extent of growth of various tissues at different ages

2. Direct anti-insulin action:

This can be brought about in the target organs in presence of Cortisol (permissive action of Cortisol is required.

i. On carbohydrate metabolism:

It is a hyperglycemic agent. Increases the blood glucose level by:

a. Decreasing the peripheral utilization of glucose.

b. Increased gluconeogensis in liver.

Metahypophyseal diabetes:

Uncontrolled secretion of GH for a long time brings about increase in blood glucose level. This leads to increase stimulation of beta cells of islets of Langerhans to secrete insulin. After sometime, due to constant stimulation, the beta cells get exhausted and lead to development of diabetes mellitus.

ii. Fat metabolism:

Acts on the adipose tissue. Neutral fats and triglycerides are broken down to release the free fatty acids. They are utilized for energy supply to the tissues.

This can lead to increased production of keto acids. Growth hormone also promotes the retention of sodium, potassium, calcium and phosphate since these substances are required for the growth of the body.

Regulation of Secretion of Growth Hormones:

It is mainly by the negative feedback control by the free form of the hormone level in circulation.

Growth hormone releasing hormone (GRH) secreted from the hypothalamus acts on anterior pituitary gland and stimulates the secretion of growth hormone, which in turn increases insulin-like growth factor (IGF) I or somatomedin C secretion from liver. When IGF I level in circulation increases, it acts on hypothalamus to stimulate the secretion of somatostatin (SS). SS on reaching anterior pituitary decreases the secretion of growth hormone (Fig. 6.12).

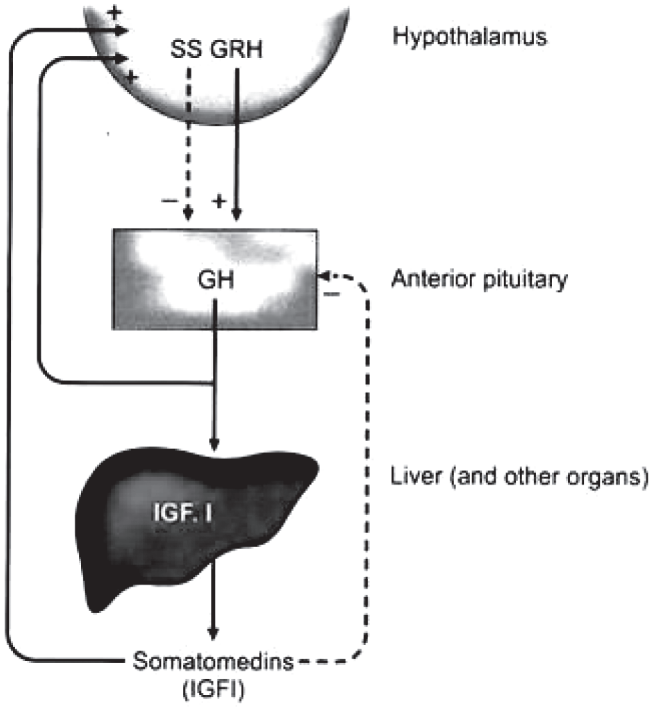


Fig. 6.12: Regulation of secretion of GH by feedback mechanism

IGF I also acts directly on anterior pituitary and exerts inhibitory influence on the secretion of growth hormone.GH secreted by the anterior pituitary gland is able to reach the hypothalamus through circulation and on reaching hypothalamus it stimulates the secretion of somatostatin. Somatostatin on reaching anterior pituitary inhibits further secretion of growth hormone.

Some of the other factors that increase the secretion of growth hormone are:

i. Increase in amino acids in circulation

ii. Hypoglycemia

iii. Free fatty acid decrease

iv. Exercise

v. At puberty

vi. Stage IV sleep.

The factors which inhibit the GH secretion are:

i. Dreaming or rapid eye movement (REM) sleep.

ii. Glucose increase.

iii. Cortisol.

iv. Obesity.

Applied Aspects of Growth Hormones:

Deficiency of GH in children:

i. Hypothalamic dysfunction

ii. Pituitary destruction

iii. Defective GHRH receptor

iv. Biologically incompetent GH or GH receptor

v. Failure to produce IGF

vi. GH receptor deficiency

vii. GH receptor unresponsiveness: Laron dwarfism

Dwarfism:

i. It's because of hyposecretion of GH from childhood.

ii. Person will have short stature. There will be a generalized stunted growth of the body.

iii. The person will have normal reproductive development.

iv. There will not be any mental abnormality and will have normal intelligent quotient (IQ).

v. Facial changes correspond with chronological age.

Achondroplasia is the most common form of dwarfism. The characteristic feature will be short limbs and normal trunk.

Laron dwarf:

i. It will be due to insensitivity of the tissues to GH.

ii. The receptors are non-responsive to GH.

iii. There can be normal or elevated level of GH in circulation.

Progeria:

Deficiency of growth hormone in adult. The person appears older at an younger age.

Dwarfism could also be due to:

i. Cretinism—thyroxine deficiency

ii. Gonadal dysgenesis

iii. Kaspar Hauser syndrome—psychosocial dwarfism

iv. Achondroplasia—child born to aged father

Frolich dwarf:

Destructive disease of part of anterior pituitary. At times may include post-pituitary and hypothalamus.

i. Stunted growth.

ii. Obesity

iii. Decreased sexual development

iv. Somnolence

v. Mentally subnormal

Deficiency of GH in adult:

i. Decreased muscle

ii. Decreased muscle strength and exercise performance

iii. Decreased lean body mass

iv. Decreased bone density

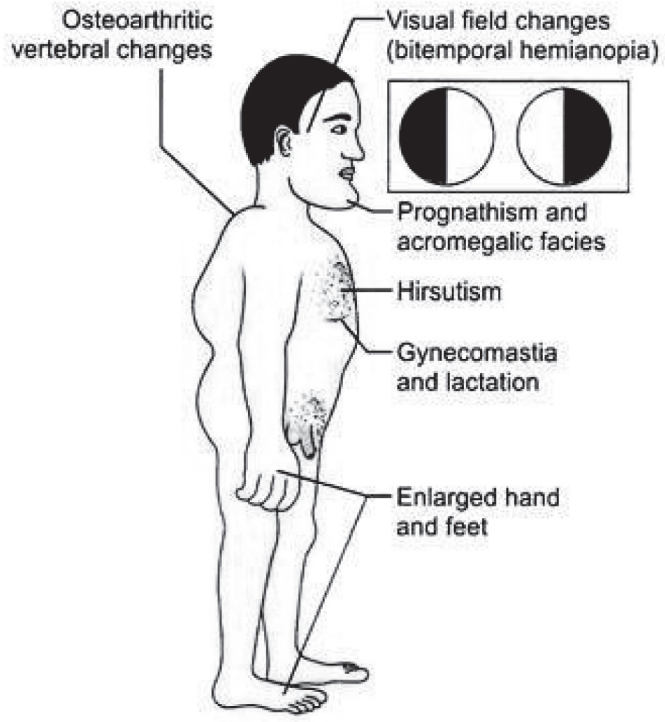


Fig. 6.13: Some of the important features of acromegaly

Acromegaly:

i. Hypersecretion of growth hormone after the puberty.

ii. Enlargement of hand and feet (acral parts of the body only can grow because of the ossification of the long bones).

iii. There will also be enlargement of mandible which results in prognatism. There will also be enlargement and protrusion of frontal bone. Because of this, the person may have gorilla-like appearance.

iv. Certain osteoarthritic changes are also observed leading to kyphosis.

v. There can be enlargement of viscera especially that of heart and may lead to cardiomegaly.

vi. There can be hirsutism (increased hair growth on anterior part of trunk) and gynecomastia (enlargement of breasts even in males) and lactation (secretion of milk).

vii. The person may suffer from bitemporal hemianopia (a type of visual field defect) due to the compressing on the medial part of optic chiasma by enlarged pituitary gland.

Gigantism:

i. Hypersecretion of hormone from childhood.

ii. Size of the person is pathologically big, but the person will be weak. Hence, the

person is known as weak giant. There will not be proportionate growth of the contractile proteins in the muscles. Hence muscles are weak.

iii. The person is prone to develop early diabetes. This is because since growth hormone has hyperglycemic action, the sustained increase in blood glucose level may lead to exhaustion of beta cells of islets of Langerhans. So the person develops diabetes.

iv. The longevity of these people is restricted and die early.

Sheehan's syndrome:

i. Observed in female. Due to postpartum hemorrhage, there can be ischemic necrosis of pituitary gland.

ii. The pituitary gland secretion in general gets decreased.

iii. Symptoms include lethargy, sexually inactive, unable to withstand stress. Growth is inhibited and thyroid function is depressed.

iv. There can be atrophy of gonads. The menstrual cycle stops.

v. When there is general deficiency of all the hormones of anterior pituitary gland, this condition is known as panhypopituitarism.

Hyperprolactinemia:

It could be due to administration of dopamine antagonist/prolactin secreting adenomas.

Features:

a. Amenorrhea, b. Galactorrhea, c. Decreased libido, d. Impotence, e. Hypogonadism, f. Testosterone level low

Insulin:

Insulin is a type of protein hormone, which is synthesized in the β-cells of islets of Langerhans. The term insulin is derived from Latin word "Insula" means island. Banting and Best (1916) observed the role of insulin in glucose metabolism.

Structure of Insulin:

Insulin is a peptide hormone and its molecular weight is 5.7 Kdt. It is made up of two polypeptide chains α and β. Insulin is constituted by 51 amino acids, of which a-chain contains 21 amino acids and β-chain contains 30 amino acid residues. Besides the primary peptide bonds, the polypeptide chains are strengthened by disulphide bonds (-S-S-).

One intra -S-S- bond occurs in the a-chain in between 6 and 11 positions of cystine. Two inter -S-S- bonds are found in between a and p chain one in between 7^{th} position of both the chains and other in between 20^{th} position of á-chain and 19^{th} position of â-chain (Fig. 6.1).

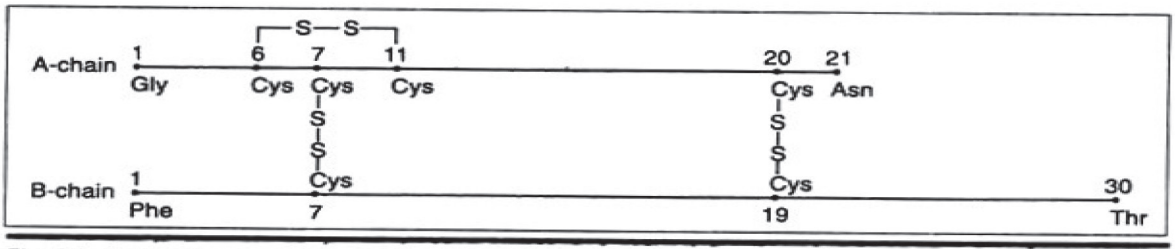


Fig. 6.1: Schematic representation of human insulin molecule

In different species of vertebrates, structure of insulin varies according to variation of amino acid residues. Variations occur at 8^{th}, 9^{th} and 10^{th} position of a-chain and 30^{th} position of â-chain.

Species	*α-chain*			*β-chain*
	8^{th}	9^{th}	10^{th}	30^{th}
Horse	Thr	Gly	Ile	Ala
Goat & Cattle	Ala	Ser	Val	Ala
Man	Thr	Ser	Ile	Thr
Rabbit	Thr	Ser	Ila	Ser

Biosynthesis of Insulin:

The synthesis of insulin takes place in p-cells of islets of Langerhans.

It is a complex phenomenon and it occurs in following ways:

1. Transcription of code:

Genes on chromosome 11 coding for insulin and are transcribed to mRNA in the nucleus.

2. Translation of the code:

After moving to the cytoplasm, mRNA is translated by the polysome attach to GER. Polypeptide synthesis is initiated with the formation of N-terminal signal peptide (leading sequence) which penetrates through the membrane of GER.

3. Synthesis of preproinsulin:

Further elongation directs the polypeptide chain into the lumen of GER, resulting in the formation of preproinsulin. It is constituted by 109 amino acid residues and mol. wt. is 11.5 kdt.

4. Separation of signal sequence:

In the lumen of GER, N-terminal signal peptide is hydrolysed away by signal peptidase. Thus signal peptide is cleaved and pro- insulin is formed in the cysternal space of GER. Pro-insulin consists of 86 amino acid residues and its mol. wt. is about 9 kd. Pro-insulin has disulphide bonds.

5. Transfer of pro-insulin:

Pro-insulin is transported from GER to the Golgi complex

6. Splitting of pro-insulin:

In Golgi cisternae pro-insulin is hydrolysed by trypsin like peptidase to yield a 53 amino acid insulin precursor and pro-c-peptide has 33 amino acids.

Under condition of excessive stimulation pro-insulin is secreted by vesicular exocytosis along with the insulin from p-cells (Fig. 6.2).

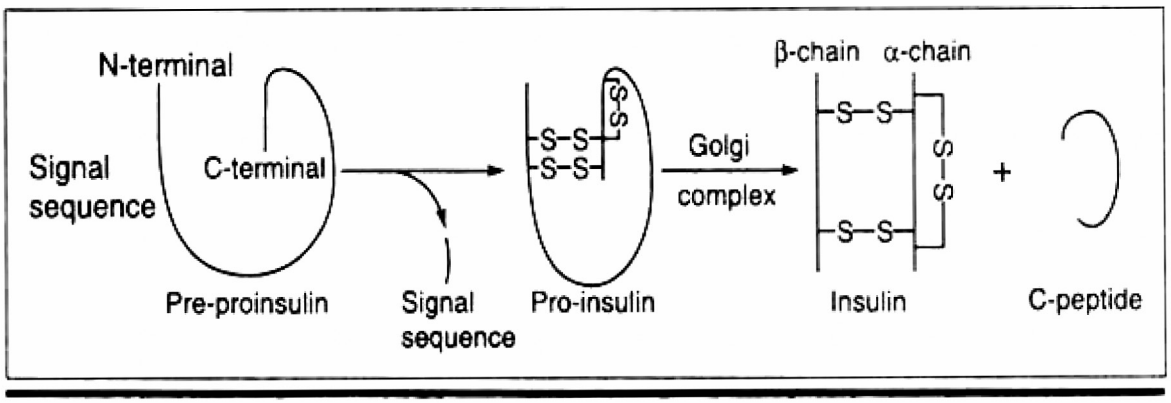


Fig. 6.2: Formation of insulin

7. Formation of insulin:

In the Golgi complex about 95% of the pro-insulin is converted to active insulin. Enzyme carboxylase peptidase hydropses c-terminal peptide bonds in the pro-c- peptide and the insulin precursor to release 2-c-terminal basic amino acids from each. Two molecules of Arg. are driven out from the insulin precursor and lead to the formation of active insulin (consists of 51 amino acids).From pro-c-peptide two amino acids Lys and Arg are separate out and leads to the formation of connective peptide or c-pep- tide (consists of 31 amino acids). Insulin and c-peptide are present in secretory granules

of Golgi complex. In some species, insulin is combined with Zn within P-cells. After stimulation insulin is secreted by exocytosis (Fig. 6.3).

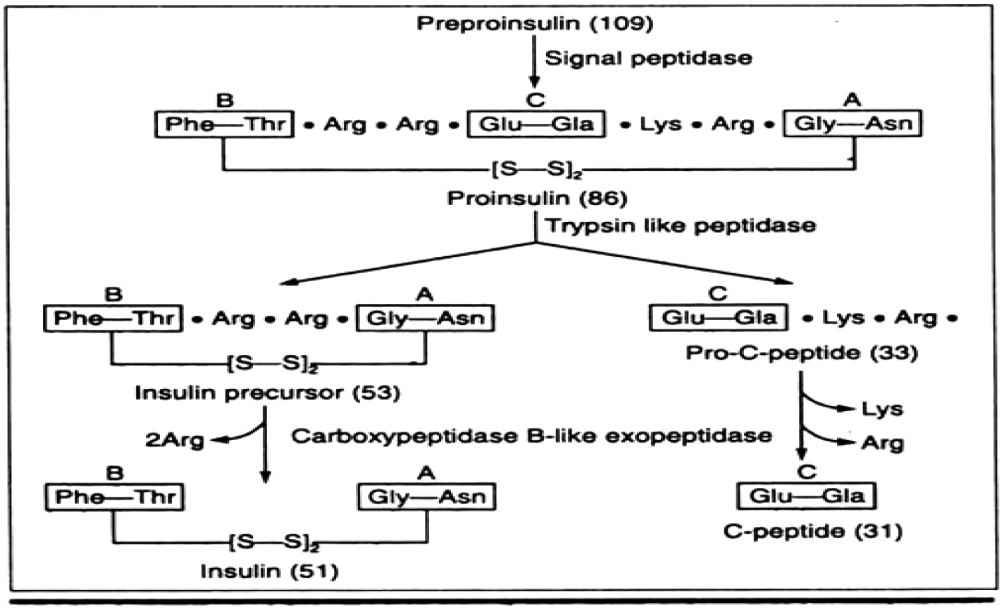


Fig. 6.3: Schematic representation of insulin synthesis

Transport of insulin:

Insulin is directly diffused within the blood sinusoids of the islets and is transported to the target organ.

Catabolism of insulin:

After biochemical reaction, insulin is degraded within the liver, kidney, skeletal muscles and placenta in presence of enzyme insulinase.

Control of secretion:

Insulin synthesis and secretion is controlled by following factors:

1. Carbohydrate meal:

Intake of carbohydrate rich food leads to raise the blood glucose which is signal for increased insulin secretion.

2. Amino acids:

Ingestion of protein causes an increase in plasma amino acids level. Elevated plasma arginine is particularly potent stimulus for insulin secretion.

3. Gastrointestinal hormone:

Intestinal hormones (GIP & VIP) secretion stimulates the insulin synthesis and secretion.

4. Epinephrine:

The synthesis and release of insulin are degraded by negative feedback mechanism of epinephrine in stress condition.

5. Glucagon:

Low blood sugar level stimulates the secretion of glucagon for glycogenesis, which in-turn inhibits the synthesis of insulin (Fig. 6.4).

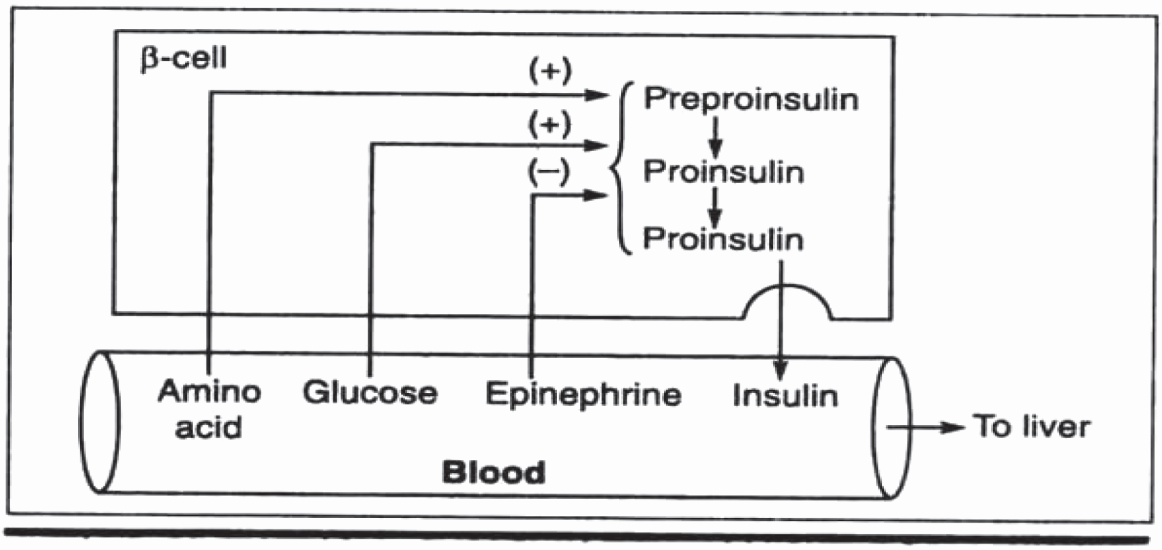


Fig. 6.4: Regulation of insulin release from β-cell

6. Somatostatin:

This hormone is secreted from D-cells of pancreatic islets and regulates the secretion á and â-cells.

Insulin Receptor:

a. Insulin acts on target tissues by binding to specific insulin receptors which are glycoproteins.

b. The human insulin receptor gene is found on chromosome 19. The insulin receptors are being constantly synthesized and degraded. Their half-life is 6 to 12 hours only.

c. It is synthesized as a single chain polypeptide, pro-receptor in the rough endoplasmic reticulum and is rapidly glycosylated in Golgi region.

d. The pro-receptor is cleaved to form mature á and â subunits ($\alpha_2\beta_2$) which is heterodimer, linked by S- S bonds.

e. Both subunits are extensively glycosylated and removal of sialic acid and galactose decreases insulin binding and insulin action.

f. Insulin receptors are found in target cell membrane.

g. Though insulin receptor is a heterodimer consisting of 2 subunits, designated α and β ($\alpha_2\beta_2$) linked by disulphide bonds.

h. The á subunit is entirely extracellular and it binds insulin, probably via a cystine rich domain.

i. The â subunit is a trans-membrane protein that performs the second major function of a receptor, i.e. signal transduction and insulin action.

j. Binding of insulin to the receptor stimulates its tyrosine kinase activity. Tyrosine kinase enzyme phosphorylates the phenolic -OH group of tyrosine residues in specific protein including that of a tyrosine in the chain of insulin receptor itself to modulate their activities, ATP + tyrosine protein → ADP + phosphotyrosine protein.

The cytoplasmic portion of p subunit has tyrosine kinase activity and an auto-phosphorylation site.

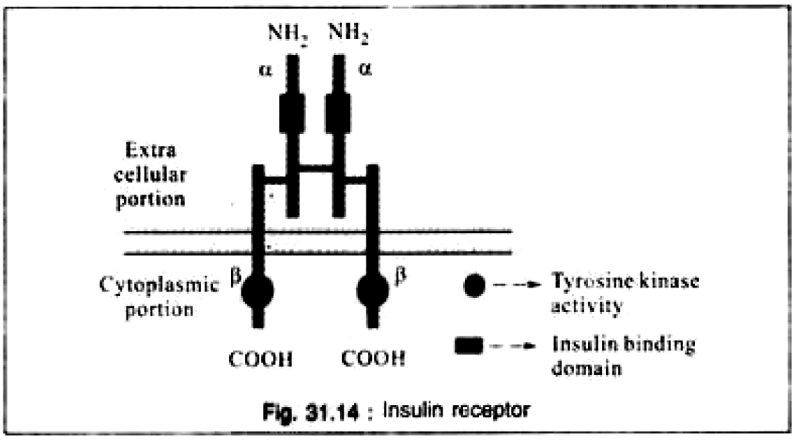


Fig. 31.14 : Insulin receptor

Insulin Secretion:

About 50 units of insulin are required per day. The human pancreas stores about 250 units. Normal concentration of insulin (fasting) in plasma: 6-126 μU/ml.

Factors Stimulating Insulin Secretion:

a. Increased blood glucose level causes an increase in insulin secretion and decreased blood glucose level depresses insulin secretion.

b. The hyperglycemia produced by glucagon enhances insulin production.

c. Since the growth hormone and glucocorticoids cause hyperglycemia they also stimulate insulin secretion.

d. Sugars which are readily metabolized— e.g., mannose and fructose—can stimulate insulin release. But non-metabolised sugars such as glactose, L-arabinose and xylose do not stimulate.

e. Many agents, such as amino acids, fatty acids and some gastro—intestinal products can stimulate insulin release only in presence of glucose.

f. Insulin secretion is enhanced by cAMP, ACTH and thyrotropin.

g. Amino acids particularly leucine and arginine can stimulate pancreas to produce insulin in both vivo and vitro. Proteins like casein also increases secretion of insulin.

h. Central nervous system indirectly influences the release of insulin. Vagal stimulation causes an increase in insulin secretion.

i. Sulfonylureas, the hypoglycemic agent, may act on insulin secretion by a different mechanism than that of glucose.

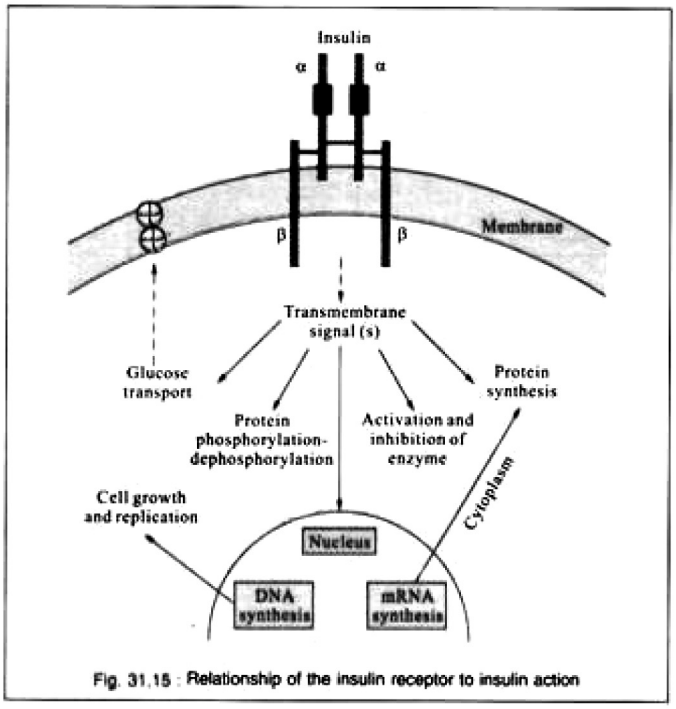


Fig. 31.15 : Relationship of the insulin receptor to insulin action

Factors Inhibiting Insulin Secretion:

a. Epinephrine is the highly effective inhibitor of insulin secretion.

b. Starvation reduces insulin secretion.

c. Magnesium also inhibits insulin secretion.

d. Vagotomy reduces insulin secretion.

Metabolism of Insulin:

a. Insulin is degraded in liver and kidney by the enzyme glutathinone insulin trans-hydrogenase which brings about reductive cleavage of the S-S bonds that connect A and B chains of the insulin molecule. Reduced glutathione acts as a coenzyme.

b. The A and B chains are further degraded by proteolysis. But when insulin is bound to antibody, it is much less sensitive to enzymic degradation.

Functions of Insulin:

a. Insulin is firmly bound to the highly specific receptor site present in the cell membrane. The receptor may probably be a glycoprotein. The biologic activities of insulin's are proportionate to their binding affinities. Insulin, thus, may carry out most of its function without entering the cell. The number of receptors declines where insulin levels are high.

b. Insulin exhibits transport at the membrane site, RNA synthesis at the nuclear site, translation at the ribosome for protein synthesis, and influence on tissue levels of cAMP. It is active in skeletal and heart muscle, adipose tissue, liver, the lens of the eye and leukocytes. It is inactive in renal tissue, red blood cells and gastrointestinal tract. The most metabolic function is centered in the muscle, adipose tissue and liver.

c. It facilitates the transport of glucose and related monosaccharides, amino acids, potassium ion, nucleosides, inorganic phosphate, and calcium ion in muscle and adipose tissue.

d. In muscle for adipose tissue, insulin increases the entry of glucose and thus leads to increased glycogen deposition, stimulation of HMP shunt resulting in increased production of NADPH, increased glycolysis, increased oxidation (Increase in oxygen uptake and CO_2 production), and increased fatty acid synthesis.

e. In adipose tissue, it increases lipid synthesis by means of fatty acid synthesis and glycerophosphate for triacylglycerol synthesis.

f. Insulin increases intracellular concentration of non-metabolized sugars such as galactose, L-arabinose, and xylose. The hormone facilitates the entry of those sugars having the same configuration at carbons, 1, 2, and 3 as D-glucose. Since fructose having a ketone group at position 2 is not transported by insulin. Intracellular transport of glucose is enhanced by anoxia indicating that glucose transport requires energy.

g. It also increases the uptake of nonmetabolizable amino acids such as alpha-aminoisobutyrate. It maintains muscle protein by decreasing protein degradation.

h. In adipose tissue, it quickly depresses the liberation of fatty acids caused by epinephrine or glucagon.

i. Insulin directly increases protein synthesis as the hormone promotes the incorporation of labelled intracellular amino acids into protein. At the ribosomal level, it increases the capacity of this organelle to translate information from messenger RNA to the protein-synthesizing machinery.

j. In the liver, it stimulates glycolysis by increasing the synthesis of glucokinase, phosphofructokinase. and pyruvate kinase. It also depresses the enzymes controlling gluconeogenesis such as pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-di-phosphatase, and glucose-6-phosphatase. Enzymes which are unimportant in the control of gluconeogenesis as well as glycolysis are not affected by insulin.

Pathophysiology of Insulin:

a. About 90% of persons with diabetes have non-insulin dependent (Type II) diabetes mellitus (NIDDM). Such patients are usually obese, have elevated plasma insulin levels.

b. The other 10% have insulin dependent (Type 1) diabetes mellitus (IDDM).

c. A few individuals produce antibodies directed against their insulin receptors. These antibodies prevent insulin from binding to the receptor so that such persons develop a syndrome of severe insulin resistance.

d. Tumors of â-cell origin cause hyperinsulinism thereby hypoglycemia. Leprechaunism is caused by the role of insulin in organogenesis. The syndrome is characterized by low birth weight, decreased muscle mass, decreased subcutaneous fat, and early death.

Abnormal Metabolism in Diabetic States:

a. In diabetes, hyperglycemia occurs due to the impaired transport and uptake of glucose into muscle and adipose tissue. Transport and uptake of amino acids are also depressed causing the raised level of amino acids into the blood, particularly, alanine, which supply fuel for gluconeogenesis in the liver. The amino acid breakdown during gluconeogenesis increases the production of urea nitrogen.

b. Lipid and fatty acid synthesis is decreased due to the decrease in acetyl-CoA, ATP, NADPH and glycerophosphate in all tissues. Stored lipids are hydrolysed by increased lipolysis and the liberated fatty acids interfere the carbohydrate phosphorylation in muscle and liver developing hyperglycemia.

c. Fatty acids in high concentration reaching the liver inhibit fatty acid synthesis by a feedback inhibition at the acetyl-CoA carboxylase step. Increased acetyl-CoA from fatty acids activates pyruvate carboxylase, stimulating gluconeogenic pathway for the conversion of amino acid carbon skeletons to glucose. Fatty acids also stimulate gluconeogenesis by entering the citric acid cycle and increasing production of citrate which is an inhibitor

of glycolysis (at phosphofructokinase).Thus, the fatty acid cycle at the level of citrate synthetase and pyruvate and isocitrate dehydrogenases. The acetyl CoA, which cannot enter the citric acid cycle or cannot be used for fatty acids synthesis, is utilized in the synthesis of cholesterol or ketones or both. The rise in ketone bodies concentration in body fluids and tissues leads to acidosis.

d. Glycogen synthesis is diminished due to decreased glycogen synthetase activity, increased phosphorylase activity and increased ADP: ATP ratio. The phosphorylase activity is stimulated by epinephrine or glucagon.

e. The insulin deficiency causes hormonal imbalance and favours the action of corticosteroids, growth hormone and glucagon which enhance gluconeogenesis, lipolysis, and decreased intracellular metabolism of glucose. The excess glucose in the urine requires water to be excreted out causing dehydration.

f. In the degradation in insulin, both liver and kidney are required. Therefore, in renal or hepatic disease, insulin requirement is decreased. This is observed in some diabetics with associated kidney or liver disease.

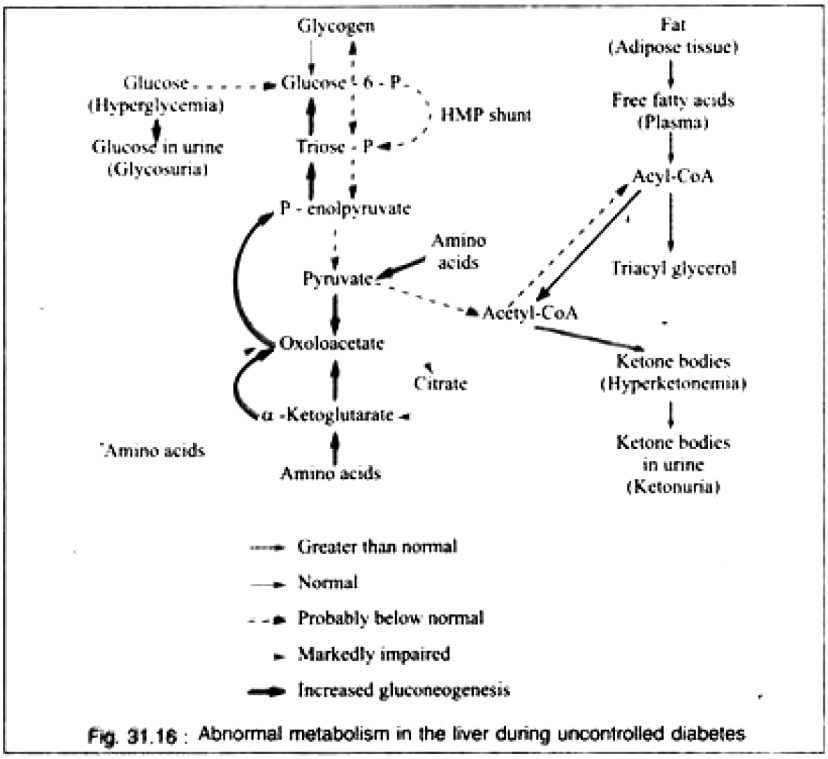


Fig. 31.16 : Abnormal metabolism in the liver during uncontrolled diabetes

Antibodies in Insulin:

a. The repeated injection of insulin produces low levels of an antibody to insulin in all subjects after 2 or 3 months of treatment.

b. The antibodies can produce lesions in the islet cells and severe diabetes.

c. Antibody-bound insulin is only slowly degraded; thus much of the insulin is actually wasted.

Experimental Diabetes:

a. Experimental diabetes can be produced by total pancreatectomy or by a single injection of alloxan, a substance related to the pyrimidine's or with streptozocin, an N-nitroso derivative of glucosamine.

b. Diabetes can also be produced by injection of diazoxide, a sulfonamide derivative which inhibits insulin secretion.

c. The injection of large amounts of antibodies to insulin is also considered to produce experimental diabetes.

d. Phlorhizin diabetes can be produced by the injection of the drug phlorhizin. This is actually a renal diabetes in which glycosuria is only produced by the failure of the reabsorption of glucose by the renal tubules.

Regulation of Insulin Secretion:

40 to 50 units of insulin is daily secreted from human pancreas. This represents about 15 to 20 per cent of the hormone stored in the gland. Insulin secretion is an energy-requiring process. Different factors are involved in insulin release.

a. Glucose:

(a) The increased concentration of glucose is the best regulator of insulin secretion.

(b) Among two ideas, one idea suggests that glucose combines "with a receptor which" is located on the B cell membrane that activates the release mechanism. The second idea suggests that intracellular metabolites pass through a pathway.such as the HMP shunt, the TCA cycle, etc.

b. Hormonal Factors:

(i) Epinephrine inhibits insulin release.

(ii) Beta adrenergic agonists stimulate insulin release by increasing intracellular cAMP.

(iii) Cortisol, estrogens, and progestin's also increase insulin secretion. Hence, insulin secretion is markedly increased during the later stages of pregnancy.

c. Pharmacologic Agents:

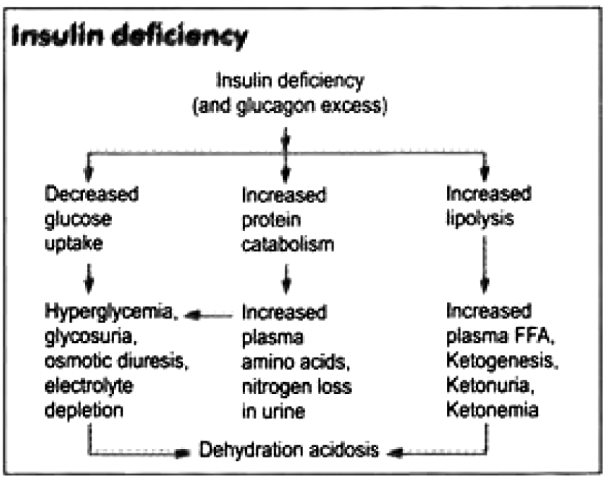
(i) Many drugs stimulate insulin secretion, but the sulfonyl urea compounds are used for therapy in humans.

(ii) Drugs such as tolbutamide stimulate insulin release and effectively used in the treatment of type II (non-insulin-dependent) diabetes mellitus. This class of drug is binded by a receptor which has been derived from the pancreatic P cells.

Effect of Insulin on Gene Expression:

(i) The actions of insulin are found to occur at the plasma membrane level or in the cytoplasm.

(ii) The synthesis of phosphoenolpyruvate carboxykinase (PEPCK) which catalyses a rate-limiting step in gluconeogenesis is decreased by insulin and hence gluconeogenesis is decreased.



(iii) Transcription is decreased due to the decreased amount of the primary transcript and of mature mRNA PEPCK which in turn is directly related to the decreased rate of PEPCK synthesis.

(iv) More than 100 specific mRNAs are affected by insulin, and a number of mRNAs in liver, adipose tissue, skeletal muscle, and cardiac muscle.

Probable Questions:

1. Write down the structure of Growth hormone.
2. What are the main functions of GH?
3. How hGH synthesis is regulated?
4. What is the mechanism of action of hGH?
5. State the effect of high and low secretion of hGH?
6. Write down the structure of insulin.

7. How biosynthesis is occurred of Insulin.
8. State the factors which stimulates insulin secretion.
9. State the factors which inhibits insulin secretion.
10. What are the functions of Insulin.
11. Describe the pathophysiology of insulin.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XIII

Biosynthesis and function of steroid and thyroid hormones (T3 and T4) and their regulations

Objective: In this unit we will discuss about various aspects of steroid and thyroid hormones.

Steroid hormones:

Steroid, any of a class of natural or synthetic organic compounds characterized by a molecular structure of 17 carbon atoms arranged in four rings. Steroids are important in biology, chemistry, and medicine. The steroid group includes all the sex hormones, adrenal cortical hormones, bile acids, and sterols of vertebrates, as well as the moulting hormones of insects and many other physiologically active substances of animals and plants. Among the synthetic steroids of therapeutic value are a large number of anti-inflammatory agents, anabolic (growth-stimulating) agents, and oral contraceptives.

Different categories of steroids are frequently distinguished from each other by names that relate to their biological source—e.g., phytosterols (found in plants), adrenal steroids, and bile acids—or to some important physiological function—e.g., progesterones (promoting gestation), androgens (favouring development of masculine characteristics), and cardiotonic steroids (facilitating proper heart function).Steroids vary from one another in the nature of attached groups, the position of the groups, and the configuration of the steroid nucleus (or gonane). Small modifications in the molecular structures of steroids can produce remarkable differences in their biological activities.

Transport of Steroid Hormones:

Steroid hormones are transported through the blood by being bound to carrier proteins—serum proteins that bind them and increase the hormones' solubility in water. Some examples are sex hormone-binding globulin (SHBG), corticosteroid-binding globulin, and albumin. Most studies say that hormones can only affect cells when they are not bound by serum proteins. In order to be active, steroid hormones must free themselves from their blood-solubilizing proteins and either bind to extracellular receptors, or passively cross the cell membrane and bind to nuclear receptors. This idea is known as the free hormone hypothesis. This idea is shown in Figure 1 to the right.

One study has found that these steroid-carrier complexes are bound by megalin, a membrane receptor, and are then taken into cells via endocytosis. One possible pathway

is that once inside the cell these complexes are taken to the lysosome, where the carrier protein is degraded and the steroid hormone is released into the cytoplasm of the target cell. The hormone then follows a genomic pathway of action. This process is shown in Figure 2 to the right. The role of endocytosis in steroid hormone transport is not well understood and is under further investigation.

In order for steroid hormones to cross the lipid bilayer of cells they must overcome energetic barriers that would prevent their entering or exiting the membrane. Gibbs free energy is an important concept here. These hormones, which are all derived from cholesterol, have hydrophilic functional groups at either end and hydrophobic carbon backbones. When steroid hormones are entering membranes free energy barriers exist when the functional groups are entering the hydrophobic interior of membrane, but it is energetically favourable for the hydrophobic core of these hormones to enter lipid bilayers. These energy barriers and wells are reversed for hormones exiting membranes. Steroid hormones easily enter and exit the membrane at physiologic conditions. They have been shown experimentally to cross membranes near a rate of 20 μm/s, depending on the hormone.

Though it is energetically more favourable for hormones to be in the membrane than in the ECF or ICF, they do in fact leave the membrane once they have entered it. This is an important consideration because cholesterol—the precursor to all steroid hormones—does not leave the membrane once it has embedded itself inside. The difference between cholesterol and these hormones is that cholesterol is in a much larger negative Gibb's free energy well once inside the membrane, as compared to these hormones. This is because the aliphatic tail on cholesterol has a very favourable interaction with the interior of lipid bilayers.

Mechanisms of action and effects:

There are many different mechanisms through which steroid hormones affect their target cells. All of these different pathways can be classified as having either a genomic effect, or a non-genomic effect. Genomic pathways are slow and result in altering transcription levels of certain proteins in the cell; non-genomic pathways are much faster.

a. Genomic pathways

The first identified mechanisms of steroid hormone action were the genomic effects. In this pathway, the free hormones first pass through the cell membrane because they are fat soluble. In the cytoplasm, the steroid may or may not undergo an enzyme-mediated alteration such as reduction, hydroxylation, or aromatization. Then the steroid binds to a specific steroid hormone receptor, also known as a nuclear receptor, which is a large metalloprotein. Upon steroid binding, many kinds of steroid receptors dimerize: two receptor subunits join together to form one functional DNA-binding unit that can

enter the cell nucleus. Once in the nucleus, the steroid-receptor ligand complex binds to specific DNA sequences and induces transcription of its target genes.

b. Non-genomic pathways

Because non-genomic pathways include any mechanism that is not a genomic effect, there are various non-genomic pathways. However, all of these pathways are mediated by some type of steroid hormone receptor found at the plasma membrane. Ion channels, transporters, G-protein coupled receptors (GPCR), and membrane fluidity have all been shown to be affected by steroid hormones. Of these, GPCR linked proteins are the most common. For more information on these proteins and pathways, visit the steroid hormone receptor page.

Biosynthesis of Cholesterol:

The main steps of the biosynthesis of cholesterol are diagrammatically represented in figure 5-22. The first reaction consists of the condensation of 2 molecules of acetyl-coA. It is the reverse of the reaction which takes place during the last turn of the helix in β-oxidation.

Then a third molecule of acetyl-coA binds to the acetoacetyl-coenzyme A thus formed which gives β-hydroxy-β-methyl-glutaryl coenzyme A (HMG coA). This binding of an acetyl-coenzyme A to a carbonyl group is similar to the reaction permitting the entry of acetyl-coenzyme A in the Krebs cycle by condensation on oxaloacetic acid. The reduction of the acid group (engaged in a thioester linkage) to alcohol, catalyzed by HMG coA reductase, gives mevalonic acid. It must be noted that all the carbon atoms of cholesterol originate from acetyl- coenzyme A.

A pyrophosphate group will then bind to the primary alcohol group of mevalonic acid. Mevalonyl-pyrophosphate will react with a third molecule of ATP; this reaction gives an unstable compound which decomposes spontaneously, losing the tertiary alcohol group and the free carbonyl group. An isoprene derivative with 5 carbon atoms is formed, isopentenyl-pyrophosphate which can be isomerized to dimethyl-allyl-pyrophosphale. The condensation of 2 fragments in C_5 gives geranyl-pyrophosphate (C_{10}), and after the binding of a third fragment in C_5, farnesyl-pyrophosphate (C_{15}) is obtained. The dimerization of the latter leads to squalene (C_{30}).

In vertebrates, the cyclization of squalene by squalene-oxidocyclase takes place by a series of reactions requiring molecular oxygen and a reducing coenzyme like NADPH, and leads to lanosterol. The passage from lanosterol to cholesterol lakes place through several parallel pathways. The most important intermediates are desmosterol and 7-dehydrocholesterol, immediate precursors of cholesterol. The isoprene derivatives with 5 carbon atoms are the precursors of dolichols, of the side chain of ubiquinone and vitamin K, of the isopentenyl group of some tRNAs.

In vertebrates, the biosynthesis of cholesterol is microsomal. It is regulated by a feedback inhibition mechanism by a metabolite of cholesterol (most probably the 25 hydroxycholesterol) acting on the HMG coA reductase. This feedback inhibition is never total to always permit the synthesis of polyisoprenoids important for other metabolisms, like the dolichols. The liver is one of the principal sites of synthesis. Cholesterol is then carried to other organs in the form of lipoproteins. It enters the cells by binding of the lipoprotein to a specific receptor. In physiological conditions the exogenous input of hepatic cholesterol to various tissues is sufficient to inhibit endogenous synthesis in these tissues.

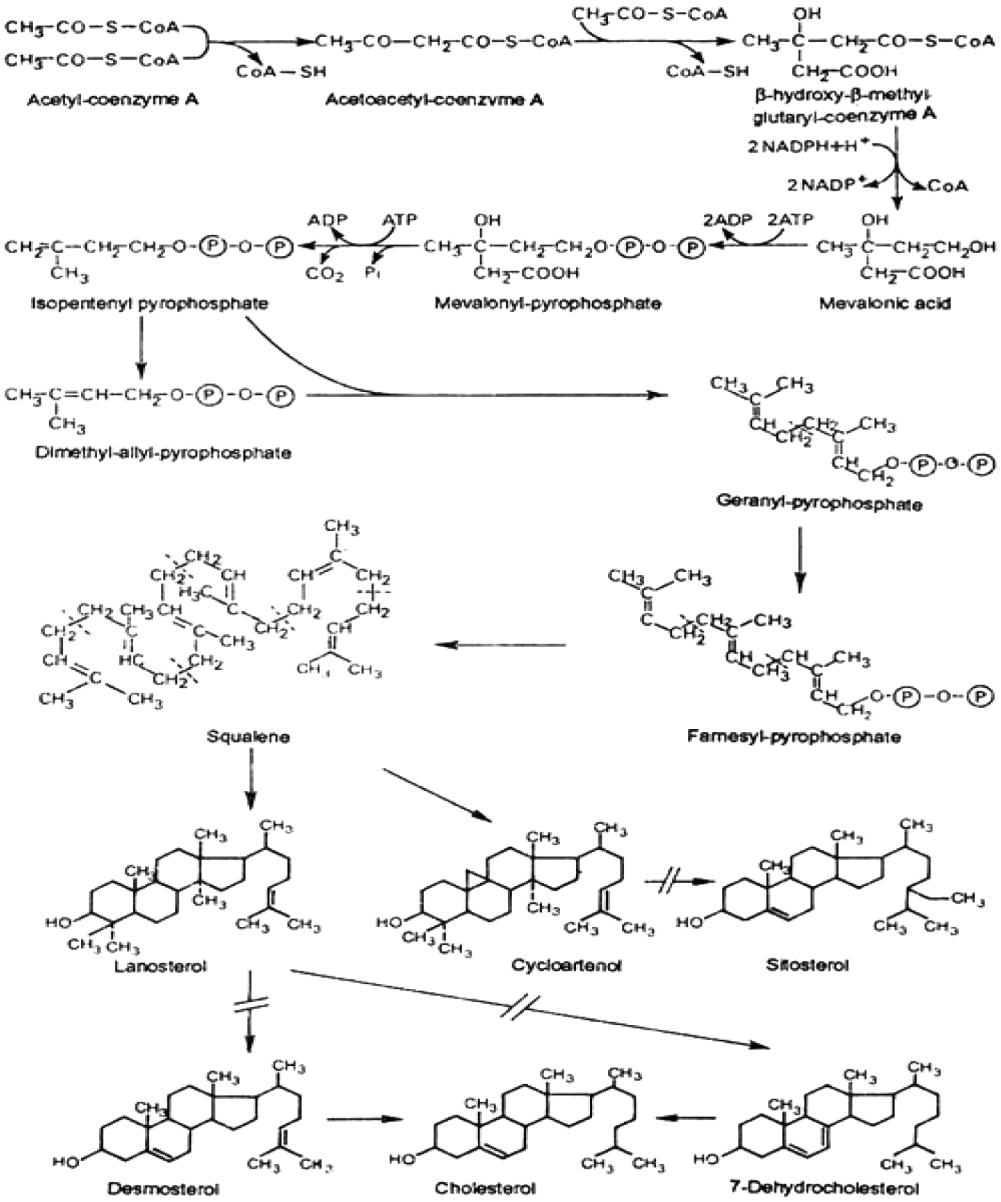


FIG. 5-22. — The principal steps of the biosynthesis of cholesterol.

Insects are capable of synthesizing squalene, but they cannot cyclize it. They use the sterols (animal or plant) present in their food and are capable of metabolizing them to cholesterol, precursor of hormonal derivatives like ecdysone.

Prokaryotes also synthesize squalene. Squalcne-hopene cyclase which carries out the cyclization is an enzyme which does not require oxygen.

Formation of Other Steroids:

Cholesterol is the starting point of the synthesis of various steroids:

1. Progesterone is secreted by the corpus luteum, the placenta and the cortex of the adrenal gland, and acts mainly in the uterus to permit implantation and gestation;

2. Aldosterone, a hormone secreted by the adrenal cortex, which permits the reabsorption of sodium (and secondarily of chlorine and water) in the kidney, hence its name, mineralocorticosteroid;

3. Cortisol and cortisone, also secreted by the adrenal cortex, sometimes called glucocorticosteroids, because they stimulate protein catabolism and neoglucogenesis in the liver (they are therefore hyperglycemic). They also act on conjunctive and limphoid tissues by depressing membrane permeability and opposing the inflammatory processes (which explains their use in therapeutics).

The synthesis of cortisol (and therefore of cortisone) is stimulated by the corticotropic hormone of the anterior lobe of the pituitary gland or ACTH (Adreno Cortico Tropic Hormone):

1. Testosterone, secreted mainly by the testicles, is responsible for the various male sexual characters;

2. Estrogenic hormones (estradiol and estrone), responsible for the various female sexual characters, synthesized mainly in the ovary and placenta, and characterized — from the structural point of view — by a phenolic ring.

It may be observed that it is relatively easy to pass (in few steps) from progesterone to other hormones having very different physiological properties; in other words, in this family of steroid hormones, small structures modifications correspond to large differences in biological activity.

We mentioned that the synthesis of Cortisol and cortisone by the adrenal glands is influenced by ACTH, a hormone of anterior lobe of the pituitary gland; we must indicate that the secretory activities of ovaries and testicles are also controlled by the hormones

of the anterior lobe of the pituitary gland called gonadotropins, like FSH (Follicule Stimulating Hormone) and LH (Luteinizing Hormone), for example.

Moreover, this anterior lobe also secretes other stimulines, like the growth hormone or somatotropic hormone, and thyrotropic hormone (TSH), which stimulates the synthesis of thyroid hormones by the thyroid gland.

Chemists sometimes classify the steroid hormones according to the number of carbon atoms contained in their molecules and thus distinguish:

1. C_{18} hormones (estradiol, estrone);

2. C_{19} hormones (testosterone);

3. C_{21} hormones (progesterone and most of the hormones secreted by the cortical part of adrenal glands, the mineralocorticoids like aldosterone as well as the glucocorticoids like Cortisol and cortisone).

Vitamins D are formed by the opening of the B cycle due to ultraviolet light, either from ergosterol (vitamin D_2), or from 7-dehydro-cholesterol (which gives vitamin D_3).

Mode of Steroid Hormone Action through Intracellular Receptors:

Steroid hormones are lipid-soluble and easily pass through the cell membrane of a target cell into the cytoplasm. In the cytoplasm they bind to specific intracellular receptors (proteins) to form a hormone receptor complex that enters the nucleus.

In the nucleus, hormones which interact with intracellular receptors (e.g., steroid hormones, iodothyromines, etc.) mostly regulate gene expression or chromosome function by the interaction of hormone-receptor complex with the genome.

Biochemical actions result in physiological and developmental effects (tissue growth and differentiation, etc.). In-fact the hormone receptor complex binds to a specific regulatory site on the chromosome and activates certain genes (DNA).

The activated gene transcribes mRNA which directs the synthesis of proteins and usually enzymes in the cytoplasm. The enzymes promote the metabolic reactions in the cell. The actions of lipid soluble hormones are slower and last longer than the action of water- soluble hormones.

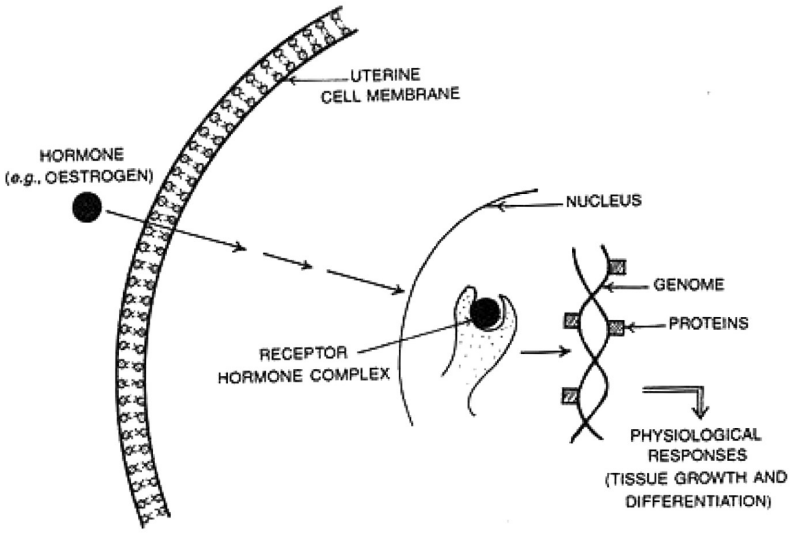


Fig. 22.20. Diagrammatic representation of the mechanism of Steroid hormone.

Thyroid Hormones:

Location and Structure of Thyroid Gland:

The thyroid gland is the largest endocrine gland located anterior to the thyroid cartilage of the larynx in the neck. The gland is well supplied with blood vessels. It is bilobed organ. The two lobes are connected by a narrow structure called the isthmus. The microscopic structure of the thyroid gland shows thyroid follicles composed of cubical epithelium and filled with a homogenous material called colloid.

Small amount of loose connective tissue forms stroma of the gland. Besides containing blood capillaries, the stroma contains small clusters of specialized Para follicular cells or 'C' cells. The thyroid gland is the only gland that stores hormones in large quantities for about two months.

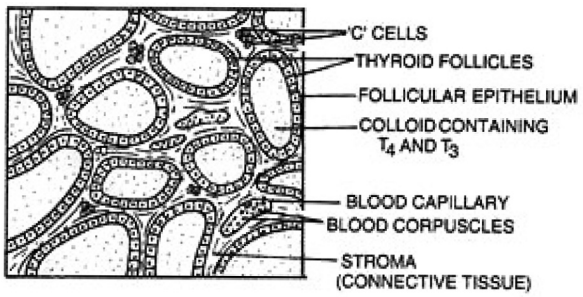


Fig. 22.2. T.S. Thyroid gland.

Hormones of Thyroid Gland:

The thyroid gland secretes three hormones. Thyroxine (tetraiodothyronine or T_4), and triiodothyronine or T_3 are secreted by the thyroid follicular cells. Thyrocalcitonin

is secreted by the Ñ-cells of the thyroid gland. This gland is stimulated to secrete its hormones by thyroid stimulating hormone (also called thyrotropin) secreted by the anterior lobe of pituitary gland.

(I) Thyroxine (T_4) and Triiodothyronine (T_3):

T_4 and T_3 contain four and three atoms of iodine respectively, therefore, they are named so. T_3 is secreted in smaller amounts but it is more active and several times more potent than T_4. T_4 is converted to T_3 by removal of one iodine in the liver, kidneys and some other tissues. Since both T_4 and T_3 have similar effects on the target cells, they are generally considered together under the name, thyroid hormone (TH).

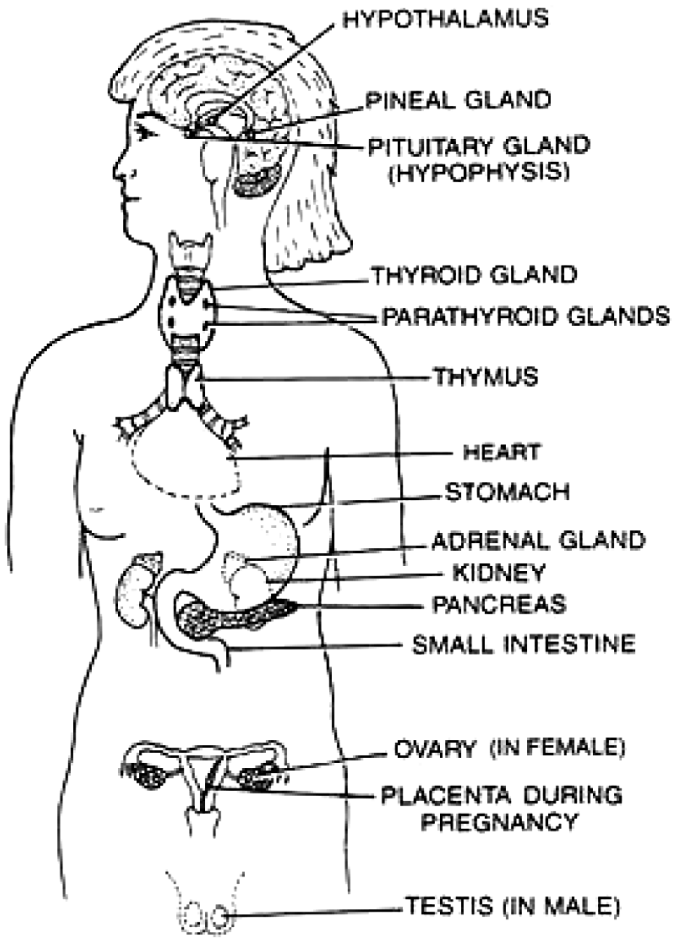


Fig. 22.3. Human endocrine glands.

The thyroid gland is the only gland that stores its hormones in large quantity. T_4 and T_3 are synthesised by attaching iodine to tyrosine amino acid.

The steps in the biosynthesis of the hormone are (Fig. 6.33):

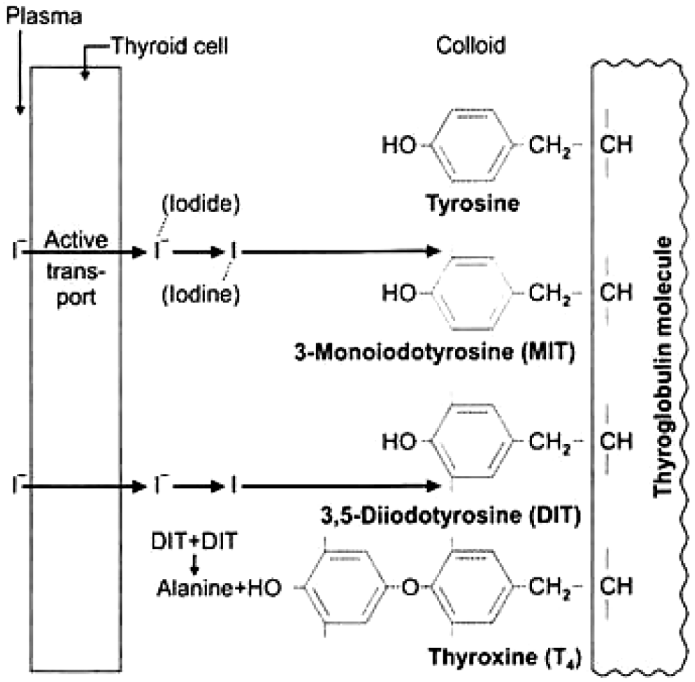


Fig. 6.33: Steps in the biosynthesis of thyroxine

i. Iodide trappingthat is the uptake of iodide by the follicular cells from the plasma against the electrochemical gradient. The hormone TSH secreted by the anterior pituitary gland affects this step. Substances, like thiocyanate, pertechnetate and perchlorate that are examples of antithyroid drugs can inhibit iodide trapping.

ii. Oxidation of iodine: It occurs inside the follicular cells by the action of the enzyme peroxidase. Drugs like thiouracil and carbimazole can inhibit this step and act as antithyroid drugs.

iii. Organification: Iodine gets incorporated to tyrosine amino acid present in the colloid and leads to the formation of MIT (Monoiodotyrosine). On further iodination of MIT, there is formation of DIT (Di- iodotyrosine).

iv. Coupling: Coupling of 2 DIT will lead to the formation of T_4 and 1 MIT with 1 DIT will results in T_3. After the synthesis, the hormone with thyroglobulin is stored in the colloid. There are many substances which have the ability to decrease the amount of thyroxin secreted by the gland. These drugs will be of choice when there is a necessity to decrease the amount of thyroxine secretion in certain pathological situations.

Steps involved in hormonopoiesis of thyroxine:

1. Iodide trapping (active process)

2. Conversion of iodide to molecular iodine. Peroxidase is the enzyme involved.

3. Organification of tyrosine to form MIT and DIT—iodinase.

4. Oxidative coupling of

MIT + DIT—to form T_3

DIT + DIT—to form T_4

5. Proteolytic separation of T_3 and T_4 from thyroglobulin— deiodinase

Table 6.6: Transport of thyroxine in plasma

Proteins available	Quantity of proteins (mg/dl)	Affinity	Transported bound %	
			T4	T3
TBG	2	+++++	67	46
TBPA	15	++	20	1
Albumin	3500	+	13	53

At the time of release of the hormones into circulation, the acinar cells will engulf the thyroglobulin along with the hormones by endocytosis.In the cells, the hormone will be separated by proteolysis and released into the circulation and thyroglobulin will be retained for further use.Most of the hormone in circulation is in protein bound form along with thyroid binding globulin (TBG), albumin (TBA), thyroid binding pre-albumin (TBPA) (Table 6.6).

Protein Bound Iodine (PBI) in Blood:

The term PBI in blood represents iodine present in thyroid hormones. The PBI values for normal adults are 3.5-7.5 mg/100 ml of plasma. PBI is a reliable measure of thyroxine content of plasma

The values for PBI in hypo- and hyperthyroidism are given below:

Myxoedema (hyperthyroidism)	**0.2-2.5 mg/100 ml**
Grave's disease (hyperthyroidism)	**8-18 mg/100 ml**

The functions of thyroxine (T_4) and tri-iodothyronine (T_3) are as follows.

(a) They regulate the metabolic rate of the body and thus maintain basal metabolic rate (BMR).

(b) They stimulate protein synthesis and, therefore, promote growth of the body tissues.

(c) They regulate the development of mental faculties.

(d) As they increase heat production, thus they maintain body temperature.

(e) They help in metamorphosis of tadpole into adult frog. If thyroid gland of the tadpole (larva) is removed, the larva fails to change into an adult.

(f) They increase action of neurotransmitters like adrenaline and noradrenaline.

(II) Thyrocalcitonin (TCT):

It is secreted when calcium level is high in the blood. It then lowers the calcium level by suppressing release of calcium ions from the bones. Thus calciton has an action opposite to that of the parathyroid hormone on calcium metabolism. Calcitonin is a peptide which contains 32 amino acids.

(f) They increase action of neurotransmitters like adrenaline and noradrenaline.

(III) Thyrocalcitonin (TCT):

It is secreted when calcium level is high in the blood. It then lowers the calcium level by suppressing release of calcium ions from the bones. Thuscalciton has an action opposite to that of the parathyroid hormone on calcium metabolism. Calcitonin is a peptide which contains 32 amino acids.

Regulation of Thyroid Hormone:

It is brought about by the negative feedback mechanism. There is involvement of hypothalamo- pituitary-thyroid axis (Fig. 6.35).

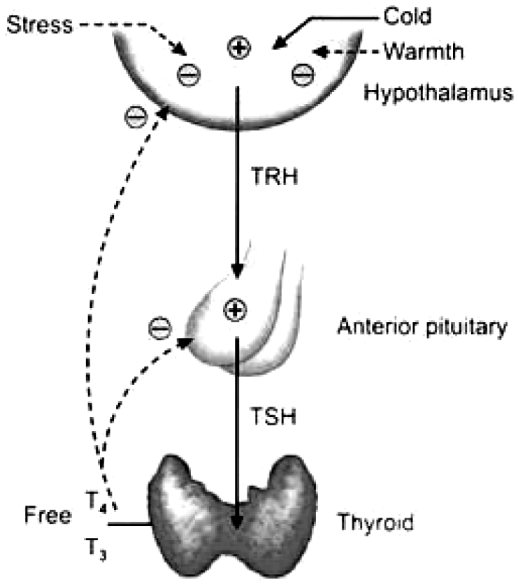


Fig. 6.35: Regulation of secretion of thyroxine (by negative feedback mechanism)

Increase in free form of hormone in circulation acts on hypothalamus and anterior pituitary gland. Acting on hypothalamus, it decreases the secretion of thyrotropin-

releasing hormone (TRF/TRH) and this acts on anterior pituitary decreases secretion of TSH.Net effect will be decreased TSH from anterior pituitary gland. This decreases the secretion of thyroid hormones from the gland.

Many of the other chemical influences acting on TRH-TSH-Thyroxine (hypothalamo-pituitary-thyroid axis) secretions have been shown in Table 6.7.

Table 6.7: Thyroid hormone feedback

Hypothalamus	*Stimulatory*	*Inhibitory*
Decreased TRH	Alpha adrenergic agonists	Alpha adrenergic blockers
		Tumors
Anterior pituitary	*TRH*	*Somatostatin*
Decreased TSH	Estrogen	Dopamine
		Glucocorticoids
		Chronic illness
Thyroid gland	*TSH*	*TSH receptor blocking antibody*
Decreased T_3 and T_4	TSH receptor stimulating antibody	Iodine, lithium

Alteration in the temperature can directly act on the hypothalamus to alter the secretion of the hormone

Disorders related to thyroid Hormones:

(A) Hyperthyroidism (Hyper secretion of thyroid hormone).

a. Exophthalmic goitre or Graves' disease or Basedow's disease or Parry's disease:

It is a thyroid enlargement (goitre) in which the thyroid secretes excessive amount of thyroid hormone. It is characterised by exophthalmia (protrusion of eye balls because of fluid accumulation behind them), loss of weight, slightly rise in the body temperature, excitability, rapid heartbeat, nervousness and restlessness.

Fig. 22.4. Graves' disease.

(B) Hypothyroidism (Hypo secretion of thyroid hormone):

(a) Cretinism:

This disorder is caused by deficiency of thyroid hormone in infants. A cretin has slow body growth and mental development of reduced metabolic rate.

Other symptoms of this disorder are slow heart beat, lower blood pressure, decrease in temperature, stunted growth, pot-belly, pigeon chest and protruding tongue and retarded sexual development. This disease can be treated by an early administration of thyroid hormones.

Fig. 22.5. Cretinism.

(b) Myxoedema or Gull's disease:

It is caused by deficiency of thyroid hormone in adults. This disease is characterized by puffy appearance due to accumulation of fat in the subcutaneous tissue because of low metabolic rate. The patient lacks alertness, intelligence and initiative. He also suffers from slowheart beat, low body temperature and regarded sexual development. This disease can be treated by administration of thyroid hormones.

Fig. 22.6. Myxoedema.

(c) Simple Goitre:

It is caused by deficiency of iodine in diet because iodine is needed for the synthesis of thyroid hormone. It causes thyroid enlargement. It may lead to cretinism or myxoedema. This disease is common in hilly areas. Addition of iodine to the table salt prevents this disease.

Fig. 22.7. Simple goitre.

(d) Hashimoto's disease:

In this disease all the aspects of thyroid function are impaired. It is an autoimmune disease in which the thyroid gland is destroyed by autoimmunity.

Probable Questions:

1. Describe the steps of cholesterol biosynthesis.
2. How steroid hormones are transported?
3. What are the mechanism of action of steroid hormones?
4. How secretion of steroid hormones are regulated?
5. Briefly describe the location and structure of thyroid gland.
6. How T3 and T4 hormones aresynthesised in thyroid gland?
7. How thyroid hormone secretions are regulated?
8. What are the effects of thyroxine hypo secretion?
9. What are the effects of thyroxine hyper secretion?

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XIV

Physiological role of hormones: hormonal regulation of mineral metabolism and fluid volume

Objective: In this unit you will learn about role of hormones in mineral metabolism and fluid volume regulation.

Water Metabolism:

Distribution of water in the body:

Water is the major constituent of human body. The average body water is 50-70% of the body weight. Females have little less water than males.

1. The water content of intracellular fluid is 50% of total body weight.
2. The water content of extracellular fluid is 20% of the body weight, which is distributed as follows:

Plasma	4.5%
Interstitial and lymph fluid	8%
Dense connective tissue, cartilage and bone	6%
Transcellular fluids (found in salivary glands, liver, pancreas, thyroid gland, gonads, skin, mucous membranes of the respiratory and gastrointestinal tracts, kidneys, fluid spaces in eye, CSF etc)	

Factors influencing the distribution of body water:

The distribution of water is continuously changing. Osmotic forces are the principal factors for controlling the amount of fluid in various compartments of the body. These are maintained by the solutes of the body. Solutes are of three types.

1. Organic molecules of small molecular size (glucose, urea, amino acids etc.):

Since these diffuse freely across the cell membrane, they are not important in the distribution of water. If they are present in large quantities, they can help retaining water.

2. Organic substances of large molecular size (proteins):

These substances can throw effect in the transport of fluids from one compartment to the other.

These inorganic electrolytes are the most important both in the distribution and in the retention of body water.

Intake and Loss of Body Water:

A. Water intake:

Water is supplied to the body by the following processes:

1. Water taken orally.

2. Along with food.

3. Oxidation of food stuffs i.e. fats, proteins and carbohydrates yield water after combustion.

B. Water loss:

Water is lost from the body by 4 routes

1. Evaporation from lungs.

2. Kidneys eliminate water as urine.

3. The intestines excrete in the feces.

4. Perspiration.

C. Additional water loss in diseases:

1. Water loss is more in diarrhea and vomiting. These losses can be fatal in infants.

2. In kidney disease, renal water loss is more.

3. In fever, insensible losses may rise much higher than normal.

4. Patients in high environmental temperatures sustain extremely high external water loss.

Water Balance:

An equilibrium persists between the intake and output of water in the body. In addition to other factors, certain hormones such as ADH, vasopressin, oxytocin and aldosterone influence the regulatory mechanism.

Balance sheet of water

Water intake (ml/day)		Water loss (ml/day)	
Drinks	1350	Urine	1500
Solid food	900	Lungs	500
Oxidation of food	450	Skin	600
		Feces	100
TOTAL	2700	TOTAL	2700

There is a continuous excretion of water in the form of digestive juices from the body into the alimentary canal. This water (except 100 ml) is reabsorbed with the water of the food and drinks. The amount of this internal secretion is 7 to 10 liters/day.

Physiological Functions of Water:

1. Specific heat:

Heat is required to raise the temperature of 1 gm. of water through one degree Celsius is more than for almost any other solid or liquid. The high specific heat of water helps in minimizing the rise in body temperature due to the heat emitted out of chemical reactions.

2. Latent heat of evaporation:

Water has the highest latent heat of evaporation than any other liquid. A certain amount of water can cause maximum cooling by evaporation, so that body temperature does not rise.

3. Solvent power:

Water forms true solutions as well as colloidal solutions. Even water insoluble substances are made water soluble by the hydrotropic action. Therefore, it is the most suitable solvent for cellular components; water thus brings various substances in contact for chemical reactions to proceed.

4. Dielectric constant:

Oppositely charge particles can coexist in water. Therefore, it is a good ionizing medium. This stimulates the chemical reactions.

5. Catalytic action:

A large number of chemical reactions in the body are accelerated by water due to its ionizing power. All chemical reactions in the body proceed in presence of water only.

6. Lubricating action:

Water acts as a lubricant in the body to prevent friction in joints, pleura, conjunctiva and peritoneum.

Regulation of Passage of Water:

1. If capillary pressure is increased, more water will flow into the tissues.
2. A fall in blood pressure helps in passage of water form the tissues to the blood.
3. If the plasma proteins are decreased, water will flow into the tissues.

4. Dilution of blood by excessive ingestion of water can lower the osmotic pressure of the plasma proteins and thus may increase capillary pressure.

Dehydration:

When the loss of water exceeds the intake, the body's water content is reduced. This means that the body is in negative water balance and the condition is known as dehydration.

Causes:

1. Primary dehydration:

(a) Deprivation of water during desert travel, extreme weakness and mental patients refraining to drinking water causes dehydration. Occurs more quickly in fever and in high environmental temperatures.

(b) Excessive water loss due to vomiting, prolonged diarrhoea, excretion of large quantities of urine and sweat. In water depletion, the concentration of extracellular fluid increases. Water is drawn from the cells and both extracellular and intracellular compartments shrink. Extreme thirst results; individual complains of hot and dry body. The tongue becomes dry.

2. Secondary dehydration:

Concentration of electrolytes of the body fluids is maintained constant through the elimination or retention of water. The reduction or increase in the total electrolytes which affects, chiefly the basic radical Na^+ (extracellular) or K^+ (intracellular) and the acid radicals HCO_3 and CI^- is accompanied by a corresponding increase or decrease in the volume of body water. This causes intracellular edema, slowing of circulation and impairment of urinal function. The individual becomes weak.

3. Dehydration due to injection of hypertonic solution:

When a highly concentrated sugar or salt solution is injected into the body, the osmotic pressure of blood will increase. This results in the flow of fluid from the tissues into the blood until equilibrium sets in. Consequently, the blood volume increases. This increased blood volume soon returns to normal by the loss of excess material through urination. This causes a net loss of body water producing dehydration.

Effects of dehydration:

1. Loss of weight due to the reduction in tissue water.
2. Disturbances in acid-base balance.

3. Rise in the non-protein nitrogen of blood.
4. Rise in the plasma protein concentration and of chloride.
5. Rise in body temperature due to reduction in circulating fluid.
6. Increased pulse rate and reduced cardiac output.
7. Dryness, wrinkling and looseness of skin.
8. Exhaustion and collapse.

Correction of dehydration:

1. Ordinary NaCl solution may be given parenterally to repair the losses.
2. In case of excretion of fluid high in Na and HCO_3 resulting in fluid and electrolyte loss, a mixture of 2/3 isotonic saline and 1/3 Na lactate should be administered intravenously.
3. Dehydration in diabetes mellitus, Addison's disease, uremia, extensive burns and shock cannot be corrected by the above methods.

Water intoxication:

Caused by excessive water retention due to renal failure, hyper secretion of ADH, excessive administration of fluids parenterally

Symptoms:

Headache, nausea and muscular weakness

Hormonal Control of water volume:

While the kidneys operate to maintain osmotic balance and blood pressure in the body, they also act in concert with hormones. Hormones are small molecules that act as messengers within the body. Hormones are typically secreted from one cell and travel in the bloodstream to affect a target cell in another portion of the body. Different regions of the nephron bear specialized cells that have receptors to respond to chemical messengers and hormones. Table 22.1 summarizes the hormones that control the osmoregulatory functions.

Table 22.1.Hormones That Affect Osmoregulation

Hormone	Where produced	Function
Epinephrine and Norepinephrine	Adrenal medulla	Can decrease kidney function temporarily by vasoconstriction
Renin	Kidney nephrons	Increases blood pressure by acting on angiotensinogen
Angiotensin	Liver	Angiotensin II affects multiple processes and increases blood pressure
Aldosterone	Adrenal cortex	Prevents loss of sodium and water
Anti-diuretic hormone (vasopressin)	Hypothalamus (stored in the posterior pituitary)	Prevents water loss
Atrial natriuretic peptide	Heart atrium	Decreases blood pressure by acting as a vasodilator and increasing glomerular filtration rate; decreases sodium reabsorption in kidneys

Epinephrine and Norepinephrine

Epinephrine and norepinephrine are released by the adrenal medulla and nervous system respectively. They are the flight/fight hormones that are released when the body is under extreme stress. During stress, much of the body's energy is used to combat imminent danger. Kidney function is halted temporarily by epinephrine and norepinephrine. These hormones function by acting directly on the smooth muscles of blood vessels to constrict them. Once the afferent arterioles are constricted, blood flow into the nephrons stops. These hormones go one step further and trigger the **renin-angiotensin-aldosterone** system.

Renin-Angiotensin-Aldosterone

The renin-angiotensin-aldosterone system, illustrated in Figure 22.15 proceeds through several steps to produce **angiotensin II**, which acts to stabilize blood pressure and volume. Renin (secreted by a part of the juxtaglomerular complex) is produced by the granular cells of the afferent and efferent arterioles. Thus, the kidneys control blood pressure and volume directly. Renin acts on angiotensinogen, which is made in the liver and converts it to **angiotensin I**. **Angiotensin converting enzyme (ACE)** converts angiotensin I to angiotensin II. Angiotensin II raises blood pressure by constricting blood

vessels. It also triggers the release of the mineralocorticoid aldosterone from the adrenal cortex, which in turn stimulates the renal tubules to reabsorb more sodium. Angiotensin II also triggers the release of **anti-diuretic hormone (ADH)** from the hypothalamus, leading to water retention in the kidneys. It acts directly on the nephrons and decreases glomerular filtration rate. Medically, blood pressure can be controlled by drugs that inhibit ACE (called ACE inhibitors).

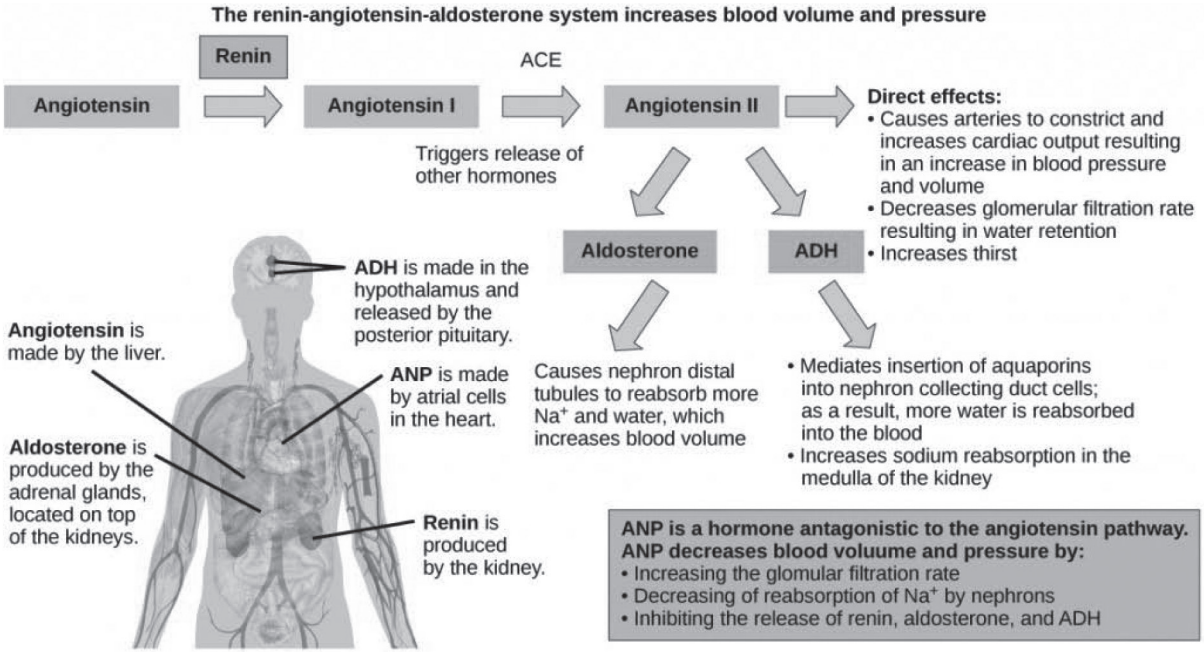


Figure 22.15. The renin-angiotensin-aldosterone system increases blood pressure and volume. The hormone ANP has antagonistic effects. (credit: modification of work by Mikael Häggström)

Mineralocorticoids

Mineralocorticoids are hormones synthesized by the adrenal cortex that affect osmotic balance. Aldosterone is a mineralocorticoid that regulates sodium levels in the blood. Almost all of the sodium in the blood is reclaimed by the renal tubules under the influence of aldosterone. Because sodium is always reabsorbed by active transport and water follows sodium to maintain osmotic balance, aldosterone manages not only sodium levels but also the water levels in body fluids. In contrast, the aldosterone also stimulates potassium secretion concurrently with sodium reabsorption. In contrast, absence of aldosterone means that no sodium gets reabsorbed in the renal tubules and all of it gets excreted in the urine. In addition, the daily dietary potassium load is not secreted and the retention of K^+ can cause a dangerous increase in plasma K^+ concentration. Patients who have Addison's disease have a failing adrenal cortex

and cannot produce aldosterone. They lose sodium in their urine constantly, and if the supply is not replenished, the consequences can be fatal.

Antidiurectic Hormone:

As previously discussed, antidiuretic hormone or ADH (also called **vasopressin**), as the name suggests, helps the body conserve water when body fluid volume, especially that of blood, is low. It is formed by the hypothalamus and is stored and released from the posterior pituitary. It acts by inserting aquaporins in the collecting ducts and promotes reabsorption of water. ADH also acts as a vasoconstrictor and increases blood pressure during hemorrhaging.

Atrial Natriuretic Peptide Hormone:

The atrial natriuretic peptide (ANP) lowers blood pressure by acting as a **vasodilator**. It is released by cells in the atrium of the heart in response to high blood pressure and in patients with sleep apnea. ANP affects salt release, and because water passively follows salt to maintain osmotic balance, it also has a diuretic effect. ANP also prevents sodium reabsorption by the renal tubules, decreasing water reabsorption (thus acting as a diuretic) and lowering blood pressure. Its actions suppress the actions of aldosterone, ADH, and renin.

Mineral Metabolism

Living beings have organic and inorganic types of chemical constituents. The organic constituents i.e. proteins, carbohydrates, fats etc. are made up of C, H, O and N. The inorganic constituents described as 'minerals' comprise of the elements present in the body other than C, H, O and N. Although they constitute a relatively small amount of the total body tissues, they are essential for many vital processes.

There are 31 elements present in the body.

They are divided into two classes:

(1) Essential elements and

(2) Non-essential elements.

Essential elements:

Those which are essential to maintain the normal living state of a tissue.

They are again divided into two sub groups:

Macro elements:

They are required to be present in the diet, more than 1 mg.

Ex. Ca, P, Mg, Na, K, CI and S.

Micro elements:

They are 8 in number and utilized in trace quantities (in microgram or Nano-gram). Hence they are called trace elements. These are Fe, Cu, Zn, Co, Mo, F, I and Mn.

Non-essential elements:

They are 8 in number. They are present in tissues but their functions if any are not clearly defined. They include Al, B, Se, Cr, Br, As, Ti and Pb. Four additional elements, Ni, Tin, Vanadium and Silicon have been suggested as essential trace elements in nutrition but their implications for human nutrition are unknown.

The mineral elements present in the body are supplied in the diet. In poor diets consumed by a large majority of people, calcium and iron deficiency occur commonly. Iodine deficiency occurs in people living in certain hilly tracts, where the soil and water are deficient in iodine. In tropical countries, addition of sodium chloride in the diet is of great importance, because of the loss of NaCl in sweat. The deficiencies of other minerals do not occur normally in average diets.

i. Sodium, potassium and chlorine are involved mainly in the maintenance of acid-base balance and osmotic control of water metabolism.

ii. Calcium, phosphorus and magnesium are constituents of bone and teeth.

iii. Phosphorus is the constituent of body cells of the tissues, such as muscle, liver etc.

iv. Sulphur is present in cysteine, methionine, thiamine, biotin, lipoic acid and coenzyme A.

I. Calcium:

Source:

Milk (0.2 gm./100 ml) and cheese are important dietary sources. Other sources-are egg yolk, lentils, nuts, cabbage, cauliflower and asparagus, etc.

Requirement:

(1) Men and women after 18 years of age require 800 mg/day.

(2) During lactation and in pregnancy of 2^{nd} and 3^{rd} term 1.2 gm./day is required.

(3) Infants under 1 year require-360-540 mg/day.

(4) Children of 1-18 years need 800-1200 mg/day.

Absorption:

Calcium is taken in the diet as calcium phosphate, carbonate, tartarate and oxalate. Calcium is absorbed actively in the upper small intestine. The active process is regulated by 1,25 dihyrocholecalciferol, a metabolite of vitamin D which is produced in the kidney in response to low plasma Ca^{++} concentrations. Absorption of calcium by the intestine is never complete. Ca is absorbed by an active transport process occurring mainly in the upper small intestine.

Calcium absorption is influenced by the following factors:

1. Vitamin D promotes absorption of Ca.
2. Acidic pH favous calcium absorption because Ca salts (phosphate and carbonates) are quite soluble in acid solution arid are relatively insoluble in alkaline solutions. Hence an increase in acidophilic flora, e.g. lactobacilli is recommended to lower pH which favours the absorption of Calcium.
3. Organic acids, lactose and basic amino acids in the diet favour calcium absorption.
4. Higher levels of proteins in the diet help to increase the absorption of calcium. On a high protein diet, about 15% of the dietary calcium is absorbed, compared with 5% absorption on a low protein diet. Certain calcium salts are much more soluble in aqueous solution of amino acids than in water and thus absorption of calcium is increased in presence of amino acids.
5. If calcium: phosphorus ratio is much high, $Ca_3(PO_4)_2$ will be formed and absorption of calcium is reduced. The optimal ratio for both elements is about 1:1 (1:2 to 2:1) and with ratios outside these limits, absorption is decreased. This is because of formation of insoluble calcium phosphate.
6. When fat absorption is impaired much free fatty acids are formed due to hydrolysis. These fatty acids react with free calcium to form insoluble calcium soap and then Ca is lost in faeces.
7. Absorption of calcium is inhibited by a number of dietary factors that cause formation of insoluble calcium salts, i.e. phytate (cereal grain), oxalate, phosphate and iron, etc.
8. High concentration of Mg in the diet decreases absorption of Ca.
9. Presence of excess fibre in the diet interferes with the absorption of Ca.

10. Percentage of calcium absorption decreases as its intake increases.
11. Parathyroid hormone increases the intestinal absorption of calcium.
12. Adrenal glucocorticoids diminish intestinal transport of Ca.
13. After the age of 55 to 60 there is gradual diminution of intestinal transport of calcium. During menopause many women develop negative calcium-phosphorus balance leading to a type of osteoporosis. This is usually accompanied by pain and fractures. The negative balance of calcium and phosphorous are markedly improved by administration of estrogen or by androgens such as testosterone. A combination of estrogen and androgen is more effective.
14. Kidney threshold regulates the blood calcium level. In a normal adult any extra calcium absorbed from the intestine is readily excreted in the urine. In hypocalcaemia kidney threshold also becomes abnormal.
15. Excess of iron also dis-favours absorption of calcium and phosphorus, as ferric phosphate is highly insoluble. The net result is an upset in the Ca:P ratio.
16. Oxalate in certain foods precipitate calcium in the intestine as insoluble calcium oxalate. The phytic acids of food form insoluble salt with calcium and reduce calcium absorption.
17. Vitamin D increases calcium and phosphorus absorption from the intestine. Vitamin D promotes synthesis of specific calcium binding protein which participates in the active transport of calcium across the small intestinal mucosa. Lack of vitamin D, excess of phytates, low Ca/P ratio in diet, increased pH of upper intestine and malabsorption syndromes influence the amount of calcium absorption adversely.

Biological role:

Calcium is involved in the following biological processes:

1. Constituent of bones and teeth:

Calcium along with phosphate constitutes the mineral part of the skeleton and teeth where it is present to the extent of 99% of the total calcium present in the body. It is primarily in the form of crystals of hydroxyapatite, while some is in combination with phosphate (calcium phosphate) in the form of amorphous crystals.

2. Neuromuscular functions:

This involves excitability of nerve function, neural transmission, and contractility of cardiac and skeletal muscle. Normal concentration of calcium ions is required for the normal excitability of heart muscle.

3. Blood coagulation:

It plays a vital role in blood clotting process since it activates the enzymic conversion of prothrombin into thrombin and production of thromboplastin. The removal of calcium from the blood can prevent blood coagulation and because of this reason EDTA, oxalates, citrates are used as anticoagulant because these ions can precipitate calcium into the respective insoluble salts.

4. Membrane function:

It controls the permeability of all membranes and is often bound by lecithine in the membrane, i.e. it decreases the permeability and balances the opposite action of sodium and potassium capillary permeability. This involves transfer of inorganic ions across cell membranes and release of neurotransmitters at synaptic junction.

5. Selected enzymatic reactions:

Calcium acts as activator for number of enzymes like ATPase, succinic dehydrogenase, lipase, etc. It also antagonizes the effect of magnesium on many enzymes. It releases cellular enzymes such as amylase from the parotid and increases the level of activity of intracellular enzymes such as—Isocitric dehydrogenase, phosphorylase and phosphofructokinase.

6. Regulation of secretion of certain peptide hormones:

Pituitary hormones, parathyroid hormone, calcitonin and vasopressin are regulated through calcium ionic concentration. Calcium along with zinc plays a vital role in release of insulin from pancreas. Calcium homeostasis: Normal blood values are 9.5-10.5 mg/100 ml. 35-45% of this is bound to proteins, mostly to the albumin fraction. In the extracellular fluid nearly all the calcium is in ionized form (55-65%). 0.5 (5-10%) mg is complexed to organic acids, phosphate, citrate, etc., while in renal failure, it may be complexed to other organic ions as well.

The skeleton is in a dynamic state of equilibrium to maintain calcium homeostasis. 4-8 gm. of calcium in bone is rapidly exchangeable with that in plasma and is present on the surface of the bone crystals—labile calcium storage pool. The remaining 99% of bone calcium is more firmly fixed in bone tissue and exchanges at a very slow rate.

Metabolism:

The blood cells contain very little amount of calcium, most of the blood calcium is therefore, in the plasma, where it is present in 3 fractions:

(1) Ionized about 2 mg/100 ml.

(2) Non-diffusible (protein bound) above 3.5 mg/100 ml.

(3) A small amount as calcium complex of citrate and phosphate.

All these forms of calcium in the serum are in equilibrium with one another. A decrease in ionized calcium in the serum causes tetany. This may be due to an increase in the pH of blood or lack of calcium because of poor absorption from the intestine, decreased dietary intake, increased renal excretion as in nephritis or parathyroid deficiency.

Factors influencing blood calcium level:

1. Parathyroid hormone:

In fasting condition or state there is no absorption from the intestine, the normal plasma Ca^{2+} concentration is maintained by its rate of excretion and its mobilization from bones through the action of the parathyroid hormone.

2. Vitamin D:

It enhances absorption of Ca^{2+} from the intestine and thus maintains normal Ca^{2+} concentration.

3. Plasma proteins:

Half of the blood Ca^{2+} (non-diffusible) is bound to plasma proteins and thus any decrease in these proteins will be accompanied by a decrease in the total calcium level.

4. Plasma phosphate:

A reciprocal relationship exists between the concentration of Ca^{2+} and phosphate ions in plasma. The marked increase in serum phosphate causes a fall in serum calcium concentration.

5. Calcitonin:

An increase in the ionized Ca^{2+} levels in the plasma is the stimulus for the production of calcitonin which then causes a deposition of Ca^{2+} in bone.

Excretion:

Calcium is excreted in the urine, bile and digestive secretion. About 75% of dietary calcium is absorbed and rest is excreted as fecal calcium. Nearly 10 g of Ca^{2+} is filtered by the renal glomeruli in 24 hours. But only 200 mg appear in the urine, which is in the ionic state as well as in the complexes with citrate and other organic anions. A very small amount of Ca^{2+} is excreted into the intestine after absorption. About 15 mg of Ca is excreted in the sweat. Vigorous physical exercise increases the loss of Ca by way of sweat.

Disease state:

Calcium metabolism is highly influenced by parathyroid hormones. In hyperparathyroidism serum calcium rises (12-22 mg/100 ml) (hypercalcaemia), phosphatase activity is increased, urinary calcium is decreased and phosphorus rises in serum. The calcium, phosphorus ratio is important in ossification. In the serum the product of calcium and phosphorus (in mg/100 ml) is normally 50 in children and may be below 30 during rickets.

The following are the diseases related to calcium in the body:

(a) Effects of parathyroid:

1. In hyperparathyroidism, the following changes occur:

(i) Hypercalcemia (12-22 mg/dl).

(ii) Decrease in serum phosphate.

(iii) Diminished renal tubular reabsorption of phosphate.

(iv) Increased phosphatase activity.

(v) Renal urinary Ca and phosphorus found from bone decalcification and dehydration.

(vi) Extra Ca and P are lost from soft tissue and bones by increased bone destroying activity.

2. In hypoparathyroidism, the following changes occur:

(i) The concentration of serum Ca may drop below 7 mg/100 ml.

(ii) Increased serum phosphate and decreased urinary excretion of calcium and phosphorus.

(iii) Normal or occasionally raised serum phosphatase activity.

(iv) Normal acid-base equilibrium.

(v) Probably increased bone density.

(b) Tetany:

Decreased ionized fraction of serum Ca causes tetany.

This may be due to:

1. Increase in the pH of blood.

2. Poor absorption of Ca from the intestine.

3. Decreased dietary intake of Ca.

4. Increased excretion of Ca as in hepatitis.

5. Parathyroid deficiency.

6. Increased retention of phosphorus as in renal tubular disease.

Symptoms:

Muscles lose tone and become flabby.

Affects the face, hands and feet.

(c) Rickets:

This is characterized by faulty calcification of bones in children showing serum phosphate values of 1 to 2 mg/100 ml.

This may be due to:

1. Vitamin D deficiency.
2. A deficiency of Ca and P in the diet or a combination of both.
3. Poor absorption of Ca from the intestine.
4. Parathyroid deficiency.
5. Increased alkaline phosphatase activity.

(d) Osteoporosis:

This disease occurs in adults due to the following causes:

1. Decalcification of bones as a result of Ca deficiency in the diet.
2. Hypoparathyroidism.
3. Low vitamin D content of the body.

Symptoms:

Fractures of the brittle bones occur even after minor accidents.

Pain due to fracture of vertebrae (may radiate round the trunk, to the buttocks or down the legs).

Renal rickets:

It is a hereditary disease. It is called familial hypophosphatemia rickets. Affected persons show severe rickets with hypophosphatemia.

The causes are:

(i) Defective transport of phosphate by the intestine and the renal tubules

(ii) Lowered serum phosphorus and hyperphosphaturia

(iii) Reduced intestinal absorption of calcium and phosphorus. Vitamin D in ordinary doses does not relieve the disease. Hence, it is referred to as vitamin D resistant rickets.

II. Phosphorus:

Source:

Phosphorus is present in nearly all foods therefore a dietary deficiency is not known to occur in man. Dairy products, cereals, egg yolk, meat, beans and nuts are usually rich sources. The daily average intake is 800-1000 mg and is about twice that of calcium.

Absorption:

Like calcium, phosphorus is also absorbed by upper small intestine and factors influencing the absorption are also similar. The normal range for plasma inorganic phosphorus is 3.0-4.5 mg/dl. In children values are higher (5-6 mg/dl) and remain so up-till puberty.

Distribution:

Phosphorus is distributed more widely than calcium. 15% is found in muscle and other soft tissues and 85% in the inorganic mineral phase of bone. It is an integral part of many macromolecules. Ex. Phospholipids, phosphoproteins and nucleic acids.

Functions:

It has no physiological effects comparable to that of calcium but it has many other functions which are as follows:

1. Formation of bone and teeth.

2. Formation of phospholipids essential to every cell.

3. Formation of nucleic acids and derivatives.

Ex. Adenylic acid and is thus significant in (RNA and DNA) protein synthesis and from genetics point of view.

4. Formation of organic phosphates as intermediate in metabolic processes.

Ex. In glycolysis, Glucose + ATP $\rightarrow$ G-6-P + ADP.

5. Formation of energy rich phosphate compounds.

Ex. ATP (energy currency of the cell).

6. Both inorganic and organic phosphates can take part in buffering the cell.

Ex, Sodium-potassium-phosphates.

7. Formation of coenzymes.

Ex. TPP, NADP.

8. Formation of phosphoprotein.

Ex. Casein.

Excretion:

Urinary excretion is equivalent to dietary phosphate intake. It varies diurnally, more being excreted at night. The usual daily loss is 600-800 mg, tubular resorption being 85-95%. Renal loss of phosphate can be of significant magnitude to lower serum phosphorus values and enhance osteoid demineralization.

Homeostasis:

There is a greater fluctuation observed in blood phosphate values due to easy shift between extracellular fluid and intracellular compartments. Thus it is quite dependent on dietary phosphorus. Inorganic phosphate affects the net movement of calcium into and out of bone.

Raised phosphate will lead to depression of the solubility of the calcium of bone crystals and thus shift equilibrium towards bone. In this manner it opposes the effect of the parathyroids. Ingestion of heavy dose of phosphate can lower serum calcium and increase excretion of calcium in urine. Lowered phosphorus on the other hand will make parathyroid activity more apparent.

Hormonal factors are not directly linked. However renal phosphate clearance is very vital in homeostasis and seems to be secondarily involved in certain endocrinopathies, e.g. involving parathormone, growth hormone and corticosteroids.

Disease state:

The following are the disease states of phosphorus in the body:

1. In rickets, serum phosphate is as low as 1-2 mg/100 ml (There is a temporary decrease in serum P during absorption of carbohydrates and some fats).
2. Organic P content is low but inorganic content is high in the serum in diabetes.
3. P retention causes acidosis in severe renal diseases. This results in increase of serum P.

4. Serum P levels are increased in hypoparathyroidism and decreased in hyperparathyroidism and celiac disease.
5. In renal rickets, blood P is very low with an increased alkaline phosphatase activity.
6. The deficiency of vitamin D is the cause of low serum P and the defects in the calcification of bones (referred to as vitamin D resistant rickets).

III. Magnesium:

Source:

Magnesium is present in milk, egg, cabbage, cauliflower etc.

Daily requirement:

Infants—100-150 mg; Children—150-200 mg and Adults—200-300 mg.

Absorption:

A greater part of the daily ingested Mg is not absorbed. A very high intake of fat, phosphate, calcium and alkalies diminish its absorption. Parathyroid hormone increases its absorption.

Distribution:

Whole blood it is 2-4 mg/dl, CSF it is 3 mg/100 ml and muscle it is 2 mg/100 ml.

Functions:

1. 70% of the total magnesium content (21g) of the body is combined with calcium and phosphorus in the complex salts of bone. The remainder is in the soft tissues and body fluids. It is the principal cation of the soft tissue.
2. Magnesium ions act as activators for many of the phosphate group transfer enzymes.
3. It is found in certain enzymes, such as co-carboxylase.
4. It functions as a cofactor for oxidative phosphorylation.

Disease state:

The following are the disease states of magnesium in the body:

1. Magnesium deficiency causes depression, muscular weakness and liability to convulsions. Its deficiency has also been observed in chronic alcoholics with low serum mg and muscular weakness.

2. Low in Kwashiorkor, causing weakness.

Low levels of Mg are reported in uremia, normal and abnormal pregnancy, rickets, growth hormone treatment, hypercalcemia and recovery phase of diabetic coma.

IV. Sodium, Potassium, Chloride:

Substances whose solutions conduct an electric current are called 'electrolytes'. They are about 11 in general. Na, K, Ca and Mg are cations whereas CI, HCO_3, HPO_4, SO_4, organic acids and proteins are anions. Among these sodium, potassium and chloride are important in the distribution and the retention of body water, thus have close relationship among them. Hence these three elements appear as a single question in the university exams.

Source:

The most important source of Na and CI in the diet is common table salt (NaCl). The good source of K are chicken, calf flesh, beef liver, dried apricot, dried peaches, bananas, the juice of orange and pineapple, potatoes etc.

Absorption:

Normally Na, K and CI are completely absorbed from the gastro-intestinal tract. About 95% of sodium which leaves the body is excreted in the urine.

Distribution:

In the tissues both Na and K occur in a relatively large amount as compared to chloride and other inorganic salts as well as protein and organic salts. Sodium is present in extra cellular fluid and in a very low concentration inside the cells whereas potassium is mainly found inside the cells and in a very low concentration in the extracellular fluid.

Functions of sodium and potassium:

These electrolytes maintain normal osmotic pressure in the body and protect the body against excessive loss of fluid.

1. They maintain the acid base balance in the body. Sodium bicarbonate, sodium phosphate, potassium phosphate form the buffer system of extracellular and intracellular fluids.
2. They maintain normal water balance.
3. Na also functions in the preservation of normal excitability of muscle and the permeability of the cells. K inhibits 'muscular contraction' in general.

4. High intracellular potassium concentrations are essential for several important metabolic functions, including protein biosynthesis by ribosomes.
5. Sodium and Potassium chlorides maintain the viscosity of blood. A number of enzymes including glycolytic enzymes, such as pyruvate kinase, require K^+ for maximal activity.
6. Na helps in the formation of the gastric juice. NaCl takes part in the series of reactions as a result of which HC1 is manufactured by the stomach.
7. K of KHb in the red cells helps in carbon dioxide transport.
8. K ions inhibit cardiac contraction and prolong relaxation.
9. K ions exert important effect on the function of nervous system.

Functions of chloride:

1. It provides $2/3^{rd}$ of the anion of plasma and is the main factor for regulating body reactions.
2. NaCl and KCl are important agents in regulation of osmotic pressure in the body.
3. HCl of gastric juice is ultimately derived from the blood chlorides.
4. Chloride ions are essential for the action of ptyalin and pancreatic amylase.
5. It is essential in acid-base regulation. Chloride plays a role in the body by chloride shift mechanism.

Metabolism:

The metabolism of these elements is influenced by the following factors:

Hormones:

Mainly adrenocortical steroids and some of the sex hormones facilitate the retention of sodium and chloride in the body and excretion of potassium by kidneys in the urine. In adrenocortical deficiency, serum sodium decreases because excretion increases.

Temperature:

When atmospheric temperature is high as in summer, large amounts of sodium and chloride are lost in perspiration (sweating) and this loss may be checked when temperature is low (in winter).

Renal function:

In renal disease, with acidosis, Na and CI ion excretion in urine is increased due to poor tubular reabsorption of sodium whereas that of K ion is decreased leading to

hyponatraemia and hypochloraemia but hyperkalaemia. Average requirement of Na and K in human body is 5-15 and 4 gm. per day, respectively.

Disorders:

Hyponatraemia:

On sodium deficient diet, young ones grow slowly, lack fat deposit, there is muscle and testicular atrophy, lung infection and deficiency of osteoid tissues. There will also be loss of water, which will be evident by rapid weight loss.

Hypokalaemia:

Extreme potassium depletion in circulating blood causes hypokalemia in young one, they grow slowly and both sexes become sterile. The heart rate is slow, muscle weakness, irritability and paralysis are seen. Bone growth is retarded and in becomes excessively fragile and kidney hypertrophy is exhibited.

Hyperkalemia:

Hyperkalemia paralysis occurs due to excessive amount of potassium in blood. The disease is characterized by periodical attacks of weakness or paralysis. The symptoms of hyperkalaemia are chiefly cardiac and central nervous system depression. They are related to the elevated plasma potassium level and not to increase in intracellular potassium levels.

A dietary chlorine deficiency produces no symptom except a subnormal growth rate. Under normal dietary condition human beings are not subject to a deficiency of sodium, potassium or chlorine. However excessive diarrhoea, vomiting or extreme sweating over long period may bring about a NaCl deficiency. Sometimes the metabolism of individual minerals is asked as a separate question in the university exams. Hence each one is described separately in detail, hereunder.

V. Sodium:

Physiological functions:

1. Major component of extracellular fluids and exists in the body in association with anions chloride, bicarbonate, phosphate and lactate.
2. In association with chloride and bicarbonate it plays a role in acid base equilibrium.
3. Maintains osmotic pressure of the body fluids and thus protects the body against excessive fluid loss.

4. Plays an important role in the absorption of glucose and galactose from small intestine.
5. Maintains normal water balance and distribution.
6. Maintains the normal neuromuscular function.
7. Functions in permeability of cells.

Distribution:

About 1/3rd of the total sodium content of the body is present in the inorganic portion of the skeleton. Most of the sodium is present in the extracellular fluid.

Plasma — 330 mg/100 ml

Muscles — 60 to 160 mg/100 gm.

Cells — 85 mg/100 gm.

Nerve — 312 mg/100 gm.

Daily requirement:

Adults require 5-15 gms/day. In temperate region, NaCl intake is less. In tropical region, NaCl intake is more. Hypertension patients should not take more than 1 gm. of Na per day.

Absorption:

Normally, Na is completely absorbed from gastro-intestinal tract. Less than 2% is eliminated in feces. In persons suffering from diarrhoea, large amounts are lost in feces.

Excretion:

Urine — 5-35 gm.

Skin — 25-50 mg

Stool — 10-125 mg

Excessive loss of Na by sweating causes heat arrays.

Disease state:

1. Adrenal cortical steroids regulate the metabolism of Na. Insufficiency of adrenal cortical steroids decreases serum Na level with an increase in sodium excretion.

2. In chronic renal disease when acidosis exists, Na depletion occurs due to poor tubular reabsorption of Na as well as to the loss of Na in the buffering acids.

3. In persons not adapted to high environmental temperature large amount of Na is lost in the sweat, developing muscular cramps of extremities, oedema, headache, nausea and diarrhoea.

4. Hyponatremia causes dehydration and reduced blood pressure, decreased blood volume and circulatory failure.

This may be due to:

(a) Prolonged vomiting and diarrhoea resulting in excessive loss of digestive fluid.

(b) Chronic renal disease with acidosis due to poor tubular reabsorption of Na.

(c) Adrenocortical insufficiency.

(d) Loss of weight due to loss of water.

5. In Hypernatremia, serum Na is high.

This occurs in:

(a) Hyperactivity of adrenal cortex as in Cushing's syndrome.

(b) Prolonged treatment with cortisone and ACTH as well as sex hormones, this results in—

(i) Increased retention of water in the body.

(ii) Increase in blood volume,

(iii) Increase in blood pressure.

6. Steroid hormones cause retention of Na and water in pregnancy.

VI. Potassium:

Physiological junctions:

1. Potassium is largely present in intracellular fluid and it is also present in small amounts in the extra cellular fluid because it influences the cardiac muscle activity.

2. It plays an important role in the regulation of acid-base balance in the cell.

3. It maintains osmotic pressure.

4. It functions in water retention.

5. It is essential for protein biosynthesis by ribosomes.

6. The glycolytic enzyme pyruvate kinase requires K^+ for maximal activity.

Sources:

High content of potassium is found in chicken, beef, liver, bananas, orange juice, pineapple, yam, potatoes etc.

Distribution:

Plasma — 20 mg/100 ml

Cells — 440 mg/100 gm.

Muscles — 250-400 mg/100g

Nerves — 530 mg/100g.

Daily requirement:

Normal intake of K^+ in food is about 4 gm. It is so widely distributed that its deficiency is rare except in pathological condition.

Blood potassium:

Normal level of serum K is 14-20 mg/100 ml. Erythrocytes contain large amounts of K which avoids hemolysis. Serum K decreases during increased carbohydrate utilization following glucose or insulin administration. Aldosterone decreases serum K.

Absorption:

Normally, K is practically completely absorbed from gastrointestinal tract and less than 10% of K is eliminated in the feces. In subjects with diarrhea large amounts are lost in feces.

Excretion:

K is normally eliminated almost entirely in urine and a small amount in the feces. Aldosterone exerts an influence on potassium excretion. In normal kidney function; K is very promptly and efficiently removed from the blood.

Disease state:

1. K is not only filtered by the kidney but is also secreted by the renal tubules. Excretion of K is greatly influenced by changes in acid-base balance and also by adrenal cortex. The capacity of kidney to excrete K is very great and therefore hyperkalaemia does not occur even after ingestion of K, if kidney function is impaired K should not be given intravenously unless, circulatory collapse and dehydration are corrected.

2. Hyperkalaemia occurs in patients in the following conditions.

(a) Renal failure

(b) Severe dehydration

(c) Addison's disease due to decreased excretion of K by the kidney

K deficiency occurs in chronic wasting diseases like malnutrition, prolonged negative nitrogen balance, gastrointestinal losses and metabolic alkalosis.

VII. Chlorine:

Physiological functions:

1. As a component of sodium chloride, chloride ion is essential in acid-base balance.
2. As Cl^- it is also essential in water balance and osmotic pressure regulation.
3. It is also important in the production of HCl in the gastric juice.
4. Cl^- ion is an activator of amylase.

Sources:

Mainly as NaCl salt (table salt).

Distribution:

Plasma — 365 mg/100ml

Cells — 190 mg/ 100mg

CSF — 440 mg/100ml

Muscle — 40 mg/100g

Nerve — 171 mg/100g

Daily requirement:

5-20 gms. Excess consumption of NaCl increases blood pressure in hypertensive patients. Causes edema in protein deficiency.

Absorption:

Normally CI is practically completely absorbed from the GI tract.

Excretion:

CI is chiefly eliminated in the urine, also in sweat. Its concentration in sweat is increased in hot climates and decreased by aldosterone.

Diseases state:

1. CI deficit also occurs when losses of Na are excessive in diarrhoea, sweating and certain endocrine disturbances.

2. Loss of CI due to loss of gastric juice by vomiting or pyloric or duodenal obstruction.

3. Hypochloremia alkalosis may develop in Cushing's syndrome or after administration of ACTH or cortisone.

VIII. Sulphur:

Sources:

Sulphur is taken mainly as cysteine and methionine present in proteins. Other compounds in the diet contribute small amounts of sulphur.

Absorption:

Inorganic sulphate is absorbed as such from intestine into the portal circulation. Small amount of sulphide may be formed in the bowel by the action of bacteria, but if absorbed into the blood stream, it is rapidly oxidized to sulphate.

Sulphur in blood (serum):

Inorganic — 0.5-1.1 mg/100 ml

Ethereal sulphate — 0.1-1.0 mg/100 ml

Neutral sulphur — 1.7-3.5 mg/100 ml

Physiological functions:

1. Sulphur is present primarily in the cell protein in the form of cysteine and methionine.

2. Cysteine plays important part in the protein structure and enzyme activity.

3. Methionine is the principal methyl group donor in the body. The 'activated' form of methionine, s-adenosyl methionine is the precursor in the synthesis of a large number of methylated compounds which are involved in intermediary metabolism and detoxification mechanism.

4. Sulphur is a constituent of coenzyme A and lipoic acid which are utilized in the synthesis of acetyl-CoA, malonyl CoA, Acyl-CoA and S-acetyl lipoate (involved in fatty acid oxidation and synthesis).

5. It is a component of a number of other organic compounds such as heparin, glutathione, thiamine, pantothenic acid, biotin, ergothionine, taurocholic acids, sulphocyamides, indoxyl sulphate, chondroitin sulphate, insulin, penicillin, anterior pituitary hormones and melanin.

Excretion:

Excreted in urine in 3 forms. Total sulphate excretion may be diminished in renal function impairment and is increased in condition accompanied by excessive tissue breakdown as in high fever and increased metabolism.

Disease state:

Serum sulphate is increased in renal function impairment, pyloric and intestinal obstruction and leukemia.Marked sulphate retention in advanced glomerulo-nephritis causes the development of acidosis.Increase in blood indica (indoxyl potassium sulphate) may occur in uremia.

IX. Iron:

Iron is present in all organisms and in all the cells. It does not exist in the free state, instead is always present in organic combination, usually with proteins. It exists in two forms i.e. Fe^{2+} (ferrous) and Fe^{3+} (ferric). It serves as an oxygen and electron carrier and is incorporated into redox enzymes and substances which carry out the function of oxygen transport such as haemoglobin and cytochromes.

Total iron content in normal adult is 4 to 5 grams. 60-70% is present in hemoglobin, 3% in myoglobin and 0.1% in plasma combined with β-globulin transport protein transferrin. Hemoprotein and flavoprotein make up to less than 1% of total iron. Rest is stored as ferritin.

Source:

Rich – Liver, heart, kidney, spleen.

Good – Egg yolk, fish, nuts, dates, beans, spinach, molasses, apples, bananas, etc.

Poor — Milk, wheat flour, polished rice, potatoes etc.

Daily requirement:

Only about 10% of ingested iron is absorbed.

i. Infants – 10-15 mg.

ii. Children – 1-3 years 15 mg.

iii. 4-10 years – 10 mg.

iv. Older children and adults of 11 to 18 years — 18 mg.

v. 19 years and above — 10 mg.

vi. Females between 11 and 50 years of age and during pregnancy or lactation – 18 mg.

vii. After 51 years of age — 10 mg.

viii. In adult women the average loss of iron with blood during menstrual period is 16-32 mg per month or an additional loss of 0.5 to 1.0 mg per day. This amount is easily obtained from diet.

ix. In excessive menstrual blood loss and in chronic iron-deficiency anemia, a supplement of 100 mg of iron per day is sufficient to replenish.

x. During growth, pregnancy and lactation iron demand is more.

xi. In healthy adult male or post menopause women dietary iron requirement is negligible unless any deficiency or loss of iron occurs.

xii. Iron deficiency occurs as a result of malabsorption from gastro-intestinal tract.

xiii. A defect in hemoglobin synthesis in anemia is commonly found in copper deficiency.

Biologically active compounds that contain iron:

1. Haemic compounds:

In these compounds the protoporphyrin is combined with iron to form haem (divalent iron) and haematin.

Ex. Hemoglobin, myoglobin, cytochromes, catalases and peroxidases.

2. Non-haemic compounds:

These include Transferrin (siderophilin) to transport iron, ferritin and haemosiderin which are the stored forms of iron and miscellaneous compounds like enzymes.

Absorption:

Very little (less than 10%) of dietary iron is absorbed. Excretion in the urine is minimal. Infants and children absorb more iron as compared to adults. Iron deficiency in infants is due to dietary deficiency. Iron deficient children absorb approximately twice as much as normal children do. Absorption mainly occurs in the duodenum and the proximal jejunum.

(a) Most of the iron in food occurs in the ferric form (Fe^{3+}), ex. either as ferric hydroxide or as ferric organic compounds. Acidic pH of the gastrointestinal tract favours the absorption whereas alkaline pH decreases it. In an acid medium, these compounds are broken down into free ferric ions or loosely bound organic iron, reducing substances such as —SH groups ex. cysteine and ascorbic acid which convert ferric iron into the reduced (ferrous) state, in this form iron is more soluble and should therefore be more readily absorbed.

(b) A diet high in phosphate, phytic acid and oxalic acid decreases iron absorption since these substances form the insoluble compounds with iron. Conversely, a diet very low in phosphate markedly increases iron absorption.

(c) The extent of absorption depends on the degree of saturation of the tissue, ex. anemic individuals absorb more than normal individuals.

(d) Iron absorption is enhanced by protein, possibly as a result of the formation of low molecular weight digestive products (peptides, amino acids) which can form soluble iron chelates.

(e) It is also increased in pernicious anaemia and in hypo plastic anaemia.

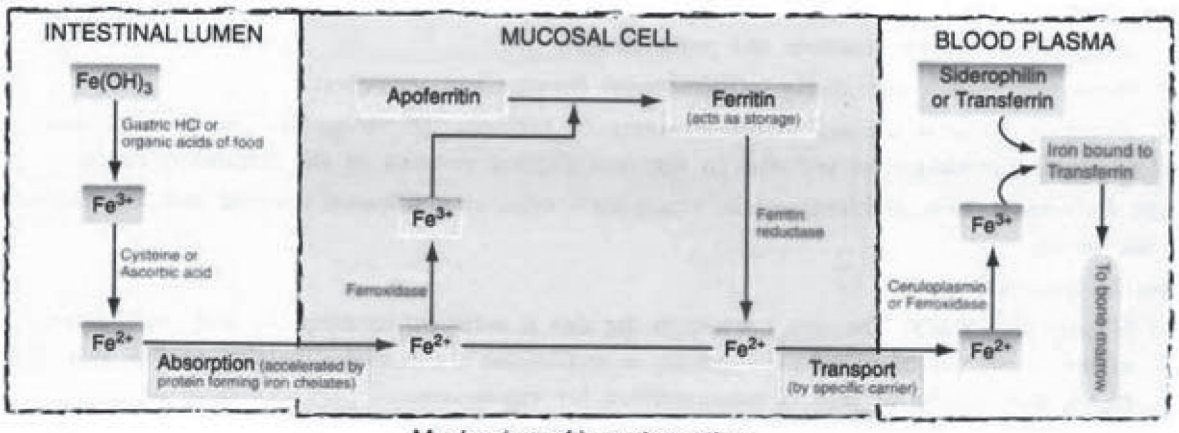
(f) Impaired absorption takes place in patients who have total removal of stomach or a removal of considerable amount of the intestine.

(g) Achlorhydria, administration of alkali, copper deficiency decrease iron absorption.

(h) Alcohol ingestion favours iron absorption.

Mechanism of Iron Absorption:

Ferrous ion on entering the mucosal cells is oxidized to ferric state and then combines with apoferritin forming ferritin which contains 23% of iron by weight. When apoferritin gets saturated with iron no further iron can be taken up by the mucosal cells to store it in the form of ferritin. Heme enters the mucosal cells without being released from the porphyrin ring. Heme is broken down in the mucosa and iron appears in the plasma transferrin.



Mechanism of iron absorption

Transport:

In the plasma, the iron is bound to transferrin which is only partially saturated. Plasma iron is also in exchange with interstitial and intra-cellular compartments. The

iron in these compartments is generally referred to as 'labile iron pool' and is estimated to be in the order of 80 to 90 mg. Here the iron may stay briefly on the cell membrane before its incorporation into haem or storage compounds. Nearly all the iron released from the mucosal cell enter the portal blood mostly in the ferrous state (Fe^{2+}). In the plasma, Fe^{2+} is oxidized rapidly to the ferric state (Fe^{3+}) and then incorporated into a specific protein.

Storage:

Stores of iron are maintained chiefly in the liver, spleen and bone marrow in the form of ferritin and haemosiderin. Women have lower stores than men and therefore, develop anaemia much more frequently than men. Iron stores are increased in haemochromatosis, severe haemolytic anaemias, aplastic anaemia and in persons receiving multiple blood transfusions, prolonged oral or parenteral iron therapy.The normal content of protein bound iron (FBI) in plasma of males is 120-140 jig/100 ml; in females it is 90-120 pg/100ml. However, the total iron binding capacity (TIBC) is about the same in both sexes i.e. 300-360 pg/100 ml.

Excretion:

Physiological excretion of iron is minimal. The normal routes of excretion are urine, bile, faeces, cellular desquamation, and sweat. Daily excretion in an adult male is estimated to be about 1 mg. In women of reproductive age, additional loss through menstruation averages to 1 mg per day.

Abnormal iron metabolism:

Ferritin and hemosiderin, the storage forms of iron act as internal iron reserve to protect against sudden loss of iron by bleeding. Ferritin is present not only in the intestine but also in liver (about 700 mg) spleen and bone marrow. If excess iron is administered parenterally exceeding the capacity of the body to store it as ferritin, it accumulates in the liver as hemosiderin, a form of colloidal iron oxide in association with protein.

Iron metabolism is disturbed mainly by the following causes:

(a) Decreased formation of hemoglobin.

(b) Decrease in circulating hemoglobin.

(c) Abnormalities in the serum iron concentration

(d) Abnormal deposition of iron-combining pigments in the tissues.

Physiological functions:

1. Iron functions mainly in the transport of oxygen to the tissues.

2. Involved in the process of cellular respiration.

3. Essential component of hemoglobin, myoglobin, cytochromes and the respiratory enzyme systems (cytochrome oxidase, catalase and peroxidase).

4. Non-heme iron is completely protein-bound (storage and transport).

5. Non-heme iron is utilized in the structure of xanthine dehydrogenase (xanthine oxidase) and succinate dehydrogenase and also in the iron sulphur proteins of the respiratory chain.

Iron deficiency:

Iron deficiency is the commonest cause of nutritional anaemia and is prevalent all over the world. Causes of iron deficiency:

(1) Dietary deficiency:

The iron content in the diet is sufficient to meet the daily requirements, but excessive amount of phytates in cereals, is responsible for non-absorbability of this iron. Hence higher daily intake of iron is recommended for vegetarians.

(2) Lack of absorption:

This may be seen in malabsorptive syndromes.

(3) Increased demand:

This occurs during rapid growth in infancy and pregnancy.

(4) Poor stores at birth:

These are found in premature birth and twin pregnancy.

(5) Pathological blood loss:

With loss of 1g of haemoglobin 3.4 mg of iron is lost. Hook-worm infestation is the most important factor responsible for blood loss. Other sources of blood loss are bleeding piles, peptic ulcer, hiatus hernia, cancer of gastrointestinal tract, chronic aspirin ingestion, and oesophageal varies.

(6) Iron deficiency anemia:

Iron deficiency anemia is widely prevalent among children, adolescent girls and nursing mothers. The hemoglobin content of the blood during iron deficiency anemia is 5 to 9 g/100 ml.

(a) Women of child bearing age:

The clinical symptoms are breathlessness on exertion, giddiness and pallor of the skin. In severe cases, there may be edema of the ankles.

(b) Weaned infants and young children:

The hemoglobin level is 5 to 9 g/100 ml of blood. The children are dull and inactive and show pallor of the skin. The appetite is poor and growth and development are retarded.

Treatment of iron deficiency anaemia:

Anaemia responds to oral iron therapy. The commonly used preparations are ferrous sulphate, ferrous fumarate and ferrous gluconate. Iron dextran can be administered both intramuscularly and intravenously, iron sorbitex is given intramuscularly, and saccharide iron oxide is given intravenously. Anemic women should take ferrous sulphate tablet. For a child below 12 months, a mixture of ferrous ammonium citrate sweetened with glycerine and for children of 1 to 5 years ferrous ammonium citrate mixture should be given for curing.

Iron overload:

Hypersiderosis may occur as a primary disorder (Idiopathic haemochromatosis) or secondary with excessive entry of exogenous, iron into the body.

1. Siderosis:

When excessive amounts of iron are released in or introduced into the body beyond the capacity for its utilization, the excess is deposited in various tissues, mainly in the liver. This may occur due to repeated blood transfusions, excessive breakdown of erythrocytes in hemolytic types of anaemia and inadequate synthesis of haemoglobin as in pernicious anaemia.

2. Nutritional siderosis:

This disorder is found among Bantus in South Africa. Bantus cook their food in large iron pots and consume iron-rich food. The absorption of iron appears to be high, leading to the development of nutritional siderosis. Livers of the Bantus contain large amounts of iron.

Hemochromatosis:

Hemochromatosis is a rare disease in which large amounts of iron are deposited in the tissues, especially the liver, pancreas, spleen and skin producing various disorders.

Accumulation of iron in the liver, pancreas and skin produces hepatic cirrhosis, bronze diabetes and bronze-state pigment respectively.

X. Copper:

Source:

Richest sources:

Liver, kidney, other meats, shell fish, nuts and dried legumes.

Poor sources:

Milk and milk products. The concentration of copper in the fetal liver is 5-10 times higher than that in liver of an adult.

Daily requirements:

Infants and children – 0.05 mg/kg body weight

Adults – 2.5 mg

A nutritional deficiency of copper has never been demonstrated in man, although it has been suspected in case of nephrosis.

Absorption:

About 30% of the normal daily diet of copper is absorbed in the duodenum.

Blood copper:

The normal concentration of copper in serum is 90 μg/100 ml. Both RBC and serum contain copper. 80% of RBC copper is present as superoxide dismutase (erythrocuperin), Plasma copper occurs as firmly bound form and loosely bound forms. The firmly bound copper consists of ceruloplas-min. Loosely bound copper is called 'directly reacting copper' and is bound to serum albumin. The plasma copper levels increase in pregnancy because of their estrogen content. Oral contraceptives have a similar effect

Physiological functions:

1. It has important role in hemoglobin synthesis.

2. It is required for melanin formation, phospholipids synthesis and collagen synthesis.

3. It has a role in bone formation and in maintenance of the integrity of myelin sheath.

4. It is a constituent of several enzymes such as tyrosinase, cytochrome oxidase, ascorbic acid oxidase, uricase, ferroxidase I (ceruloplasmin), ferroxidase II, superoxide dismutase, amino oxidase and dopamine hydroxylase.

5. Three copper containing proteins namely cerebrocuperin, erythrocuperin and hepatocuperin are present in brain, RBC and liver respectively.

Excretion:

Only 10 to 60 mg of copper is excreted in the urine. 0.5 to 1.3 mg is excreted through bile and 0.1 to 0.3 mg is excreted by intestinal mucosa into the bowel lumen.

Effects of copper deficiency:

1. Although iron absorption is not disturbed but the release of iron into the plasma is prevented due to the decreased synthesis of ceruloplasmin. As a result, hypoferremia occurs which leads to the depressed synthesis of heme developing anemia in severe deficiency of copper.

2. The experimental animals on a copper deficient diet lose weight and die.

3. In copper deficient lambs, low cytochrome oxidase activity results in neonatal ataxia.

4. Copper deficiency produces marked skeletal changes, osteoporosis and spontaneous fractures.

5. Elastin formation is impaired in the deficiency of copper. Because a copper containing enzyme plays an important role in the connective tissue metabolism, especially in the oxidation of lysine into aldehyde group which is necessary for cross linkage of the polypeptide chains of elastin and collagen.

6. Copper deficiency results in myocardial fibrosis in cows. It is suggested that reduction in cytochrome oxidase activity may lead to cardiac hypertrophy.

Disorders of copper metabolism:

Wilson's disease (hepatoreticular degeneration):

Wilson's disease is a rare hereditary disorder of copper metabolism.

The following disorders have been observed in this disease:

(a) The absorption of copper from the intestine is very high (about 50 percent); whereas 2 to 5 percent copper is absorbed in normal subjects.

(b) Ceruloplasmin formation is very less. Hence a greater part of serum copper remains loosely bound to serum protein-notably albumin and therefore, copper can be transported to the tissues, such as brain and liver or to the urine.

(c) Excessive deposition of copper in the liver and the kidney causes hepatic cirrhosis and renal tubular damage respectively. The renal tubular damage results in the increased urinary excretion of amino acids, peptides and glucose.

XI. Iodine:

Source:

Rich sources are sea water, marine vegetation and vegetables as well as fruits grown on the sea board. Plants grown at high altitudes are deficient in iodine because of its low concentration in the water. In such regions, iodide is commonly added to the drinking water or table salt in concentrations of 1:5000 to 1:200000.

Daily requirement:

Adults – 100 to 150 μg

In adolescence and in pregnancy – 200 μg

Distribution:

Normal iodine content of body is 10 to 20 mg. 70 to 80% of this is present in thyroid gland. Muscles contain large amount of iodine. The concentration of iodine in the salivary glands, ovaries, pituitary gland, brain and bile is greater than that in muscle. Iodine in saliva is inorganic iodide, while most of the iodine present in tissue is in the organic form.

Blood Iodine:

Practically all the iodine in the blood is in the plasma. The normal concentration in plasma or serum is 4 to 10 μg/100 ml. 0.06 to 0.08 μg/100 ml is in inorganic form, 4 to 8 μg/100 ml is in the organic form bound to protein, precipitated by protein precipitating agents. 90% of the organic form consists of thyroxine and the remainder tri and di-iodothyronine. About 0.05% of thyroxine is in the free state. RBC contains no organic iodine.

Absorption:

Iodine and iodide are absorbed most readily from the small intestine. Organic iodide compounds (di-iodothyronine and thyroxine) are partly absorbed as such and a part is broken down in the stomach and intestines with the formation of iodides. Absorption also takes place from outer mucus membrane and skin.

Storage:

90% of the iodine of the thyroid gland is in organic combination and stored in the follicular colloid as 'thyroglobulin' a glycoprotein containing thyroxine, di-iodothyronine and smaller amounts of triiodothyronine.

On demand these substances are mobilized and thyroxine as well as triiodothyronine is passed into the systemic circulation. They undergo metabolic degradation in the liver.

Excretion:

1. Inorganic iodine is mostly excreted by the kidney, liver, skin, lungs and intestine and in milk.

2. About 10% of circulating organic iodine is excreted in feces. This is entirely unabsorbed food iodine.

3. 40 to 80 % is usually excreted in the urine, 20 to 70 μg daily in adults, 20 to 35 μg in children. The urinary elimination is largest when the intake is lowest.

4. Urinary iodine is increased by exercise and other metabolic factors.

Physiological functions:

Iodine is required for the formation of thyroxine and triiodothyronine hormones of the thyroid gland. These thyroid hormones are involved in cellular oxidation, growth, reproduction and the activity of the central and autonomic nervous systems. Triiodothyronine is more active than thyroxine in many respects.

Iodine deficiency:

1. In adults the thyroid gland is enlarged producing goiter. If treatment is started very early, the thyroid becomes normal. If treatment is delayed, the enlargement persists.

2. In children, severe iodine deficiency results in the extreme retardation of growth causing cretinism.

Prevention of goiter:

Goiter can be prevented by the regular use of iodized salt or iodine added to the drinking water.

Goitrogenic substances in foods:

Cabbage, cauliflower and radish contain substance like vinyl-2- thiooxazolidone which makes iodide present in the food unavailable by reacting with it. Such substances are called ‘goitrogenic’ substances.

Selenium:

i. Good dietary sources are kidney cortex, pancreas, pituitary and liver.

ii. It is rapidly absorbed mainly in duodenum.

iii. It is distributed in liver 0.44 μg/gm in skin 0.27 μg/gm and in muscle 0.37 μg/gm.

iv. In the cells it is present as selenocystinenadselenomethionine.

v. Selenium along with Vitamin E plays an important role in tissue respiration.

vi. Selenium is involved in biosynthesis of coenzyme Q (ubiquinone), which is involved in respiratory chain.

vii. Selenium acts as an antioxidant providing protection against peroxidation in tissues and membrane.

viii. It is an essential component of glutathione peroxidase, an enzyme which catalyzes the conversion of reduced glutathione to its oxidized form.

ix. Selenium is excreted in faeces, urine and via exhalation.

x. It causes toxic effect called selenosis.

Probable Questions:

1. State the distribution of water in the body. What factors affect distribution of water?
2. state the procedure of water intake and water loss from the body.
3. What are the physiological functions of water?
4. What is primary and secondary dehydration?
5. Write down the effect of dehydration.
6. How hormones regulate water balance in the body.
7. What is macro elements and micro elements. Give examples.
8. How calcium balance is regulated by hormones.
9. What factors control calcium absorption?
10. What factor control calcium level in blood.
11. State the diseases associated with problems in calcium metabolism.
12. Write down the physiological role of Phosphorous in the body.
13. How hormone controls phosphorous metabolism.
14. How iron get absorbed in the body.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

Unit-XV

GI tract hormone source, composition and function

Objective: In this unit you will know about source, composition and function of different gastro intestinal hormones.

Introduction:

The gastrointestinal hormones (or gut hormones) constitute a group of hormones secreted by enteroendocrine cells in the stomach, pancreas, and small intestine that control various functions of the digestive organs. Later studies showed that most of the gut peptides, such as secretin, cholecystokinin or substance P, were found to play a role of neurotransmitters and neuromodulators in the central and peripheral nervous systems.

Enteroendocrine cells do not form glands but are spread throughout the digestive tract. They exert their autocrine and paracrine actions that integrate gastrointestinal function.

The primary function of the gastrointestinal tract is to supply nutrients to our bodies via the processes of ingestion, motility, secretion, digestion, and absorption; this occurs through complex coordination of digestive processes that are regulated by intrinsic endocrine and nervous systems. Although the nervous system exerts influence on many digestive processes, the GI tract is the largest endocrine organ in the human body and produces numerous mediators that play an integral role in regulating functions of the GI tract.

Types of GI Hormones:

I. Gastrin:

This hormone is secreted by gastrin cells (= G-cells) in the pyloric region of the stomach. It stimulates gastric glands to secrete and release the gastric juice. It also stimulates gastric mobility.

II. Enterogastrone:

(= Gastric Inhibitory Peptide— GIP). It is secreted by the duodenal epithelium. It inhibits gastric secretion and motality. It slows gastric contraction, hence it is also called gastric inhibitory peptide.

III. Secretin:

It was the first hormone to be discovered by scientists. It is secreted by the epithelium

of duodenum. It releases bicarbonates in the pancreatic juice. It increases secretion of bile. It decreasesgastric secretion and motality.

IV. Cholecystokinin pancreozymin (CCK-PZ):

The word cholecystokinin is derived from three roots: *Chol* meaning bile, *cyst* meaning bladder and *kinin* meaning to remove. The word pancreozymin is derived from pancreas and zymin, which means enzyme producer. This hormone is secreted by the epithelium of entire small intestine. It stimulates the gall bladder to release bile and pancreas to secrete and release digestive enzymes in the pancreatic juice.

V. Duocrmin:

It is secreted by the duodenal epithelium and stimulates the Brunner's glands to release mucus and enzymes into the intestinal juice.

VI. Enterocrinin:

It is secreted by the epithelium of entire small intestine. It stimulates the crypts of Lieberkuhn to release enzymes into the intestinal juice.

VII. Vasoactive Intestinal Peptide (VIP):

It is secreted by the epithelium of entire small intestine. It dilates peripheral blood vessels of the gut. It also inhibits gastric acid secretion.

VIII. Villikinin:

It is secreted by the epithelium of entire small intestine. It accelerates movement of villi.

IX. Somatostatin (SS):

Somatostatin secreted by the Delta (δ) cells of islets of Langerhans of the pancreas inhibits the secretion of glucagon by aplha cells and insulin by beta (β) cells Somatostatin produced by argentaffin cells of gastric and intestinal glands suppresses the release of hormones from the digestive tract.

X. Pancreatic Polypeptide (PP):

It is secreted by the pancreatic polypeptide cells (also ailed PP cells or F-cells) of islets of Langerhans. It inhibits the release of pancreatic juice from the pancreas.

Bio synthesis of GI Hormones:

The GI hormones classify as endocrines, paracrine, or neurocrine based on the method by which the molecule gets delivered to its target cell(s). Endocrine hormones are secreted from enteroendocrine cells directly into the bloodstream, passing from the portal circulation to the systemic circulation, before being delivered to target cells with receptor-specificity for the hormone. The five GI hormones that qualify as endocrines are

gastrin, cholecystokinin (CCK), secretin, glucose-dependent insulinotropic peptide (GIP), and motilin. Enteroendocrine cells also secrete paracrine hormones, but they diffuse through the extracellular space to act locally on target tissues and do not enter the systemic circulation. Two examples of paracrine hormones are somatostatin and histamine. Additionally, some hormones may operate via a combination of endocrine and paracrine mechanisms. These "candidate" hormones are glucagon-like peptide-1 (GLP-1), pancreatic polypeptide, and peptide YY. Lastly, neurocrine hormones get secreted by postganglionic non-cholinergic neurons of the enteric nervous system. Three neurocrine hormones with significant physiologic functions in the gut are vasoactive intestinal peptide (VIP), gastrin release peptide (GRP), and enkephalins.

Gastrointestinal hormones undergo synthesis in specialized cells of the GI tract mucosa known as enteroendocrine cells. Enteroendocrine cells are specialized endoderm-derived epithelial cells that originate from stem cells located at the base of intestinal crypts. These cells are dispersed throughout the GI mucosa, sprinkled in between epithelial cells from the stomach all the way through to the colon. Also, these enteroendocrine cells possess hormone-containing granules concentrated at the basolateral membrane, adjacent to capillaries, that secrete their hormones via exocytosis in response to a wide range of stimuli related to food intake. These stimuli include small peptides, amino acids, fatty acids, oral glucose, distension of an organ, and vagal stimulation.

G cells secrete gastrin in the antrum of the stomach and the duodenum in response to the presence of breakdown products of protein digestion (such as amino acids and small peptides), distention by food, and vagal nerve stimulation via GRP. More specifically, phenylalanine and tryptophan are the most potent stimulators of gastrin secretion among the protein digestion products. The vagal nerve stimulation of gastrin secretion is unique because gastrin and motilin are the only hormones released directly by neural stimulation.

CCK is secreted from I cells in the duodenum and jejunum in response to acids and monoglycerides (but not triglycerides), as well as the presence of protein digestion products.Secretin is secreted from S cells in the duodenum in response to H+ and fatty acids in the lumen. Specifically, a pH less than 4.5 signals arrival of gastric contents, which initiates the release of secretin.

GIP is secreted by K cells in the duodenum and jejunum in response to glucose, amino acids, and fatty acids. GIP is the only GI hormone with a response to all three macronutrient types, and newer studies suggest that changes in intraluminal osmolarity may be what stimulates GIP secretion.GLP-1 is also produced in the small intestine and secreted from L cells. The presence of hexose and fat stimulate its release. Pancreatic polypeptide and peptide YY are secreted by protein and fat, respectively, although their functions are still relatively unknown.

Organ Systems Involved:

The digestive system is the primary site of action for most GI hormones and related polypeptides. The stomach is the primary site of gastrin production with some D-cells also populating the duodenum. Somatostatin and histamine are also produced in the stomach by enterochromaffin-like (ECL) cells, which is an enteroendocrine cell subtype. The small intestines, namely the duodenum and jejunum handle secretion of CCK, secretin, GIP, and motilin.

Function

The two gastrointestinal hormone families discussed above are responsible for most of the regulation of gastrointestinal function. The main actions of the gastrin-CCK family and the secretin family of hormones are listed below.

a. Gastrin

- Stimulates H+ (acid) secretion by parietal cells in the stomach
- Trophic (growth) effects on the mucosa of the small intestine, colon, and stomach
- Inhibits the actions of Secretin and GIP
- Inhibited by H+

b. CCK

- Contraction of the gallbladder with simultaneous relaxation of the sphincter of Oddi
- Inhibits gastric emptying
- Stimulates secretion of pancreatic enzymes: lipases, amylase, and proteases
- Secretion of bicarbonate from the pancreas
- Trophic effects on the exocrine pancreas and gallbladder

c. Secretin

- Inhibits gastrin, H+ secretion, and growth of stomach mucosa
- Stimulates biliary secretion of bicarbonate and fluid
- Secretion of bicarbonate from the pancreas
- Trophic effect on the exocrine pancreas

d. GIP

- Stimulation of insulin secretion
- Induces satiety
- In large doses, decreases gastric acid secretion
- In large doses, decreases the motor activity of the stomach and therefore slows gastric emptying when the upper small intestine is already full of food products.

- Stimulates the activity of lipoprotein lipase in adipocytes
- Protects beta-cells of the pancreas from destruction by apoptosis

e. GLP-1

- Decreases gastric emptying
- Induces satiety
- Increases sensitivity of pancreatic beta-cells to glucose.

f. Motilin

- Increases gastrointestinal motility by stimulating the "migrating motility" or "myoelectric complex" that moves through the fasting stomach and small intestines every 90 minutes. This cyclical release and action get inhibited by the ingestion of food. Not much is known about this peptide, except for this essential function.

Mechanism of action of GI hormones:

The release of GI hormones is in response to input from G-protein-coupled receptors that detect changes in luminal contents. Some of these receptors only respond to selective luminal substances and subsequently release GI hormones from their respective enteroendocrine cells through unknown mechanisms. Overall, gastrointestinal hormones manage a diverse set of actions in the body including:

- Contraction and relaxation of smooth muscle wall and sphincters
- Secretion of enzymes for digestion
- Secretion of fluid and electrolytes
- Trophic (growth) effects on tissues of GI tract
- Regulating secretion of other GI peptides (i.e., somatostatin inhibits secretion of all GI hormones)

To better understand how these actions are carried out by GI hormones, it is best to use gastrin's functions as an example. Gastrin is an interesting hormone because it acts through two mechanisms that ultimately increase the secretion of gastric acid (hydrogen ions) into the stomach. The first mechanism involves gastrin binding to CCK-2 receptors on parietal cells, causing increased expression of K^+/H^+ ATPase enzymes that are directly responsible for increased hydrogen ion secretion into the stomach. The second mechanism is mediated by enterochromaffin-like cells, which secrete histamine in response to activation by gastrin. Histamine then binds H2 receptors on nearby parietal cells, which further stimulates secretion of hydrogen ions. In addition to stimulating ECL cells to produce acid, gastrin also stimulates these parietal cells and ECL cells to proliferate.

Hormone	Major Activities	Stimuli for Release
Gastrin	Stimulates gastric acid secretion and proliferation of gastric epithelium	Presence of peptides and amino acids in gastric lumen
Cholecystokinin	Stimulates secretion of pancreatic enzymes, and contraction and emptying of the gall bladder	Presence of fatty acids and amino acids in the small intestine
Secretin	Stimulates secretion of water and bicarbonate from the pancreas and bile ducts	Acidic pH in the lumen of the small intestine
Ghrelin	Appears to be a strong stimulant for appetite and feeding; also a potent stimulator of growth hormone secretion.	Not clear, but secretion peaks prior to feeding and diminishes with gastric filling
Motilin	Apparently involved in stimulating housekeeping patterns of motility in the stomach and small intestine	Not clear, but secretion is associated with fasting
Gastric Inhibitory Peptide	Inhibits gastric secretion and motility and potentiates release of insulin from beta cells in response to elevated blood glucose concentration	Presence of fat and glucose in the small intestine

Possible Questions:

1. Describe source and function of any five GI hormones.
2. How GI hormones are synthesized in the body.
3. State the mechanism of actions of GI hormones.
4. Describe stimuli of release of any five GI hormones.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

Unit-XVI

Neuroendocrine system and neurosecretion: neural control of glandular secretion; hypothalamic pituitary unit, neuroendocrine feedback

Objective: In this unit you will learn about Neuroendocrine system and neurosecretion: neural control of glandular secretion; hypothalamic pituitary unit, neuroendocrine feedback

Neuroendocrine system and neurosecretion:

Neuroendocrine system: The nervous system in association with endocrine system that serves as the primary control centre of the body is called neuroendocrine system.

Example: The hypothalamus (releasing factors) stimulates the pituitary gland to release various hormones that control various metabolic activities of the body.

Neurohormone: Any hormone that is produced by a specialized nerve cell (but not by endocrine gland) and is secreted from the nerve endings into the blood stream or tissues to exert its function is called neurohormone.

Example: ADH, noradrenaline, ecdyson, juvenile hormone etc.

Neurosecretion: The synthesis, storage & secretion of (hormones) neurohormones by neurosecretory cells (possess both nerve & endocrine functions) is called neurosecretion.

Example: In the hypothalamus, neurosecretory cells receive nerve impulses from the other parts of the brain or body which signal is transmitted to the pituitary gland by means of neurohormones.

Neurotransmitters: The chemicals that mediate the transmission of nerve impulse across a synapse or neuromuscular junction.

Example: Acetylcholine, adrenaline, noradrenaline, dopamine, serotonin, GABA etc.

Hypothalamus: It is a part of vertebrate forebrain situated below the thalamus & cerebrum that mainly regulates body temperature & neuroendocrine functions.

The neuroendocrine system:

The neuroendocrine system is made up of special cells called neuroendocrine cells. They are spread throughout the body. Neuroendocrine cells are like nerve cells (neurons), but they also make hormones like cells of the endocrine system (endocrine cells). They

receive messages (signals) from the nervous system and respond by making and releasing hormones. These hormones control many body functions.

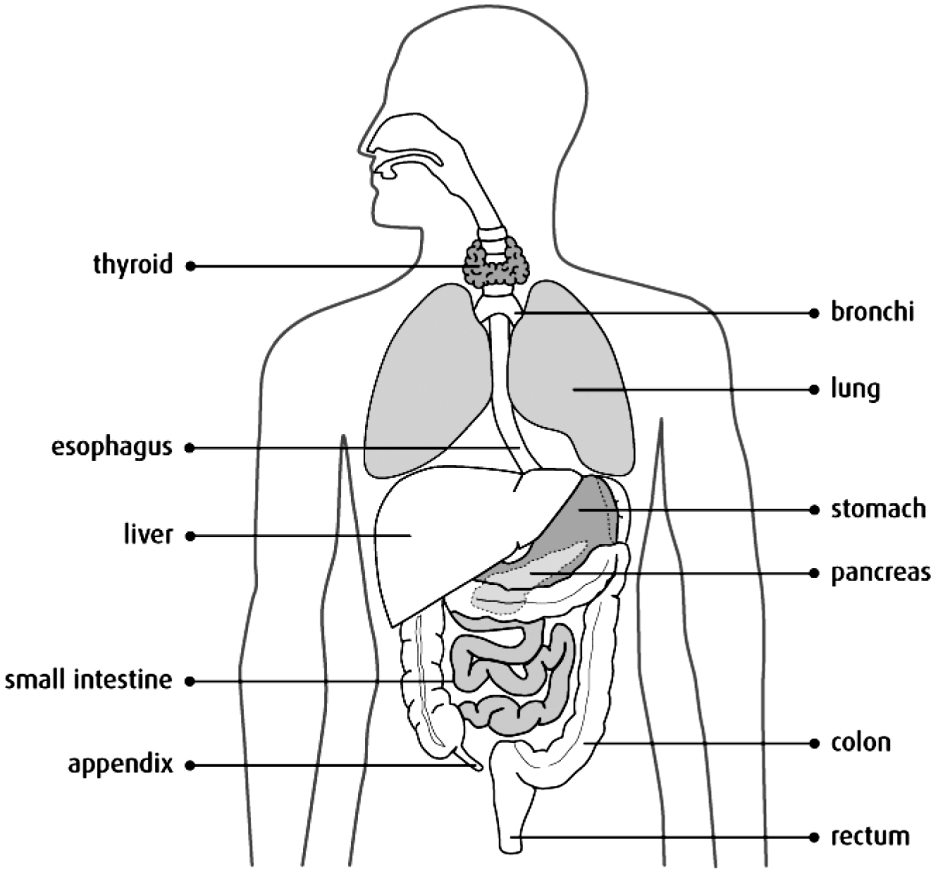
Location of neuroendocrine cells:

Neuroendocrine cells are found in almost every organ of the body. They are mainly found scattered in the gastrointestinal (GI) tract (including the small intestine, rectum, stomach, colon, esophagus and appendix), the gallbladder, the pancreas (islet cells) and the thyroid (C cells). Neuroendocrine cells are also commonly found in the lungs or airways into the lungs (bronchi), as well as the respiratory tract of the head and neck. The neuroendocrine cells scattered throughout these organs are often referred to as the diffuse neuroendocrine system.

The pituitary gland, the parathyroid glands and the inner layer of the adrenal gland (adrenal medulla) are almost all made up of neuroendocrine cells.

Other sites of neuroendocrine cells include the thymus, kidneys, liver, prostate, skin, cervix, ovaries and testicles.

Part of the Neuroendocrine System



Function of neuroendocrine cells:

Neuroendocrine cells make and release hormones and similar substances (peptides) in response to neurological or chemical signals. The hormones then enter the blood and travel throughout the body to other cells (target cells). The hormones attach to specific receptors on target cells, which cause changes in the cells and what they do.

Neuroendocrine cells have many functions, which include controlling:

- the release of digestive enzymes to break down food
- how fast food moves through the GI tract
- air and blood flow through the lungs
- blood pressure and heart rate
- the amount of sugar (glucose) in the blood
- bone and muscle growth and development

The following are examples of hormones or peptides released by neuroendocrine cells and what they do.

- Serotonin (5-HT or 5-hydroxytryptamine) is a chemical released by nerve cells (neurotransmitter) that helps with digestion. A lot of the body's serotonin is found and made in the neuroendocrine cells of the GI tract where it controls the movement of food through the GI tract.
- Gastrin tells the stomach to release acid and enzymes to help with digestion.
- Insulin is made by pancreatic islet cells. It lowers the level of sugar (glucose) in the blood when it's high. It controls when cells absorb (take up) sugar for energy.
- Epinephrine (adrenaline) is made by neuroendocrine cells of the adrenal gland. It is released during times of stress, like when you feel fear, and increases heart rate and blood pressure.
- Growth hormone is made in the pituitary gland. It promotes the growth and development of bones and muscles.

Major Neuroendocrine Systems:

Various endocrine glands are intimately associated with hypothalamus and pituitary to controlthe various physiological function of the body by means of various axes which are:

1. **Hypothalamic-pituitary-thyroid (HPT) axis**
2. **Hypothalamic-pituitary-gonodal (HPG) axis**
3. **Hypothalamic-pituitary-adrenal (HPA) axis**
4. **Hypothalamic-neurohypophyseal axis.**

Any of the systems of dual control of certain activities in the body of some higher animals by nervous and hormonal stimulation is the neuroendocrine system. For example, the posterior pituitary gland and the medulla of the adrenal gland receive direct nervous stimulation to secrete their hormones, whereas the anterior pituitary gland is stimulated by releasing hormones from the hypothalamus.

A substantial volume of scientific evidence has been accumulated demonstrating that biological aging is associated with functional deficits at the cellular, tissue, organ, and system levels. Although several theories have been proposed to explain these changes, as well as the increased risk of disease with age, no single explanation has adequately accounted for the diversity of physiological changes associated with age.

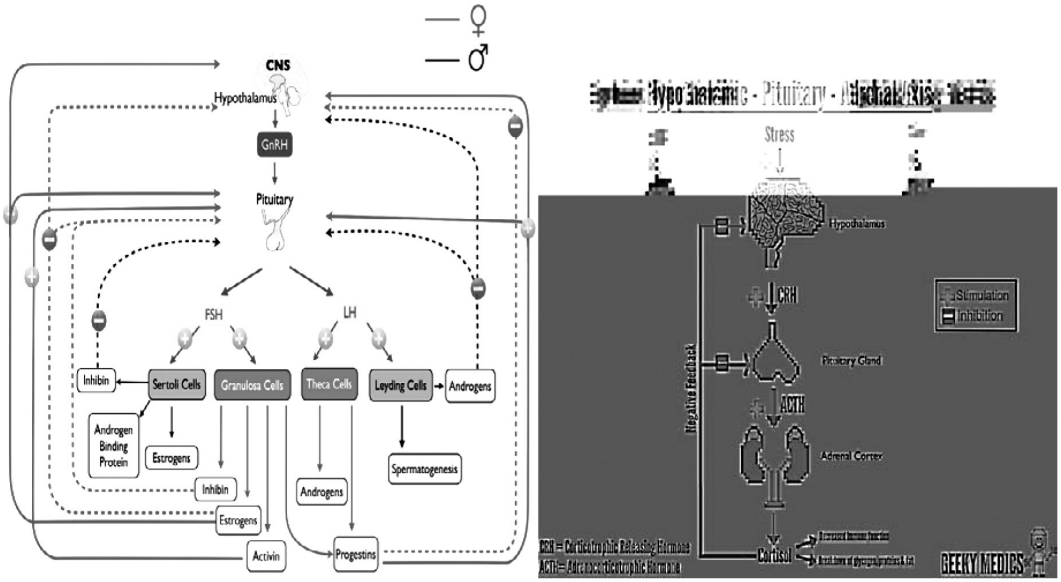
The concept that deficiencies in the neuroendocrine system contribute to aging evolved from **studies indicating that-**

(1) the endocrine system has an important role in developmental processes,

(2) hormones have an important trophic & integrative role in maintaining tissue function, and

(3) hormone deficiency results in deterioration of tissue function.

The neuroendocrine system is composed of the hypothalamus and pituitary gland and is under the influence of neurotransmitters and neuropeptides that regulate hypothalamic releasing and hypothalamic release inhibiting hormones secreted into the blood vessels that connect the hypothalamus and pituitary gland. The release of these hypothalamic hormones influences the secretion of anterior pituitary hormones that subsequently regulate tissue function. The hypothalamus and pituitary gland have the capacity to detect humoral secretions (hormones secreted) from target tissues and adjust hormone production to maintain an optimal internal “milieu” appropriate for normal function.



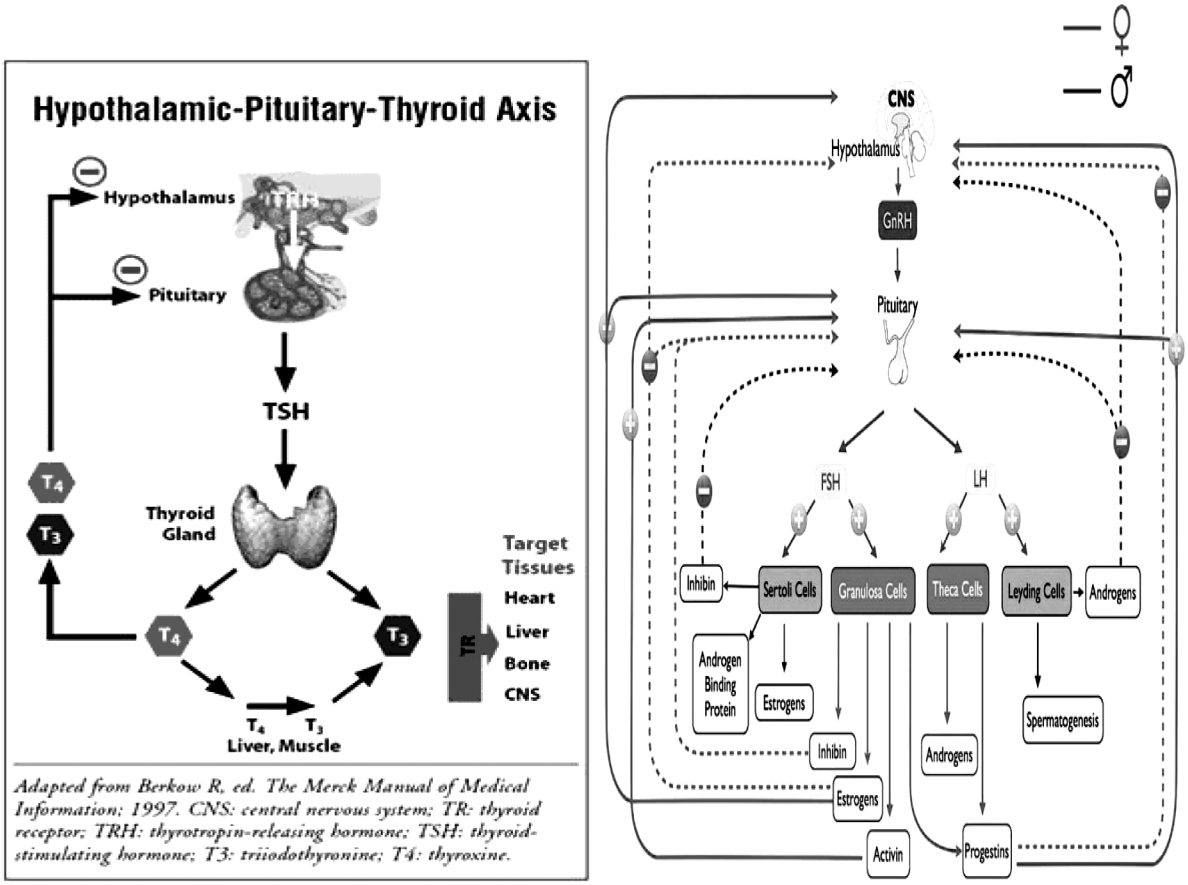


Figure: Various hypothalamic-hypophyseal axes

It is well-established that the neuroendocrine system has a critical role in integrating biological responses and influencing:

(1) cellular protein synthesis and general metabolism through the release of growth hormone andthyroid-stimulating hormone (TSH), respectively,

(2) reproductive function through the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and oxytocin, and

(3) plasma electrolytes and responses to stress through regulation of the hormones vasopressin (antidiuretic hormone, or ADH) and adrenocorticotropin (ACTH).

In addition, the hypothalamus also has an important role in the integration of parasympathetic and sympathetic nervous system activity, and can thereby influence a wide variety of functions, including heart rate, blood pressure, vascular responses, and glucose metabolism. The hypothalamus has been implicated in the regulation of biological rhythms by its interactions with hypothalamic nuclei. More recently, the regulation of fat metabolism and food intake has been shown to be regulated through the hypothalamus by its response to the protein, leptin, and its synthesis of neuropeptide Y. It should be

noted that the classification of hormones and their primary function presented here is an overly simplistic view of the neuroendocrine system, since critical interactions occur among these hormones that contribute to the coordinated regulation of cellular and tissue function.

Although the specific etiology of age-related changes in the neuroendocrine system is unknown, it has been proposed that cellular and molecular alterations in specific subpopulations of neurons within the hypothalamus and pituitary, and/or supporting structures within the brain, contribute to the decrease in tissue function. Some of the alterations may be related to loss of neurons or synapses, genetic errors, and/ or the production of free radicals, all of which lead to progressive aberrations in neurons and contribute to neuroendocrine aging. As a result, the neuroendocrine theory of aging is unique when compared to other theories of aging in that the neuroendocrine alterations are, in many cases, not considered the primary causative factors of biological aging, but rather are considered to be mediators of aging that are initiated by cellular changes in specific subpopulations of neurons or systems that closely interact with hypothalamic neurons. Three classic examples of age-associated changes in neuroendocrine regulation, and the resulting consequences for tissue function, help emphasize the importance of this system in the development of the aging phenotype.

First, with increasing age there is a decline in growth-hormone secretion that results in a decrease in insulin-like growth factor-1 (IGF-1) production in the liver and other tissues. The loss of these anabolic hormones contributes to the general decline in cellular protein synthesis, skeletal muscle mass, immune function, and cognitive ability in rodents, nonhuman primates, and humans. The decrease in growth-hormone release from the pituitary gland results from impaired release of growth-hormone-releasing hormone and increased release of somatostatin (an inhibitor of growth hormone) from hypothalamic neurons.

Second, decreased secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons results in a decline in luteinizing hormone. This is the primary factor in the loss of reproductive cycles in the female rodent, and, in conjunction with the loss of ovarian follicles, contributes to the decline in oestrogen levels in women. These latter changes result in atrophy of secondary reproductive tissues and have been implicated in the post-menopausal loss of bone and cognitive function. Decreased GnRH secretion in the male also contributes to a decrease in LH and androgen levels and to the corresponding loss of skeletal muscle mass and reproductive function.

Finally, increased secretion of ACTH and the adrenal hormone, cortisol, in response to stress have been reported to contribute to atrophy and/or loss of neurons, as well as age-related decline in cognitive function. These latter findings have contributed to the hypothesis that increased levels of glucocorticoids contribute to brain aging.

Although other mechanisms are possible, the alterations in the secretion of hypothalamic hormones with age have been traced to deficiencies in the secretion of brain neurotransmitters. For example, the activity of dopamine and norepinephrine decreases with age, and both acute and chronic procedures used to increase levels of these neurotransmitters in aged animals have been shown to restore some aspects of neuroendocrine function. Studies have shown an increase in growth hormone release and a restoration of some aspects of reproductive function in older animals in response to the L-Dopa, dopamine and norepinephrine precursor. These findings have led investigators to conclude that a decline in neurotransmitter activity is a contributing factor in the neuroendocrine decline that accompanies aging. Nevertheless, the possibility that interactions with other hypothalamic peptides, the loss of neurons, or intracellular changes within hypothalamic neurons contribute to the loss of function cannot be excluded.

In fact, the inability of hypothalamic neurons to compensate for the age-related alterations in circulating levels of hormones supports the concept that the normal feedback mechanisms that occur within the hypothalamus are impaired in aged animals. Whether these altered feedback mechanisms are related to the deficiencies in neurotransmitters or result from other aberrations within the aging neuroendocrine system remain to be established. Nevertheless, deficits in the regulation of these critical hormonal systems contribute to deterioration of tissue function and undoubtedly are an important factor in age-related disease and disability.

Probable Questions:

1. Define neurohormone. Give examples.
2. Define neuroendocrine system. State the location of neuroendocrine cells.
3. What are the functions of neuroendocrine cells?
4. Name four major neuroendocrine system.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XVII

Molecular basis of endocrinopathies I: thyrotoxicosis, hypothyroidism, Hashimoto's thyroiditis.

Objective: In this unit we will discuss different types of endocrinopathies such as thyrotoxicosis, hypothyroidism, Hashimoto's thyroiditis.

Introduction: The endocrine system is a network of glands that produce and release hormones that help control many important body functions, including the body's ability to change calories into energy that powers cells and organs. The endocrine system influences how your heart beats, how your bones and tissues grow, even your ability to make a baby. It plays a vital role in whether or not you develop diabetes, thyroid disease, growth disorders, sexual dysfunction, and a host of other hormone-related disorders.

Glands of the Endocrine System

Each gland of the endocrine system releases specific hormones into your bloodstream. These hormones travel through your blood to other cells and help control or coordinate many body processes.

Endocrine glands include:

- **Adrenal glands:** Two glands that sit on top of the kidneys that release the hormone cortisol.
- **Hypothalamus:** A part of the lower middle brain that tells the pituitary gland when to release hormones.
- **Ovaries:** The female reproductive organs that release eggs and produce sex hormones.
- **Islet cells in the** pancreas**:** Cells in the pancreas control the release of the hormones insulin and glucagon.
- **Parathyroid:** Four tiny glands in the neck that play a role in bone development.
- **Pineal gland:** A gland found near the center of the brain that may be linked to sleep patterns.
- **Pituitary gland:** A gland found at the base of brain behind the sinuses. It is often called the "master gland" because it influences many other glands, especially the thyroid. Problems with the pituitary gland can affect bone growth, a woman's menstrual cycles, and the release of breast milk.

- **Testes:** The male reproductive glands that produce sperm and sex hormones.
- **Thymus:** A gland in the upper chest that helps develop the body's immune system early in life.

Thyroid**:** A butterfly-shaped gland in the front of the neck that controls metabolism.

Even the slightest hiccup with the function of one or more of these glands can throw off the delicate balance of hormones in your body and lead to an endocrine disorder, or endocrine disease.

Causes of Endocrine Disorders

Endocrine disorders are typically grouped into two categories:

- Endocrine disease that results when a gland produces too much or too little of an endocrine hormone, called a hormone imbalance.
- Endocrine disease due to the development of lesions (such as nodules or tumors) in the endocrine system, which may or may not affect hormone levels.

The endocrine's feedback system helps control the balance of hormones in the bloodstream. If your body has too much or too little of a certain hormone, the feedback system signals the proper gland or glands to correct the problem. A hormone imbalance may occur if this feedback system has trouble keeping the right level of hormones in the bloodstream, or if your body doesn't clear them out of the bloodstream properly.

Increased or decreased levels of endocrine hormone may be caused by:

- A problem with the endocrine feedback system
- Disease
- Failure of a gland to stimulate another gland to release hormones (for example, a problem with the hypothalamus can disrupt hormone production in the pituitary gland)
- A genetic disorder, such as multiple endocrine neoplasia (MEN) or congenital hypothyroidism
- Infection
- Injury to an endocrine gland
- Tumor of an endocrine gland

Most endocrine tumors and nodules (lumps) are noncancerous. They usually do

not spread to other parts of the body. However, a tumor or nodule on the gland may interfere with the gland's hormone production.

Types of Endocrine Disorders

There are many different types of endocrine disorders. Diabetes is the most common endocrine disorder diagnosed are as follows:

- **Adrenal insufficiency.** The adrenal gland releases too little of the hormone cortisol and sometimes, aldosterone. Symptoms include fatigue, stomach upset, dehydration, and skin changes. Addison's disease is a type of adrenal insufficiency.
- **Cushing's disease.** Overproduction of a pituitary gland hormone leads to an overactive adrenal gland. A similar condition called Cushing's syndrome may occur in people, particularly children, who take high doses of corticosteroid medications.
- **Gigantism (**acromegaly**) and other growth hormone problems.** If the pituitary gland produces too much growth hormone, a child's bones and body parts may grow abnormally fast. If growth hormone levels are too low, a child can stop growing in height.
- **Hyperthyroidism.** The thyroid gland produces too much thyroid hormone, leading to weight loss, fast heart rate, sweating, and nervousness. The most common cause for an overactive thyroid is an autoimmune disorder called Grave's disease.
- **Hypothyroidism.** The thyroid gland does not produce enough thyroid hormone, leading to fatigue, constipation, dry skin, and depression. The underactive gland can cause slowed development in children. Some types of hypothyroidism are present at birth.
- **Hypopituitarism.** The pituitary gland releases little or no hormones. It may be caused by a number of different diseases. Women with this condition may stop getting their periods.
- **Multiple endocrine neoplasia I and II (MEN I and MEN II).** These rare, genetic conditions are passed down through families. They cause tumors of the parathyroid, adrenal, and thyroid glands, leading to overproduction of hormones.
- **Polycystic ovary syndrome (PCOS).** Overproduction of androgens interfere with the development of eggs and their release from the female ovaries. PCOS is a leading cause of infertility.
- **Precocious puberty.** Abnormally early puberty that occurs when glands tell the body to release sex hormones too soon in life.

Thyrotoxicosis: It refers to the clinical state of excess circulating thyroid hormones (T3 and/or T4), leading to a **hypermetabolic condition**. It may arise from various

causes, not all of which involve increased hormone production (as in **hyperthyroidism**, a specific subtype of thyrotoxicosis).

Symptoms range from mild or asymptomatic to severe, including:

- **Weight loss**
- **Heat intolerance**
- **Palpitations**
- In critical cases: **thyroid storm**, **delirium**, **atrial fibrillation**, **heart failure**, and **cardiovascular collapse**.

Complications may include:

- **Osteoporosis**
- **Muscle weakness**
- **Thromboembolic events**
- **Altered mental status**
- **Death**, if untreated

Diagnosis and management require:

- Detailed **clinical evaluation**
- **Thyroid function tests**
- **Imaging** to determine etiology

Effective treatment is **cause-specific**, and successful outcomes depend on **interdisciplinary collaboration** in patient care.

Etiology

The etiology of thyrotoxicosis can be divided into an endogenous or exogenous source of TSH.

Increased endogenous secretion of thyroid hormone

- Grave's disease
- Toxic multinodular goiter
- Toxic adenoma
- TSH-producing adenoma or pituitary adenoma
- HCG-mediated hyperthyroidism
- Thyroiditis
- Drug-induced

Increased exogenous secretion of thyroid hormone

- Factitious hyperthyroidism
- Excessive replacement therapy with levothyroxine

The most common cause of thyrotoxicosis is Graves' disease, followed by toxic multinodular goiter (TMNG) and toxic adenoma (TA)[7]. Other causes include thyroiditis, subacute thyroiditis, painless thyroiditis, and gestational hyperthyroidism. Drug-induced thyrotoxicosis has been associated with amiodarone and iodinated contrast. Rare causes of thyrotoxicosis include TSH-producing adenomas, struma ovarii, gestational trophoblastic neoplasia, thyrotoxicosis factitia, activation mutations of the TSH receptor, and functional thyroid cancer metastases.

Epidemiology: Thyrotoxicosis affects ~1.2% of the U.S. population. Graves' disease is the most common cause, especially in women aged 30–50. Toxic multinodular goiter and thyroiditis are other contributors. 1–2% of cases can progress to thyroid storm.

Pathophysiology: Excess thyroid hormone increases metabolic rate and sympathetic activity by upregulating adrenergic receptors. This leads to increased heart rate, cardiac output, and oxygen consumption, reduced vascular resistance, and expanded blood volume. Untreated, this can result in heart failure.

- *Graves' disease:* Autoantibodies stimulate TSH receptors, causing diffuse goiter and hyperthyroidism (type II hypersensitivity).
- *Toxic nodules:* Autonomous hormone production independent of TSH.
- *Thyroiditis:* Release of preformed hormone due to follicular destruction.
- *Gestational hyperthyroidism:* HCG mimics TSH action.

Symptoms: Weight loss, heat intolerance, palpitations, tremors, anxiety, muscle weakness, fatigue, amenorrhea (women), gynecomastia (rare, men). In older adults: depression, fatigue, "apathetic thyrotoxicosis."

Signs: Goiter, tachycardia/AF, tremor, hyperreflexia, ophthalmopathy (Graves'), pretibial myxedema, and thyroid acropachy. Thyroid storm presents with fever, delirium, cardiac failure.

Diagnosis:

- Low TSH with elevated free T3/T4 = overt thyrotoxicosis
- Graves': positive TSH receptor antibodies, diffuse RAI uptake
- Toxic adenoma: focal RAI uptake
- Thyroiditis: low RAI uptake, elevated ESR/CRP
- Pregnancy: adjust hormone reference ranges

Management:

- *Symptomatic relief:* Beta-blockers (e.g., propranolol)
- *Thionamides:* Methimazole or PTU to inhibit thyroid hormone synthesis
    - PTU preferred in first trimester pregnancy
    - Monitor for agranulocytosis and hepatotoxicity
- *Radioiodine therapy:* Most common in adults; contraindicated in pregnancy
- *Surgery:* Total/partial thyroidectomy; used for large goiters or intolerance to other treatments
- *Thyroiditis:* NSAIDs or steroids for inflammation; beta-blockers for symptoms
- *Pediatric and pregnancy management:* Methimazole is first-line in children; PTU used in early pregnancy

Conclusion: Thyrotoxicosis is a clinically diverse disorder with multiple etiologies. Diagnosis depends on hormonal assays and imaging, while treatment must be individualized based on the underlying cause, patient age, comorbidities, and pregnancy status.

Hypothyroidism:

Hypothyroidism happens when the thyroid gland doesn't make enough thyroid hormone. This condition also is called underactive thyroid. Hypothyroidism may not cause noticeable symptoms in its early stages. Over time, hypothyroidism that isn't treated can lead to other health problems, such as high cholesterol and heart problems. Blood tests are used to diagnose hypothyroidism. Treatment with thyroid hormone medicine usually is simple, safe and effective once you and your health care provider find the right dosage for you.

Symptoms

The symptoms of hypothyroidism depend on the severity of the condition. Problems tend to develop slowly, often over several years. At first, you may barely notice the symptoms of hypothyroidism, such as fatigue and weight gain. Or you may think they are just part of getting older. But as your metabolism continues to slow, you may develop more-obvious problems.

How does thyroid work?

The thyroid gland is a butterfly-shaped endocrine organ located anterior to the trachea and just below the larynx. It plays a pivotal role in regulating **metabolism**, the biochemical process by which the body converts food into energy. The gland synthesizes

and secretes two primary hormones: **thyroxine (T4)** and **triiodothyronine (T3)**, which modulate energy expenditure, thermoregulation, and cardiac function by influencing cellular metabolism throughout the body.Hormonal output from the thyroid is under **tight negative feedback control** by the **pituitary gland**, which secretes **thyroid-stimulating hormone (TSH)**. TSH levels adjust in response to circulating T3 and T4 concentrations to maintain metabolic homeostasis. Dysregulation of this axis leads to **hyperthyroidism** (excess hormone) or **hypothyroidism** (deficient hormone), both of which can significantly disrupt systemic physiological functions.

Who is affected by hypothyroidism?

Hypothyroidism can affect people of all ages, genders and ethnicities. It's a common condition, particularly among women over age 60. Women are generally more likely to develop hypothyroidism after menopause than earlier in life.

Difference between hypothyroidism and hyperthyroidism?

In hypothyroidism, the thyroid doesn't make enough thyroid hormone. The difference between hypothyroidism and hyperthyroidism is quantity. In hypothyroidism, the thyroid makes very little thyroid hormone. On the flip side, someone with hyperthyroidism has a thyroid that makes too much thyroid hormone. Hyperthyroidism involves higher levels of thyroid hormones, which makes your metabolism speed up. If you have hypothyroidism, your metabolism slows down.

Many things are the opposite between these two conditions. If you have hypothyroidism, you may have a difficult time dealing with the cold. If you have hyperthyroidism, you may not handle the heat. They are opposite extremes of thyroid function. Ideally, you should be in the middle. Treatments for both of these conditions work to get your thyroid function as close to that middle ground as possible.

SYMPTOMS AND CAUSES of Hypothyroidism:

Hypothyroidism symptoms may include:

- Tiredness.
- More sensitivity to cold.
- Constipation.
- Dry skin.
- Weight gain.
- Puffy face.
- Hoarse voice.

- Coarse hair and skin.
- Muscle weakness.
- Muscle aches, tenderness and stiffness.
- Menstrual cycles that are heavier than usual or irregular.
- Thinning hair.
- Slowed heart rate, also called bradycardia.
- Depression.
- Memory problems

Hypothyroidism in infants

Anyone can get hypothyroidism, including infants. Most babies born without a thyroid gland or with a gland that doesn't work correctly don't have symptoms right away. But if hypothyroidism isn't diagnosed and treated, symptoms start to appear. They may include:

- Feeding problems.
- Poor growth.
- Poor weight gain.
- Yellowing of the skin and the whites of the eyes, a condition called jaundice.
- Constipation.
- Poor muscle tone.
- Dry skin.
- Hoarse crying.
- Enlarged tongue.
- A soft swelling or bulge near the belly button, a condition called umbilical hernia.

When hypothyroidism in infants isn't treated, even mild cases can lead to severe physical and mental development problems.

Hypothyroidism in children and teens

In general, children and teens with hypothyroidism have symptoms similar to those in adults. But they also may have:

- Poor growth that leads to short stature.
- Delayed development of permanent teeth.
- Delayed puberty.
- Poor mental development.

Causes:

The thyroid is a small, butterfly-shaped gland located at the base of the neck, just below the Adam's apple. The thyroid gland makes two main hormones: thyroxine (T-4) and triiodothyronine (T-3). These hormones affect every cell in the body. They support the rate at which the body uses fats and carbohydrates. They help control body temperature. They have an effect on heart rate. And they help control how much protein the body makes.

Hypothyroidism happens when the thyroid gland doesn't make enough hormones. Conditions or problems that can lead to hypothyroidism include:

- **Autoimmune disease.** The most common cause of hypothyroidism is an autoimmune disease called Hashimoto's disease. Autoimmune diseases happen when the immune system makes antibodies that attack healthy tissues. Sometimes that process involves the thyroid gland and affects its ability to make hormones.
- **Thyroid surgery.** Surgery to remove all or part of the thyroid gland can lower the gland's ability to make thyroid hormones or stop it completely.
- **Radiation therapy.** Radiation used to treat cancers of the head and neck can affect the thyroid gland and lead to hypothyroidism.
- **Thyroiditis.** Thyroiditis happens when the thyroid gland becomes inflamed. This may be due to an infection. Or it can result from an autoimmune disorder or another medical condition affecting the thyroid. Thyroiditis can trigger the thyroid to release all of its stored thyroid hormone at once. That causes a spike in thyroid activity, a condition called hyperthyroidism. Afterward, the thyroid becomes underactive.
- **Medicine.** A number of medicines may lead to hypothyroidism. One such medicine is lithium, which is used to treat some psychiatric disorders. If you're taking medicine, ask your heath care provider about its effect on the thyroid gland.

Less often, hypothyroidism may be caused by:

- **Problems present at birth.** Some babies are born with a thyroid gland that doesn't work correctly. Others are born with no thyroid gland. In most cases, the reason the thyroid gland didn't develop properly is not clear. But some children have an inherited form of a thyroid disorder. Often, infants born with hypothyroidism don't have noticeable symptoms at first. That's one reason why most states require newborn thyroid screening.
- **Pituitary disorder.** A relatively rare cause of hypothyroidism is the failure of the pituitary gland to make enough thyroid-stimulating hormone (TSH). This is usually because of a noncancerous tumor of the pituitary gland.

- **Pregnancy.** Some people develop hypothyroidism during or after pregnancy. If hypothyroidism happens during pregnancy and isn't treated, it raises the risk of pregnancy loss, premature delivery and preeclampsia. Preeclampsia causes a significant rise in blood pressure during the last three months of pregnancy. Hypothyroidism also can seriously affect the developing fetus.
- **Not enough iodine.** The thyroid gland needs the mineral iodine to make thyroid hormones. Iodine is found mainly in seafood, seaweed, plants grown in iodine-rich soil and iodized salt. Too little iodine can lead to hypothyroidism. Too much iodine can make hypothyroidism worse in people who already have the condition. In some parts of the world, it's common for people not to get enough iodine in their diets. The addition of iodine to table salt has almost eliminated this problem in the United States.

Risk factors

Although anyone can develop hypothyroidism, you're at an increased risk if you:

- Are a woman.
- Have a family history of thyroid disease.
- Have an autoimmune disease, such as type 1 diabetes or celiac disease.
- Have received treatment for hyperthyroidism.
- Received radiation to your neck or upper chest.
- Have had thyroid surgery.

Complications:

Hypothyroidism that isn't treated can lead to other health problems, including:

- **Goiter.** Hypothyroidism may cause the thyroid gland to become larger. This condition is called a goiter. A large goiter may cause problems with swallowing or breathing.
- **Heart problems.** Hypothyroidism can lead to a higher risk of heart disease and heart failure. That's mainly because people with an underactive thyroid tend to develop high levels of low-density lipoprotein (LDL) cholesterol — the "bad" cholesterol.
- **Peripheral neuropathy.** Hypothyroidism that goes without treatment for a long time can damage the peripheral nerves. These are the nerves that carry information from the brain and spinal cord to the rest of the body. Peripheral neuropathy may cause pain, numbness and tingling in the arms and legs.

- **Infertility.** Low levels of thyroid hormone can interfere with ovulation, which can limit fertility. Some of the causes of hypothyroidism, such as autoimmune disorders, also can harm fertility.
- **Birth defects.** Babies born to people with untreated thyroid disease may have a higher risk of birth defects compared with babies born to mothers who do not have thyroid disease.

  Infants with hypothyroidism present at birth that goes untreated are at risk of serious physical and mental development problems. But if the condition is diagnosed within the first few months of life, the chances of typical development are excellent.
- **Myxedema coma.** This rare, life-threatening condition can happen when hypothyroidism goes without treatment for a long time. A myxedema coma may be triggered by sedatives, infection or other stress on the body. Its symptoms include intense cold intolerance and drowsiness, followed by an extreme lack of energy and then unconsciousness. Myxedema coma requires emergency medical treatment.

Treatment of hypothyroidism:

In most cases, hypothyroidism is treated by replacing the amount of hormone that your thyroid is no longer making. This is typically done with a medication. One medication that is commonly used is called levothyroxine. Taken orally, this medication increases the amount of thyroid hormone your body produces, evening out your levels.

Hypothyroidism is a manageable disease. However, you will need to continuously take medication to normalize the amount of hormones in your body for the rest of your life. With careful management, and follow-up appointments with your healthcare provider to make sure your treatment is working properly, you can lead a normal and healthy life.

What happens if hypothyroidism is not treated?

Hypothyroidism can become a serious and life-threatening medical condition if you do not get treatment from a healthcare provider. If you are not treated, your symptoms can become more severe and can include:

- Developing mental health problems.
- Having trouble breathing.
- Not being able to maintain a normal body temperature.
- Having heart problems.
- Developing a goiter (enlargement of the thyroid gland).

You can also develop a serious medical condition called myxedema coma. This can happen when hypothyroidism isn't treated.

Prevention of hypothyroidism:

Hypothyroidism cannot be prevented. The best way to prevent developing a serious form of the condition or having the symptoms impact your life in a serious way is to watch for signs of hypothyroidism. If you experience any of the symptoms of hypothyroidism, the best thing to do is talk to your healthcare provider. Hypothyroidism is very manageable if you catch it early and begin treatment.

Foods related to hypothyroidism?

Most foods in western diets contain iodine, so you do not have to worry about your diet. Iodine is a mineral that helps your thyroid produce hormones. One idea is that if you have low levels of thyroid hormone, eating foods rich in iodine could help increase your hormone levels. The most reliable way to increase your hormone levels is with a prescription medication from your healthcare provider. Do not try any new diets without talking to your provider first. It's important to always have a conversation before starting a new diet, especially if you have a medical condition like hypothyroidism.

Foods that are high in iodine include:

- Eggs.
- Dairy products.
- Meat, poultry and seafood.
- Edible seaweed.
- Iodized salt.

Work with your healthcare provider or a nutritionist (a healthcare provider who specializes in food) to craft a meal plan. Your food is your fuel. Making sure you are eating foods that will help your body, along with taking your medications as instructed by your healthcare provider, can keep you healthy over time. People with thyroid condition should not consume large amounts of iodine because the effect may be paradoxical (self-contradictory).

Can hypothyroidism go away on its own?

In some mild cases, you may not have symptoms of hypothyroidism or the symptoms may fade over time. In other cases, the symptoms of hypothyroidism will go away shortly

after you start treatment. For those with particularly low levels of thyroid hormones, hypothyroidism is a life-long condition that will need to be managed with medication on a regular schedule.

Hashimoto's Disease:

Hashimoto's disease is an autoimmune disorder that can cause hypothyroidism, or underactive thyroid. Rarely, the disease can cause hyperthyroidism, or overactive thyroid.

The thyroid is a small, butterfly-shaped gland in the front of your neck. In people with Hashimoto's disease

- the immune system makes antibodies that attack the thyroid gland
- large numbers of white blood cells, which are part of the immune system, build up in the thyroid
- the thyroid becomes damaged and can't make enough thyroid hormones

Thyroid hormones control how your body uses energy, so they affect nearly every organ in your body—even the way your heart beats. Hashimoto's disease is also called Hashimoto's thyroiditis, chronic lymphocytic thyroiditis, or autoimmune thyroiditis.

How common is Hashimoto's disease?

The number of people who have Hashimoto's disease in the United States is unknown. However, the disease is the most common cause of hypothyroidism, which affects about 5 in 100 Americans.

Who is more likely to have Hashimoto's disease?

Hashimoto's disease is 4 to 10 times more common in women than men. Although the disease may occur in teens or young women, it more often develops in women ages 30 to 50. Your chance of developing Hashimoto's disease increases if other family members have the disease.

Persons are more likely to develop Hashimoto's disease if they have other autoimmune disorders, including

- celiac disease, a digestive disorder that damages the small intestine
- lupus, a chronic, or long-term, disorder that can affect many parts of the body
- rheumatoid arthritis, a disorder that affects the joints
- Sjögren's syndrome, a disease that causes dry eyes and mouth
- type 1 diabetes, a disease that occurs when your blood glucose, also called blood sugar, is too high.

What are the complications of Hashimoto's disease?

Many people with Hashimoto's disease develop hypothyroidism. Untreated, hypothyroidism can lead to several health problems, including

- high cholesterol
- heart disease and heart failure *NIH external link*
- high blood pressure
- myxedema *NIH external link*, a rare condition in which the body's functions slow down to the point that it can threaten your life

What are the symptoms of Hashimoto's disease?

Many people with Hashimoto's disease have no symptoms at first. As the disease progresses, you may have one or more of the symptoms of hypothyroidism.

Some common symptoms of hypothyroidism include

- fatigue
- weight gain
- trouble tolerating cold
- joint and muscle pain
- constipation
- dry skin or dry, thinning hair
- heavy or irregular menstrual periods or fertility problems
- slowed heart rate

Hashimoto's disease causes your thyroid to become damaged. Most people with Hashimoto's disease develop hypothyroidism. Rarely, early in the course of the disease, thyroid damage may lead to the release of too much thyroid hormone into your blood, causing symptoms of hyperthyroidism. Thyroid may get larger and cause the front of the neck to look swollen. The enlarged thyroid, called a goiter, may create a feeling of fullness in your throat, though it is usually not painful. After many years, or even decades, damage to the thyroid may cause the gland to shrink and the goiter to disappear.

Causes of Hashimoto's disease:

Researchers don't know why some people develop Hashimoto's disease, but a family history of thyroid disease is common. Several factors may play a role, including[2]

- genes
- viruses, such as hepatitis C

Hypothyroidism can also be caused by

- some medicines used to treat bipolar disorder or other mental health problems
- iodine-containing medicines used to treat abnormal heart rhythm
- exposure to toxins, such as nuclear radiation

Diagnosis of Hashimoto's disease:

- **medical history and physical exam**. Your doctor will start by taking a medical history and performing a physical exam. In addition to asking about symptoms, the doctor will check your neck for a goiter, which some people with Hashimoto's disease can develop.
- **blood tests**. Your doctor will order one or more blood tests to check for hypothyroidism and its causes. Examples include tests for
    - the thyroid hormones T4 (thyroxine) and T3 (triiodothyronine)
    - thyroid-stimulating hormone, or TSH
    - thyroid peroxidase antibodies (TPO), a type of thyroid antibody that is present in most people with Hashimoto's disease

You probably won't need other tests to confirm you have Hashimoto's disease. However, if your doctor suspects Hashimoto's disease but you don't have antithyroid antibodies in your blood, you may have an ultrasound *NIH external link* of your thyroid. The ultrasound images can show the size of your thyroid and other features of Hashimoto's disease. The ultrasound also can rule out other causes of an enlarged thyroid, such as thyroid nodules—small lumps in the thyroid gland.

Treatment of Hashimoto's disease:

How doctors treat Hashimoto's disease usually depends on whether the thyroid is damaged enough to cause hypothyroidism. If you don't have hypothyroidism, your doctor may choose to simply check your symptoms and thyroid hormone levels regularly.

The medicine levothyroxine which is identical to the natural thyroid hormone thyroxine (T4), is the recommended way to treat hypothyroidism. Prescribed in pill form for many years, this medicine is now also available as a liquid and in a soft gel capsule. These newer formulas may be helpful to people with digestive problems that affect how the thyroid hormone pill is absorbed.

Some foods and supplements can affect how well your body absorbs levothyroxine. Examples include grapefruit juice, espresso coffee, soy, and multivitamins that contain iron

or calcium.Taking the medicine on an empty stomach can prevent this from happening. Your doctor may ask you to take the levothyroxine in the morning, 30 to 60 minutes before you eat your first meal.

Your doctor will give you a blood test about 6 to 8 weeks after you begin taking the medicine and adjust your dose if needed. Each time you change your dose, you'll have another blood test. Once you've reached a dose that's working for you, your doctor will likely repeat the blood test in 6 months and then once a year. Never stop taking your medicine or take a higher dose without talking with your doctor first. Taking too much thyroid hormone medicine can cause serious problems, such as atrial fibrillation or osteoporosis. Hypothyroidism can be well-controlled with thyroid hormone medicine, as long as you take the medicine as instructed by your doctor and have regular follow-up blood tests.

How does eating, diet, and nutrition affect Hashimoto's disease?

The thyroid uses iodine, a mineral in some foods, to make thyroid hormones. However, if you have Hashimoto's disease or other types of autoimmune thyroid disorders, you may be sensitive to harmful side effects from io iodine. Eating foods that have large amounts of iodine—such as kelp, dulse, or other kinds of seaweed, and certain iodine-rich medicines—may cause hypothyroidism or make it worse. Taking iodine supplements can have the same effect.

Talk with members of your health care team about

- what foods and beverages to limit or avoid
- whether you take iodine supplements
- any cough syrups you take that may contain iodine

However, if you are pregnant, you need to take enough iodine because the baby gets iodine from your diet. Too much iodine can cause problems as well, such as a goiter in the baby. If you are pregnant, talk with your doctor about how much iodine you need.

Researchers are looking at other ways in which diet and supplements—such as vitamin D and selenium may affect Hashimoto's disease. However, no specific guidance is currently available.

Probable Questions:

1. What are the causes of endocrine disorders?
2. Discuss different types of endocrine disorders?
3. Discuss symptoms of thyrotoxicosis.
4. How thyrotoxicosis is treated?
5. What are the symptoms of hypothyroidism?
6. State difference between hypothyroidism and hyperthyroidism.
7. Discuss symptoms and causes of hypothyroidism.
8. Discuss complications of hypothyroidism.
9. How hypothyroidism is treated?
10. Discuss food related to hypothyroidism.
11. Who are more prone to Hashimoto's disease?
12. State the complications of Hashimoto's disease.
13. What are the symptoms of Hashimoto's disease?
14. How Hashimoto's disease is detected and treated?

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XVIII

Molecular basis of endocrinopathies II: Addison's diseases, Cushing syndrome, androgen deficiency syndromes-testicular neoplasm

Objective: In this unit we will discuss different kinds of endocrinopathies such as Cushing syndrome, Addison's diseases, androgen deficiency syndromes-testicular neoplasm.

Addison's disease:

Thomas Addison (1855) first described this disease. It occurs due to hypo secretion of cortical glucocorticoids. It may be due to bilateral tubercular destruction or due to tubercular infection or due to autoimmune diseases. The clinical features are as follows:

Anatomical features:

1. There is increased melanin pigment synthesis in skin and shows bronze in colour.
2. Diseased person shows muscular weakness.
3. Oedema occurs due to loss of water from the capillaries.

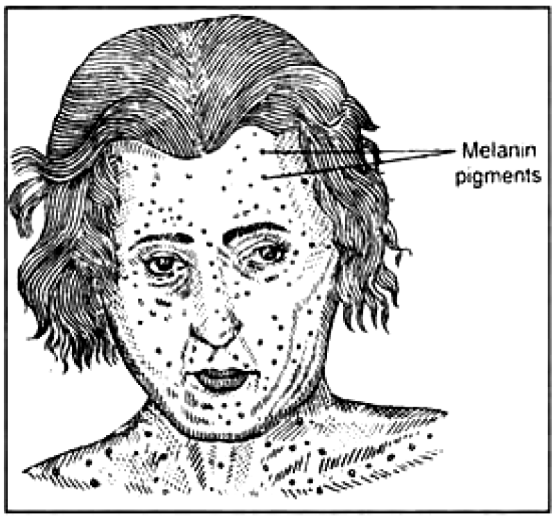


Fig. 12.6: Addison's disease

Physiological features:

1. Gastro-intestinal disturbances and vomiting occurs.
2. There will be fall of blood pressure.

3. Blood pressure becomes lowered.
4. Metabolic rate is reduced.
5. Subnormal body temperature (below 96°C).
6. Depressed glycogenesis and gluconeogenesis.
7. NaCI is excreted in greater quantity.
8. Potassium is retained.
9. Disturbances is ionic balance.

Psychological features:

1. Ionic disturbances may cause lack of mental concentration.
2. Patient shows restlessness.
3. Shows insomnia.

Sexological features:

1. Sexual activities are reduced.
2. Both primary and secondary sex characters are ill developed.

Cushing's syndrome: H. Cushing (1932) first described this disease; Excess glucocorticoids secretion takes place due to adrenocortical tumours. Pituitary tumour may cause over secretion of ACTH which in-turn causes hyperplasia of cortex.

The clinical features are as follows:

Anatomical features :

1. Excessive body hair growth (hirsutism)
2. Osteoporosis occurs due to decalcification of bones.
3. Face becomes round due to accumulation of fat.
4. Presence of buffalo hump on the back of neck.
5. Skin shows purple striate over the abdomen.
6. Obesity takes place.
7. Slow wound healing
8. Wasting of muscles occurs in the limbs.

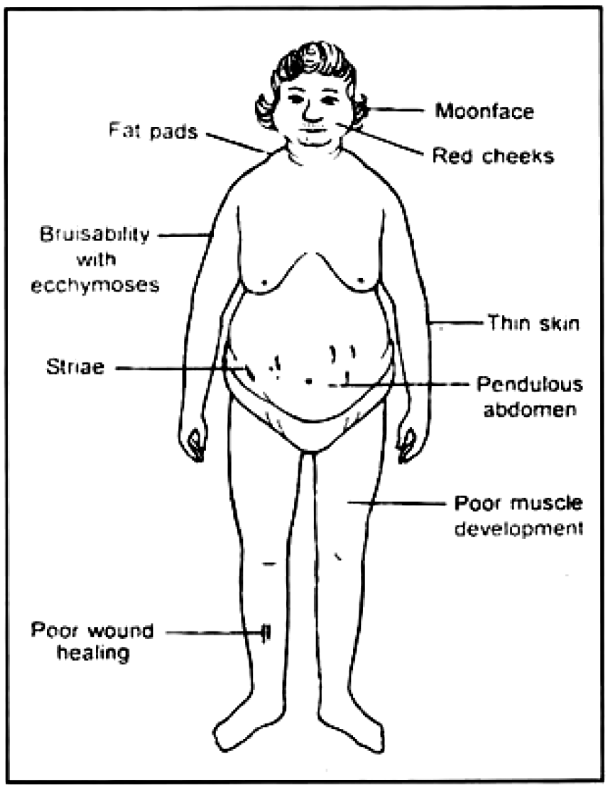


Fig. 12.7: Outstanding signs of Cushing's syndrome

Physiological features:

1. Blood sodium level becomes increased but potassium level decreases.
2. Polyuria and polydipsia occur.
3. Hyperglycemia occurs.
4. Loss of protein takes place from bony matrix.
5. Lipogenic effect occurs.

Psychological features:

1. Patients are mentally deranged.
2. Hypertension takes place.

Sexological features:

1. Female shows masculinization with the development of male secondary sex characters, like growth of beard, mustaches, under developed mammary glands etc.
2. Males suffer from impotence and atrophy of testes.

Androgen Deficiency Syndrome:

Androgen deficiency is when the body has lower levels of male sex hormones, particularly testosterone, than is needed for good health. This deficiency may be caused by problems in the areas of the brain that control the function of the testes (the pituitary gland and the hypothalamus), or by problems in the testes themselves. Treatment involves testosterone replacement therapy.

The term 'male menopause' is meaningless as it doesn't exist: there is no sudden, severe or inevitable drop in sex hormone production in men as experienced by women.

A modest and gradual drop in sex hormone levels is seen across male populations from the age of about 30 but this fall is not seen in all men. In most cases the drop in testosterone appears to be caused by them developing other illnesses along the way.

Androgens are sex hormones

Hormones can be thought of as chemical messengers. They communicate with tissues in the body to bring about many different changes. Hormones are needed for different processes like growth, reproduction and well-being.

Androgens are the group of sex hormones that give men their 'male' characteristics (collectively called virilisation). The major sex hormone in men is testosterone, which is produced mainly in the testes. The testes are controlled by a small gland in the brain called the pituitary gland, which in turn is controlled by an area of the brain called the hypothalamus.

Androgens are crucial for male sexual and reproductive function. They are also responsible for the development of secondary sexual characteristics in men, including facial and body hair growth and voice change. Androgens also affect bone and muscle development and metabolism.

The term androgen deficiency means your body is not making enough androgens, particularly testosterone, for full health. The effects of this depend on how severe the deficiency is, its cause and the age at which the deficiency begins.

Testosterone

The major sex hormone in men is testosterone. Some of the functions of testosterone in the male body include:

- starting and completing the process of puberty
- bone and muscle development
- growth of body hair, including facial hair

- change of vocal cords to produce the adult male voice
- sex drive (libido) and sexual function
- prostate gland growth and function
- sperm production.

Symptoms of androgen deficiency

When there is not enough testosterone circulating in the body, it can cause a wide range of symptoms. However, a number of these symptoms may be non-specific and can mimic the symptoms of other diseases and conditions.

Some of the symptoms of androgen deficiency include:

- reduced sexual desire
- hot flushes and sweating
- breast development (gynaecomastia)
- lethargy and fatigue
- depression
- reduced muscle mass and strength
- increased body fat, particularly around the abdomen
- weaker erections and orgasms
- reduced amount of ejaculate
- loss of body hair
- reduced bone mass, therefore increased risk of osteoporosis.

Androgen deficiency in older men

If testosterone levels decline with age, a number of factors may be causing it. In particular, any cause of poor general health, including obesity, will lower testosterone. Recent research shows that testosterone levels do not drop significantly in healthy older men.

The impact of the fall in testosterone levels in older men is still not completely understood. There has been much media coverage of ‘andropause’ or ‘male menopause’, suggesting that many older men would benefit from testosterone treatment (testosterone replacement therapy). However, there is limited evidence to suggest benefit, and the risks are not clear.

A recent study on the effects of testosterone treatment in older HYPERLINK “https://www.nejm.org/doi/full/10.1056/NEJMoa1506119” men showed a small increase in sexual function with testosterone treatment (in some cases for less than 12 months), but no significant improvement in mood, vitality or physical function.

Do not start any testosterone treatment without careful diagnosis of androgen deficiency. Make sure you have a full health assessment, and that your testosterone levels have clearly been shown to be consistently low. Often, there are other health problems at play (such as obesity and diabetes) that should be treated first, which may make testosterone replacement therapy unnecessary.

The effect of lower testosterone levels with increasing age and the effects of testosterone replacement therapy in men are currently being studied. Of concern are some studies suggesting a rise in cardiovascular disease after starting testosterone therapy in older men, but this remains controversial.

Androgen deficiency in boys

Boys who have not completed puberty should only be treated by paediatric hormone specialists (paediatric endocrinologists).

Causes of androgen deficiency

Some of the causes of androgen deficiency include conditions affecting the:

- **testes** – medical problems that affect the testes can stop them from making enough testosterone. Some of these conditions are present from birth (for example, Klinefelter’s syndrome – a genetic disorder where there is an extra sex chromosome in the body’s cells). Other conditions may occur at various stages of a boy’s or a man’s life, such as:
    - undescended testes
    - loss of testes due to trauma or ‘twisting off’ of the blood supply (torsion)
    - complications following mumps
    - side effects of chemotherapy or radiotherapy
- **pituitary gland** – the most common condition that affects the pituitary gland and leads to low testosterone levels is the presence of a benign tumour (adenoma). The tumour may interfere with the function of the pituitary gland, or it may produce the hormone prolactin, which stops the production of the gonadotrophins, which are the hormones needed to signal the testes to produce testosterone

- **hypothalamus** – particular conditions, such as tumours or a genetic disorder (Kallmann's syndrome), can prevent the hypothalamus from prompting the pituitary gland to release hormones. This will inhibit testosterone production by the testes. This is a rare cause of androgen deficiency.

Diagnosis of androgen deficiency

Androgen deficiency is diagnosed using a number of assessments, including:

- **medical history** – a full history is taken, including details about fertility, sexual function, symptoms of androgen deficiency, other medical problems, occupation, medication and drug use (prescribed and non-prescribed)
- **physical examination** – a thorough general examination is performed, including measuring the size of the testicles and checking for breast development
- **blood tests** – are taken to determine the level of testosterone in the blood. Ideally, a fasting blood test should be taken in the morning to detect the body's peak release of testosterone. Testosterone levels should be measured on two separate mornings. The pituitary hormone levels should also be measured
- **other tests** – may be required to determine if testosterone deficiency is due to another underlying medical condition. These may include blood tests to check for iron levels, genetic tests (to diagnose an underlying genetic condition, such as Klinefelter's syndrome), or MRI scans of the brain (to examine the pituitary gland). Semen analysis will help to determine the potential fertility of men with androgen deficiency.

Treatment of androgen deficiency

Treatment for proven androgen deficiency is based on testosterone replacement therapy. Testosterone is best administered by skin gels creams, or by injection (short- or long-acting).

If your testosterone deficiency is caused by your pituitary gland and you are also wishing to father a child, your doctor will probably recommend gonadotrophin injections, several times a week for many months, to stimulate both testosterone and sperm production.

Testosterone treatment is not recommended for men trying to have a child as it acts as a powerful contraceptive by suppressing the pituitary hormones that drive sperm production. If you are androgen deficient and you and your partner are trying to have a baby, see a fertility specialist.

If you are having testosterone replacement therapy you will have regular reviews with your doctor. How often you have these will depend on your age and other risk factors for prostate cancer.

Older men need to be checked for prostate cancer before testosterone replacement therapy can be started, because increased levels of testosterone could make unrecognised prostate cancer grow. However, testosterone replacement therapy is not thought to increase the risk of a new prostate cancer above that of the general population.

Other diseases associated with sex hormone abnormalities:

True hermaphrodites are individuals who have both ovarian and testicular tissue. This occurs extremely rarely. Conditions involving hormonal abnormalities that result in ambiguities of the genitalia are more common, although their precise frequency is unknown.

A generous estimate in North America and Europe would probably be that somewhere in the region of 1 in 5000 to 1 in 10,000 births involves an intersex condition characterized by dramatic abnormality of the external genitalia. If less dramatic abnormalities, such as hypospadias or gynecomastia are included, estimates could reach as high as 1 in 100 individuals.

Most of our information on the human consequences of early hormonal perturbations has come from the more dramatic, but rarer, causes of genital ambiguity, since some of these are known to involve hormonal abnormalities of prenatal onset.

The primary sources of information have included:

(1) XX individuals exposed to high levels of androgens prenatally because of congenital adrenal hyperplasia (CAH);

(2) XY individuals exposed to lower than normal levels of androgens prenatally because their cells have deficient or defective androgen receptors (androgen insensitivity syndrome-AIS);

(3) XY individuals exposed to reduced androgens prenatally because they are deficient in enzymes needed to produce particular androgens from precursor hormones.

Some other conditions that involve prenatal hormonal abnormality, usually without ambiguity of the external genitalia at birth, have also been studied.

These include:

(4) XY individuals with idiopathic hypogonadotrophic hypogonadism (IHH), a syndrome involving deficiency in the hypothalamic hormones that promotes the production of testicular hormones.

(5) XX or XO individuals exposed to lower than normal levels of ovarian hormones prenatally, because their second X chromosome is absent or imperfect, resulting in-ovarian regression (Turner syndrome).

A third set of conditions involves XY individuals who are reassigned to the female sex early in life, because of problems with the appearance of their external genitalia. Thus, their prenatal hormone environment was that of a normal male but contrasts with their female sex of rearing.

These conditions include:

(1) Cloacal exstrophy;

(2) Penile agenesis (aphallia); and

(3) Ablatio penis.

i. Congenital Adrenal Hyperplasia:

Congenital adrenal hyperplasia (CAH) is an autosomal, recessive disorder that results in overproduction of androgen, beginning prenatally. The underlying problem is a deficiency in enzymes needed to produce adrenal steroids. In 90% of cases the deficient enzyme is 21-hydroxylase (21-OH).

In this and most other forms of CAH, the negative feedback system detects the low levels of Cortisol and the adrenal attempts to compensate by producing additional metabolic precursors to it. Because of the blockage in Cortisol production, however, these precursors are shunted into the androgen pathway, resulting in an overproduction of adrenal androgens, as well as progesterone and 17-hydroxyprogesterone.

Androgen levels in female fetuses with CAH are in the range of normal males and girls with the disorder are typically born with some degree of genital virilization. In some cases the virilization is so severe that the girls are mistaken for boys at birth and reared as such. Typically, however, the girls are diagnosed with CAH near the time of birth based on genital ambiguity, and they are assigned and reared in the female sex.

They are treated with hormones to regulate the postnatal hormone milieu, and their genitalia are feminized surgically. The incidence of CAH caused by 21-OH deficiency in Europe and the United States has been estimated at between 1 in 5000 and 1 in 15,000 births, occurring in both girls and boys.

Boys appear to have normal levels of androgens prenatally, and are not born with genital ambiguity. As a consequence, their condition is usually detected because of salt-losing crises caused by aldosterone deficiency. This typically occurs within a few weeks of birth, but in some cases affected boys are not identified until the elevated adrenal androgens induce precocious puberty in early childhood.

ii. Androgen Insensitivity Syndrome:

Androgen insensitivity refers to a deficiency in the ability of androgen receptors to respond to the hormones, testosterone and DHT. This insensitivity can be complete (CAIS) or partial (PAIS). Both disorders are transmitted as X-linked, recessive traits, and thus occur predominantly in genetic males.

Individuals with CAIS appear female at birth, despite an XY chromosome complement, and typically are raised as girls with no suspicion of the underlying disorder. At puberty the breasts develop under the influence of estrogen derived from testicular androgen. Typically the disorder is detected when menstruation fails to occur, because of the lack of feminine internal reproductive structures.

Physical appearance in PAIS varies enormously, ranging from essentially that of a CAIS individual to uncomplicated hypospadias, infertility, or even gynecomastia in an otherwise healthy-appearing male. Estimates of the incidence of CAIS vary enormously, although it appears to be rarer than CAH. The incidence of PAIS is not known, perhaps because its milder manifestations go undetected.

iii. Deficiencies in Enzymes Needed to Produce Androgens:

These deficiencies are transmitted as autosomal, recessive traits. They are rare in the general population, but can occur frequently in populations where inbreeding is common. In one area of the Dominican Republic, the incidence of 5αR has been estimated at 1 in 90 males. The enzyme 5αR converts T to DHT, and patients deficient in the enzyme have low levels of DHT but normal to high levels of T.

Because DHT is needed for normal virilization of the external genitalia prenatally, 5áR deficiency results in female-appearing or ambiguous genitalia at birth, and individuals with the disorder usually are assigned and reared as girls. High levels of testosterone and DHT derived from it at puberty however, cause virilization, including growth of the phallus and scrotum, deepening of the voice and development of male-typical musculature.

The enzyme 17βHSD is needed to produce T from its immediate precursor, androstenedione. Patients deficient in this enzyme have low levels of T and DHT, but elevated levels of androstenedione. The natural history of 17PHSD is similar to that of 5aR deficiency.The genital appearance at birth is feminine or ambiguous, but physical virilization occurs at puberty. In populations where these disorders are common they sometimes have descriptive names, such as guevedoce, geuvote (penis at 12 years of age), or machihembra (first woman, then man) or Turnim Man.

iv. Idiopathic Hypogonadotrophic Hypogonadism:

Individuals with IHH have low levels of pituitary gonadotropins or their hypothalamic releasing factor. As a consequence, their gonads lack sufficient stimulation to produce

normal levels of hormones. The disorder can occur after puberty, or congenitally. If the disorder is congenital, it is usually detected when the child does not undergo normal puberty.

Males with congenital IHH usually have normal appearing genitalia at birth, perhaps because maternal gonadotropins stimulated their testes to produce hormones prenatally. Thus, it cannot be assumed that their hormone levels are lower than normal before birth. However, beginning at birth, and perhaps to some extent before, their levels of testicular hormones would be lower than in normal males.

v. Turner Syndrome:

Turner syndrome (TS) results from an absent or imperfect second X chromosome, and appears to involve a random genetic error. In 50 to 60% of cases the second X chromosome is entirely missing. Other cases involve mosaicisms or abnormalities of the second sex chromosome.

TS occur in approximately 1 in 2000 to 1 in 5000 live female births in North American and Western Europe. The external genitalia are female, but in the majority of TS girls, the ovaries regress sometime after the third month of gestation, impairing or eliminating their ability to produce hormones.

The syndrome has several stigmata, including short stature, skeletal growth disturbances, cardiovascular and renal abnormalities, otitis media, primary gonadal failure, absence of secondary sexual characteristics, and infertility. Short stature is universal in TS, and over 90% of affected females experience primary gonadal failure and infertility, but other stigmata vary dramatically from individual to individual.

vi. Cloacal Exstrophy:

Cloacal exstrophy is a severe defect of the ventral abdominal wall. It involves abnormalities and insufficiencies in the urinary and bowel systems that until 1960 were universally fatal. Now, many children born with this syndrome survive. Cloacal exstrophy occurs in approximately 1 in 200,000 to 1 in 400,000 births, and is more common in XY than XX individuals.

In XY individuals, the testes appear histologically normal but are typically undescended, and the penis is usually either absent or represented as two separate and incomplete structures. Even when present as a single structure, the penis typically is small and poorly formed. In XX individuals there are also abnormalities of the genitalia.

Because of this, most XX and XY patients with cloacal exstrophy are surgically feminized and assigned and raised as females. Those who are XY were exposed to male-typical levels of testicular hormones prenatally and neonatally, until surgical removal of the testes.

vii. Penile Agenesis (Aphallia):

In penile agenesis (aphallia), an XY individual is born without a penis, despite the presence of a normal scrotum and functioning testes. The causes of the condition are unknown, although it is usually associated with abnormalities of the urinary and gastrointestinal tracts.

Estimates of its incidence range from 1 in 50,000 to 1 in 10- 30 million, and mortality is high. As a result there are very few individuals with aphallia. However, those who do survive are often surgically feminized and reared as girls. Like XY individuals with cloacal exstrophy, their prenatal and early neonatal hormonal milieu would resemble that of healthy male fetuses.

viii. Ablatio Penis:

In rare instances accidents can cause severe damage or even complete ablation of the penis in an otherwise healthy infant. In some such cases, XY infants have been reassigned as female, surgically feminized, and reared as girls. They would have been exposed to normal male levels of testicular hormones prenatally and postnatally until the time when the testes were removed.

Testicular Neoplasm:

Testosterone controls the development of the reproductive organs and other male physical characteristics. In the United States, around 1 in 250 males develop testicular cancer during their Iifetime. In 2019, experts predict that 9,560 males will receive a diagnosis of testicular cancer. The average age at diagnosis is 33 years; the condition mostly affects young and middle aged men. In very rare cases, it can happen before puberty. Only 8% of cases occur after the age of 55.

Early signs:

Symptoms of testicular cancer often appear at an early stage, but sometimes, they do not appear until much later.

The individual may notice a change, or a doctor will find it during a routine physical exam. A common early symptom is a painless lump or swelling in a testicle. Changes occur in the testicles for many reasons. A lump does not always mean cancer, but anyone who notices a change should see a doctor.

There may also be:

- a sharp pain in the testicle or scrotum
- a heavy feeling in the scrotum

- a difference in size between the testicles

In some cases, hormonal changes will cause the breasts to grow and become sore.

Other symptoms

In the later stages, as cancer spreads to other organs, a person may notice:

- lower back pain, if cancer spreads to the lymph nodes
- difficulty breathing, if it affects the lungs
- abdominal pain, if it affects the liver

headaches and confusion, if it reaches the brain

Causes:

Most testicular cancers start in the germ cells. These are the cells in the testicles that produce immature sperm.

Doctors do not know why testicular cells become cancerous, but some genetic factors may increase the risk.

Testicular cancer is more likely to occur in people with the following risk factors:

- cryptorchidism, or an undescended testiclea family history of testicular cancer
- being white, rather than black or Asian

Having HIV might increase the risk. Having a vasectomy does not increase the risk.

It is not possible to prevent testicular cancer, as doctors do not know what causes it, and because genetic factors may play a role. A person cannot change these factors.

Treatment:

Testicular cancer is highly treatable, especially in the early stages. Most males with a diagnosis of testicular cancer will live at least another 5 years following diagnosis.

Treatment will usually involve a combination of the following:

- surgery
- radiation therapy
- chemotherapy
- stem cell treatment
- surveillance

Surgery:

A surgeon will remove one or both testicles to prevent the tumor from spreading. The person will receive a general anesthetic. The surgeon will then make a small incision in the groin and remove the testicle through the incision. Removing one testicle does not usually affect the person's sex life or fertility, but removing both testicles means that the male will not be able to conceive naturally. However, other fertility options are available. For example, the doctor might suggest banking sperm for future use, if necessary.

Other effects of removing the testicles may include:

- a loss of sex drive
- difficulty achieving an erection
- fatigue
- hot flashes
- loss of muscle mass

A doctor may prescribe testosterone supplements — as a gel, a patch, or an injection — to help with these issues.

It is also possible to restore the appearance of testicles by having a prosthesis. A surgeon will implant this in the scrotum. It is filled with salt water. A person who has surgery in the early stages may not need any further treatment.

Lymph node surgery

If cancer has reached the lymph nodes, usually those around the large blood vessels at the back of the abdomen, a surgeon will need to remove these. A surgeon can do this as open or laparoscopic surgery.

This procedure will not impact fertility directly, but any nerve damage may affect ejaculation. This may mean that sperm does not come out through the urethra but goes to the bladder instead. This is not dangerous, but a lower sperm count can affect fertility.

Radiation therapy

Radiation therapy damages the DNA inside the tumor cells and destroys their ability to reproduce. In this way, it can remove cancer and may prevent it from spreading or coming back. A person who has surgery may need radiation therapy to ensure that treatment removes any remaining cancer cells. If cancer has spread to the lymph nodes, a doctor may also recommend radiation therapy.

The following temporary side effects may occur:

- tiredness
- rashes
- muscle and joint stiffness
- loss of appetite
- nausea

These symptoms should pass once the treatment is over.

Chemotherapy

Chemotherapy uses medication to destroy cancer cells and stop them from dividing and growing.A doctor may recommend chemotherapy if a person has testicular cancer that has spread to other parts of the body. A doctor will give the treatment either orally or as an injection.Chemotherapy attacks healthy cells as well as cancerous ones, which may lead to the following side effects:

- nausea and vomiting
- hair loss
- mouth sores
- tiredness and a general feeling of being unwell

These symptoms usually resolve after treatment finishes.

Stem cell treatment:

In some cases, stem cell therapy can enable a person to receive higher doses of chemotherapy that would otherwise be too dangerous to administer. During the weeks before treatment, a special machine will harvest stem cells from the person's blood. Healthcare professionals will freeze and store these cells. The person receives a high dose of chemotherapy, and they will then receive the stem cells into a vein as in a transfusion. These cells establish themselves in the bone marrow and start making new blood cells. This enables the person's body to recover from higher doses of chemotherapy.

Disadvantages of this type of therapy include:

- Due to the high dose of chemotherapy, it is risky and may involve life threatening adverse effects.
- It can involve a long stay in the hospital.
- It can be expensive, and medical insurance may not cover it.

Surveillance:

A doctor will carry out surveillance after a person has had treatment for testicular cancer, to check for any signs that the cancer has come back.

Surveillance does not involve active treatment, but the individual will attend regular appointments and undergo tests.

Diagnosis:

To diagnose testicular cancer, a doctor will recommend:

Blood tests: These can measure levels of alpha-fetoprotein, human chorionic gonadotrophin, and lactate dehydrogenase. These are substances that may suggest the presence of a tumor.

Ultrasound: This can reveal the presence and size of a tumor.

Biopsy: The doctor takes a small tissue sample from the testicle for investigation using a microscope. A biopsy can determine whether cancer is present or not.

Types of testicular cancer

If tests show that testicular cancer is present, a doctor will also need to know what type of cancer it is and what stage it is at before discussing a treatment plan with the individual.

There are two main types **of testicular cancer:**

Seminoma: This type grows slowly and contains only seminoma cells. There are two subtypes: classic and spermatocytic.

Nonseminoma: This can involve various kinds of cancer cell. There are several subtypes, including embryonal carcinoma, yolk sac carcinoma, chiorcarcinoma, and teratoma.

Other tumors that are not cancerous include stromal tumors, Leydig cell tumors, and Sertoli cell tumors.

Staging the cancer

The stage of the cancer will also affect treatment options:

Localized: The cancer is only in the testis and has not spread.

Regional: The cancer has reached the lymph nodes in the abdomen.

Distant: The cancer has spread to other parts of the body, such as the lungs, liver, brain, and bones.

Self-examination:

The best time to check for testicular cancer is when the scrotal skin is relaxed, usually after a warm shower or bath.

To perform a self-exam:

1. Gently hold the scrotum in the palms of both hands. Stand in front of a mirror and look for any swelling on the skin of the scrotum.
2. Feel the size and weight of the testicles first.
3. Press around the testicles with the fingers and thumbs, and be aware of any lumps or unusual swellings.
4. Feel each testicle individually. Place the index and middle fingers under one testicle with the thumbs on the top. Gently roll the testicle between the thumbs and the fingers. It should be smooth, oval shaped, and somewhat firm, with no lumps or swellings. The top and back of each testicle should have a tube-like section, called the epididymis, where sperm is stored.

Repeat this process once each month, checking for changes in the size, weight, or feel of the testicles.Many males have one testicle that hangs lower than the other or one testicle that is bigger than the other, but as long as these proportions do not change over time, it is not a cause for concern.It is not currently possible to prevent testicular cancer, because there are no known lifestyle risk factors. However, if there is a family history, genetic testing may help detect iyt early, if it happens. Regular self-exams may also lead to an early diagnosis.The outlook for someone with early stage testicular cancer is excellent, with 95% of people surviving at least another 5 years after diagnosis. Around 11% of people receive a diagnosis after the cancer has spread to other organs. According to the American Society of Clinical Oncology, 74% of these people will live at least another 5 years.Being aware of any changes can make it easier to spot testicular cancer in the early stages. With prompt treatment, there is an excellent outlook for this type of cancer.

Probable Questions:

1. What are the anatomical features of Addison's disease?
2. What are physiological features of Addison's disease?
3. What are the psychological features of Addison's disease?
4. What are the sexological features of Addison's disease?
5. What are the anatomical features of Cushing's syndrome?

6. What are physiological features of Cushing's syndrome?
7. What are the psychological features of Cushing's syndrome?
8. What are the sexological features of Cushing's syndrome?
9. What is androgen deficiency syndrome?
10. What are the symptoms of androgen deficiency?
11. How androgen deficiency is diagnosed?
12. How androgen deficiency is treated?
13. Discuss Other diseases associated with sex hormone abnormalities.
14. Discuss Congenital Adrenal Hyperplasia.
15. What is Androgen Insensitivity Syndrome?
16. What is Idiopathic Hypogonadotrophic Hypogonadism
17. Discuss Cloacal Exstrophy.
18. What is Penile Agenesis?
19. What are the symptoms of testicular neoplasm?
20. Discuss different causes of testicular neoplasm
21. Discuss treatment procedures of testicular neoplasm.
22. Discuss different types of testicular neoplasm.
23. How self examination can prevent progression of testicular neoplasm?
24. Discuss diagnosis of testicular neoplasm.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XIX
Hormone Related Cancers

Objective: In this unit we will discuss about various types of endocrine cancer

Introduction:

Endocrine tumors develop when abnormal cells in an endocrine gland or organ grow and multiply uncontrollably. Over time, the new cells can develop into a solid mass of tissue known as a tumor.

In most cases, endocrine tumors are benign (noncancerous). In cases where they are malignant (cancerous), the cells that make up the tumor are capable of invading nearby tissues and spreading to other parts of the body, where they can form new tumors.

While many kinds of endocrine cancers are rare, some are common. For example, thyroid cancer, a type of endocrine cancer, is the seventh most commonly diagnosed cancer in women and the tenth most commonly diagnosed cancer in men in the United States. Other endocrine cancers include cancers of the adrenal glands, parathyroid glands, pituitary gland, hypothalamus, and pancreas. Fortunately, effective treatments, including surgery, chemotherapy, radiation therapy, and medications, among other therapies, are available for endocrine cancers.

The endocrine system is a group of glands and organs that produces and secretes hormones. Hormones are chemicals that travel through the bloodstream to tissues around the body, where they regulate and coordinate many important processes in the body, including metabolism, growth, development, sexual function, reproduction, and mood. An endocrine tumor can form when abnormal cells in an endocrine gland or organ arise and grow uncontrollably. There are two broad categories of endocrine tumors:

Functioning tumors, which produce and secrete hormones. When a tumor arises from a hormone-producing cell, the cells that make up the tumor also produce and secrete excessive levels of the hormones made by the initial cell, which can cause a range of symptoms and problems in the body, depending on which hormone is involved.

Nonfunctioning tumors which do not produce or secrete hormones.

Both functioning and nonfunctioning tumors can develop into large masses that press against nearby tissues and organs, impairing their ability to work properly.

Types of endocrine cancer

Cancer can develop in any endocrine gland or tissue in the body. Common types include the following:

- **Thyroid cancer**: The thyroid is a small, butterfly-shaped organ located at the base of the neck. It produces and secretes thyroid hormones that help regulate metabolism.
- **Pituitary tumors**: A pea-sized organ located just below the brain, the pituitary gland produces several different hormones that regulate the function of other endocrine glands, including the adrenal and thyroid glands, as well as the gonads (the ovaries and testicles). The pituitary helps control important functions of the body, including growth, blood pressure, metabolism, and sperm and egg production, among many others. Pituitary tumors are almost always benign. According to the National Cancer Institute, only around 0.1% to 0.2% of pituitary tumors are cancerous.
- **Adrenal cancers**: The two adrenal glands, located just above the kidneys, produce several different hormones that play an important role in regulating a number of essential bodily processes, including metabolism, the stress response, inflammation, blood pressure, and sexual development. Adrenal hormones include cortisol, aldosterone, and adrenaline (also known as epinephrine). A few types of cancer can occur in the adrenal gland, including adrenocortical carcinomaÿþ and pheochromocytomaÿþ, though the latter tumor is usually benign.
- **Pancreatic cancers**: Though the pancreas plays an active role in the digestive system, it's also part of the endocrine system. Specialized neuroendocrine cells in the pancreas produce and secrete hormones, including insulin and glucagon, to help regulate blood sugar levels.
- ÿþNeuroendocrine cells have features of both nerve cells and hormone-producing endocrine cells. Cancerous tumors that develop from these neuroendocrine cells are called pancreatic neuroendocrine tumors (pancreatic NETs or pNETs) or islet cell tumors. Pancreatic NETs are rare and, according to the ACS, account for just under 2% of all pancreatic cancers. (Because neuroendocrine cells are found in various tissues throughout the body, including the gastrointestinal tract and lungs, NETs can also occur in those locations.)
- **Parathyroid cancers**: The four pea-sized parathyroid glands, located behind the thyroid gland in the neck, produce parathyroid hormone (PTH). PTH helps regulate blood calcium levels. When blood calcium levels are low, the parathyroid glands secrete PTH, which stimulates the release of calcium from bones into the blood, increases the absorption of calcium from food by the intestine, and prevents the kidney from excreting too much calcium in the urine. Together, these actions increase blood calcium levels. People with parathyroid cancer have severe hypercalcemia (high blood calcium levels). Parathyroid cancer is very rare.

- **Hypothalamic endocrine tumors**: The hypothalamus is a small part of the brain connected to the pituitary gland. It produces and secretes hormones that regulate the activity of the pituitary gland (which in turn secretes hormones that regulate the activity of several other endocrine glands). Cancer of hormone-secreting cells in the hypothalamus is rare.

Causes of endocrine cancer: The cause of most endocrine cancers is usually unclear.

Normally, the growth and production of new cells are tightly regulated to ensure that only healthy cells are produced and survive. In cancer, genetic changes—or mutations—in a single cell interfere with the careful regulation of cell growth and production. As a result, the affected cell grows and multiplies uncontrollably and can invade nearby tissues and spread to other parts of the body. The genetic changes that trigger cancer may be inherited from one or both parents or may occur sporadically, meaning they are acquired during a person's life. Sporadic mutations can occur randomly or due to environmental exposures, such as smoking tobacco products or radiation exposure.

Symptoms of endocrine cancer:

Endocrine cancer symptoms vary greatly, depending on which part of the endocrine system is affected, whether the tumor has grown large enough to press against nearby tissues, and which hormones, if any, are produced and secreted in excessively high levels.

Risk factors for endocrine tumors may include:

Thyroid cancer:

- Being female
- Being between 25 and 80 years of age
- Radiation exposure, such as head or neck radiation treatments in childhood
- Personal history of goiter, thyroid nodules, or a previous thyroid cancer diagnosis
- Family history of thyroid cancer or thyroid disease
- Certain genetic conditions including multiple endocrine neoplasia type 2A (MEN2A) and type 2B (MEN2B)
- Certain inherited conditions, including familial medullary thyroid cancer (FMTC), Cowden disease, Carney complex type 1, and familial adenomatous polyposis (FAP)

Pituitary Tumors:

- Certain inherited conditions, including multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4), Carney complex, McCune-Albright syndrome, familial isolated pituitary adenoma (FIPA)

Adrenal cancer:

- Certain inherited and genetic conditions, including Li-Fraumeni syndrome, Beckwith-Wiedemann syndrome, Carney complex, MEN1, familial adenomatous polyposis (FAP), Lynch syndrome, MEN2A, MEN2B, von Hippel-Lindau (VHL) syndrome, neurofibromatosis type 1 (NF1), hereditary paraganglioma syndrome, Carney-Stratakis dyad, Carney triad

Pancreatic neuroendocrine tumors:

- Smoking
- Alcohol consumption
- Family history of pancreatic NETs
- Certain inherited genetic syndromes, including NF1, MEN1, VHL syndrome
- Diabetes
- Chronic Pancreatitis

Parathyroid Cancer:

- Radiation exposure, such as from previous treatment with radiation therapy to the head or neck
- Certain inherited conditions, including familial isolated hyperparathyroidism (FIHP), hyperparathyroidism-Jaw tumor syndrome (HPT-JT), MEN1, MEN2A

Hypothalamic endocrine tumors:

- Neurofibromatosis

Diagnosis of Endocrine tumor:

Doctors can perform a series of steps to check for endocrine cancer, including:

- A medical history to determine whether the patient has any risk factors for endocrine cancer, such as a family history of certain conditions or a medical condition associated with endocrine cancer

- A physical exam to evaluate symptoms and signs that could be caused by an endocrine cancer
- Lab tests to check for abnormal levels of hormones and/or other markers in the blood or urine
- Imaging studies, such as a computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, ultrasound, or other imaging tests to look for evidence of abnormal tissue
- A biopsy to obtain a tissue sample for analysis that can check for the presence of cancer cells
- Genetic testing to determine the genetic makeup of tumor cells. In many cases, knowing a tumor's genetic makeup helps health care providers choose the best treatment options.

Treatment:

Various treatment options are available for endocrine cancers. The treatment varies based on the type of cancer involved, how far it has progressed, and the patient's overall health, among many other factors.

Endocrine cancer treatment may involve:

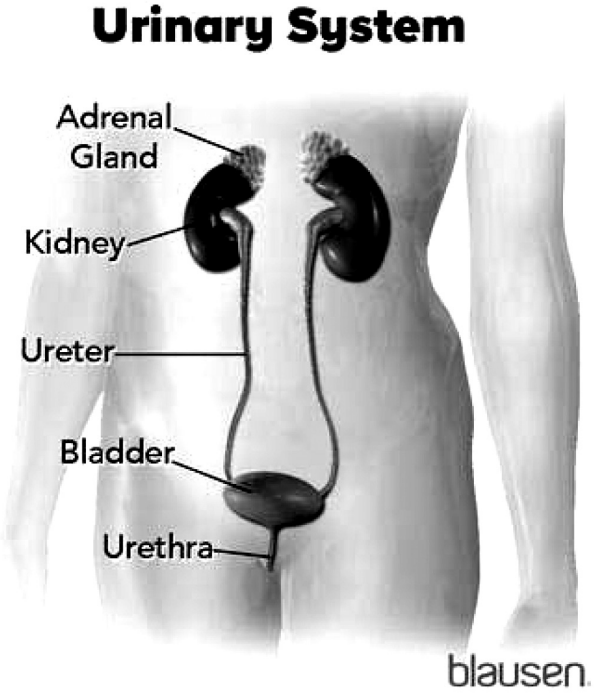
- **Surgery** to remove the cancerous tissue. In some cases, the entire affected organ may be removed. For example, adrenalectomy—the surgical removal of an adrenal gland—is frequently used to treat adrenal cancer.
- **Radiation therapy**, also known as radiotherapy, kills cancer cells by exposing them to high doses of radiation. It is often used after surgery to kill any remaining cancer cells.
- **Chemotherapy** involves the use of drugs to kill cancer cells. It may be given before surgery to reduce tumor size or after surgery to eliminate any cancer cells that remain.
- **Targeted drug therapy** uses drugs that target specific genes or proteins found in cancer cells.
- **Ablation** may be used to destroy tumors. During an ablation procedure, a probe is used to deliver heat, cold, alcohol, or other forms of energy to the tumor, thereby destroying the cancerous tissue.
- **Embolization** is a technique to cut off blood flow to—and kill—cancer cells. In an embolization procedure, substances are injected into a blood vessel to block blood

flow to a tumor. Sometimes, beads that contain chemotherapy drugs (known as chemoembolization) or a radioactive isotope (known as radioembolization) are also injected into the blood vessel to deliver chemotherapy or radiation, respectively, to the tumor.

- **Immunotherapy** involves the use of drugs to enhance the ability of the patient's immune system to fight against cancer.
- **Hormone therapy** may be used to suppress the production of—or block the effects of—excess hormones made by endocrine tumors. In other cases, cancer or cancer treatments such as surgery, chemotherapy, or radiation therapy can impair the ability of an endocrine gland to produce hormones. In these cases, patients may need to take hormones to replace those that the affected gland can no longer produce.
- **Observation** may be appropriate for select small thyroid cancers that are well differentiated and considered low risk. In such circumstances, patients may choose observation rather than immediate surgery.

1. Adrenal tumors:

The adrenal glands are the pair of small endocrine glands located above the kidneys. They respond to signals from the nervous system and secrete hormones that regulate stress. The adrenal glands also produce hormones that help maintain metabolism as well as distinguish male and female physical and sexual characteristics.



There are two varieties of tumors that can proliferate on the adrenal glands:

- Most growths are **benign** and symptoms are treatable
- **Malignant** adrenal tumors are rare, and generally grow as a result of metastasizing cancer that originated in a different organ

Risk factors:

People with certain genetic conditions are at a higher risk for developing adrenal tumors. These include:

- Li-Fraumeni syndrome
- Carney Complex
- Family history of adrenal tumors

Symptoms:

Adrenal gland tumors do not always present the same group of symptoms and may also not cause any symptoms at all. Nevertheless, the following symptoms may be warning signs:

- Headaches
- Unusual weight changes
- Unusual anxiety
- Heart palpitations or elevated blood pressure
- Unusual hair growth
- Disproportionate acne
- Diminished sex drive
- Muscle weakness
- Easy bleeding or bruising

Diagnosis and treatment

Doctor can detect the presence of adrenal tumors in the following ways:

- **Biopsy:** This is the most definitive diagnostic method. Doctors examine tissue samples for evidence of cancer.

- **Blood and Urine Tests:** The levels of hormones produced under certain circumstances are present in blood and urine and are indicative of possible tumors
- **Imaging:** With either a CT scan or MRI, your physician can verify the existence of an adrenal tumor, as well as determine its exact size and placement
- **Metaiodobenzylguanidine scan (MIBG):** This is a special test administered during the course of two days. It is designed to show adrenal tumors that are not evident on other scans. On the first day, a patient gets an injection followed by a scan with a special camera. The next day, the scan is repeated

After examining the results of one or more of these tests, your doctor may inform you that you have an adrenal tumor. Treatment is based on tumor size, location, and whether it is metastasizing.

The primary types of treatment for adrenal tumors include:

- Surgery
- Radiation Treatment
- Chemotherapy

2. Neuroendocrine tumors

The brain and the nervous system provide the signals to the endocrine system to produce hormones that regulate bodily functions. Since these two systems are so interdependent, they are often referred to as the neuroendocrine system. Tumors that affect the functioning of cells within this system are collectively called neuroendocrine tumors.

The primary types of tumors are:

- **Pheochromocytoma**, which affects production of adrenaline and often presents in the adrenal glands
- **Neuroendocrine tumors**, which is a generic term for tumors that affect hormones in major organs (such as the pancreas)

Risk factors

People are at a higher risk for developing these tumors because of certain factors that include:

- Gender: men are more likely than women to develop Pheochromocytoma
- Age: Pheochromocytoma patients are generally between 40-60 years old
- Genetics

Symptoms

Each variation of neuroendocrine tumor presents specific symptoms.

- Pheochromocytoma
- Elevated blood pressure
- Damp and sticky skin
- Unusual anxiety
- Heart palpitations
- Nausea, headaches, fever
- Neuroendocrine Carcinoma
- Hyper or hypoglycemia
- Unusual weight changes
- Unusual anxiety
- Unexplained lumps
- Jaundice
- Unexplained bleeding
- Unusual bowel or bladder changes
- Ongoing night sweats

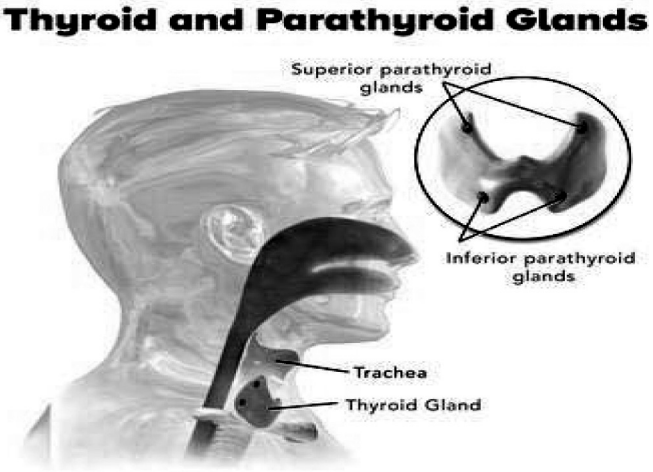
Diagnosis and treatment

Doctors can detect the presence of the tumors in the following ways:

- **Biopsy:** This is the most definitive diagnostic method. Doctors examine tissue samples for evidence of a tumor
- **Blood and Urine Tests:** The levels of hormones produced under certain circumstances are present in blood and urine and are indicative of possible tumors
- **Imaging:** With a CT scan, MRI, or X-ray, the doctor can usually verify the existence of a neuroendocrine tumor, as well as determine its exact size and placement
- After examining the results of one or more of these tests, your doctor may inform you that you have a neuroendocrine tumor. Treatment is based on where the tumor is located, how big it is, whether it is metastasizing, and the patient's general health. The primary types of treatment include:
- Surgery
- Radiation
- Chemotherapy

3. Parathyroid tumor:

The parathyroid glands are four pea-sized glands located in the neck near the thyroid. They secrete parathyroid hormone (PTH), which regulates calcium levels throughout the body. Tumors can form within the tissues of the parathyroid and tend to grow very slowly, impacting the body with over-production of PTH, also called hyperparathyroidism. The vast majority of parathyroid tumors are benign (not cancerous). In fact, parathyroid cancer has only been diagnosed in a few hundred cases.



Risk Factors

Besides genetics, there are no common characteristics that put people at higher risk for developing parathyroid cancer. Some patients with parathyroid cancer have already been suffering from parathyroid adenomas or hyperplasia.

Symptoms

The following symptoms may indicate the presence of a parathyroid tumor and the resulting hyperparathyroidism:

- Lump or nodule in the neck
- Pain in the bones or in the upper back
- Fractures
- Kidney stones
- Pancreatitis
- Muscle weakness
- Trouble speaking
- Vomiting

- Fatigue
- Weight loss
- Constipation
- Frequent urination
- Extreme thirst

Diagnosis and treatment

Cancer of the parathyroid can be difficult to detect, since symptoms are similar to those of simple hyperparathyroidism. At this time, there are no specific tests for these tumors, but an official diagnosis can emerge with the following:

- **Symptoms:** A patient's symptoms strongly indicate the presence of a parathyroid tumor. The doctor surgically identifies and removes it
- **During hyperparathyroidism surgery:** During surgery to remove various non-cancerous lesions or growths from a patient with hyperparathyroidism, the surgeon may discover cancerous lesions
- **After hyperparathyroidism surgery:** Upon removal and examination of a seemingly non-cancerous lesion or growth from a patient with hyperparathyroidism, the doctor discovers it is indeed cancerous
- **Symptoms after surgery:** If a patient with hyperparathyroidism undergoes surgery but still experiences symptoms additional imaging tests can help verify the diagnosis. Tests include:

Scintigraphy and ultrasound for neck tumors

- CT scan and MR scan

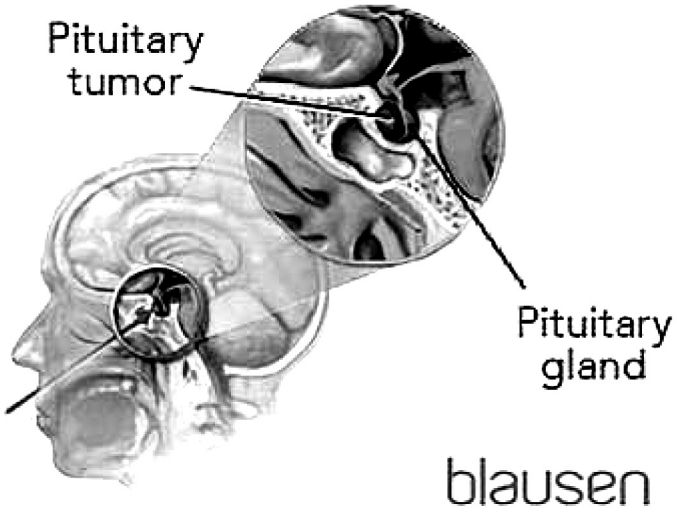
Surgery is the primary treatment for parathyroid tumors. Removal of nearby thyroid gland and lymph nodes may sometimes be performed. When cancer has metastasized, additional methods and drugs are necessary to help the body excrete excess calcium. These include:

- Intravenous saline
- Diuretics
- Bisphosphonates
- Gallium nitrate
- Cinacalcet

4. Pituitary tumors

As tiny as a pea, and located towards the bottom center of the brain, the pituitary gland secretes hormones that stimulate other endocrine glands to function properly. The pituitary gland helps regulate metabolic functions, as well as growth, reproduction, and blood pressure levels.

Pituitary tumors are growths on the gland. Pituitary tumors can cause either too much or too little hormone production. In most cases, these tumors do not spread and are not considered cancerous.



Risk factors

People are at a higher risk for developing pituitary tumors due to particular factors. These may include:

- Age: patients are generally older
- Family history

Symptoms

Since the pituitary gland regulates many other hormone-producing organs, symptoms can vary, depending on the affected area. Sometimes pituitary tumors themselves secrete hormones, causing biochemical symptoms.

Three or more of the following symptoms are generally present because of pituitary tumors:

- Sexual dysfunction
- Growth of jaw, hands, and feet
- Breast secretion

- Depression
- Infertility
- Growth issues
- Osteoporosis
- Joint pain
- Excessive bruising
- High or low blood pressure
- Obesity
- Cessation of menstrual periods
- Nausea and vomiting
- Seizures
- Fatigue and weakness
- Headaches or difficulty seeing
- Unusual weight changes

Hormone-producing pituitary tumors can result in the following symptoms:

- Weight gain in the upper back and gut
- Development of a hump on the upper back
- Unusual facial roundness or hardening facial features
- Unusual growth in hands and feet
- Leaking milky liquid from the breasts (women)
- Breast growth (men)
- Loss of muscle and body hair (men)
- Irregular heartbeat
- Unusual jittery or ill-tempered moods

Diagnosis and treatment:

Doctor can detect the presence of a pituitary tumor in the following ways:

- **Blood and Urine Samples:** Abnormal hormone levels can be detected through the blood and urine
- **Imaging:** With either a CT scan or MRI, your physician can verify the existence of a pituitary tumor, as well as determine its exact size and placement
- **Vision Tests:** With an eye test, your doctor will be able to determine whether the pituitary tumor has grown large enough to significantly affect your vision

After examining the results of one or more of these tests, your doctor may inform you that you have a pituitary tumor. Since the gland affects so many different bodily functions, the specific diagnosis is based on where the tumor is causing the majority of symptoms.

Treatment varies according to the size of the tumor, what structure it is affecting and how deeply embedded in the brain. With early detection and treatment, the prognosis for recovery is generally excellent.

- **Surgery:** This is the most common option, especially in cases where the tumor is putting pressure on the optic nerve and causing vision problems
- **Radiation:** This option can be used along with surgery or by itself. The two types of radiation therapy used are external beam radiation and gamma-knife radiosurgery
- **Medication:** Certain drugs can suppress overproduction of hormones and help reduce tumor size

Some Rare Endocrine Cancer:

1. Anaplastic Thyroid Cancer (ATC)

What is anaplastic thyroid cancer?

Anaplastic thyroid cancer, or ATC, is a type of thyroid cancer. The thyroid is a gland located in the front of your neck, just below the Adam's apple. It is responsible for sending out hormones to the rest of your body. ATC is different than other types of thyroid cancers because ATC invades other parts of the body very quickly. This type of cancer usually affects people over the age of 60. ATC can also be called anaplastic thyroid carcinoma.

How common is anaplastic thyroid cancer?

ATC is a rare type of thyroid cancer, making up 1% to 2% of thyroid cancer cases. ATC affects one to two people per one million per year in the US.

How is anaplastic thyroid cancer diagnosed?

ATC can start as a bump in the throat area. The tumor growing on the thyroid can make your voice hoarse by blocking your vocal chords, or it can make it difficult to breathe by blocking your windpipe. Sometimes people can have ATC for a while and not notice it because the tumor remains small.

Imaging: If you have symptoms of ATC, your doctor will use imaging scans such as ultrasound, CT, and MRI to look at the size of the tumor. They will also check for signs that the tumor has spread to other parts of the body.

Biopsy: To check if the tumor is ATC, your doctor will perform a biopsy, taking a small sample from the tumor with a needle. A pathologist will study cells from the sample under the microscope to see what kind of tumor it is.

How is anaplastic thyroid cancer treated?

Surgery: Once ATC is diagnosed, you may have surgery to remove the thyroid. This surgery is a called a thyroidectomy. If a thyroidectomy is not an option, your doctor will discuss other options with you.

Radiation therapy and chemotherapy: A thyroidectomy is often combined with radiation and chemotherapy treatments. Doctors and scientists are looking for ways to improve radiation therapy. For example, new ways to give radiation therapy have been developed that allow higher radiation doses over less time with more precision. The hope is to target the tumor without injuring the healthy nearby muscle and tissue.

ATC is a difficult disease to treat because of its ability to spread to the rest of the body. Research is being conducted on the different types of treatment options, and support networks are available for people with ATC.

Does anaplastic thyroid cancer run in families?

No, ATC does not run in families.

How does anaplastic thyroid cancer form?

Scientists are always working to understand how cancer forms, but it can be hard to prove. ATC often starts in a thyroid that is already unhealthy. It can form within a goiter or it can arise from another thyroid cancer. Scientists have found many different changes in ATC cells, which tells them that there are likely many ways that ATC can start. This makes it very hard to develop a single treatment that can work for all ATC patients.

What is the prognosis for someone with anaplastic thyroid cancer?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate ATC survival rates by how groups of people with ATC have done in the past. Because there are so few ATC patients, these rates may not be very accurate. They also don't consider newer treatments being developed. ATC is one of the fastest

growing cancers, with only half of people with ATC surviving 6 months after diagnosis. It is very important to work with a team of experts as soon as possible after diagnosis to improve your chances of survival.

2. Neuroendocrine Tumor (NET)

What is neuroendocrine tumor (NET)?

Carcinoid tumor is a type of neuroendocrine tumor that grows from neuroendocrine cells. Neuroendocrine cells receive and send messages through hormones to help the body function. Neuroendocrine cells are found in organs throughout the body.

Carcinoid tumors often grow very slowly. In children and young adults, carcinoid tumors are most often found in the appendix, called appendiceal carcinoid tumors, or in the lungs, called bronchial tumors. In adults, carcinoid tumors are most often found in the digestive tract. This tumor may spread to other parts of the body but does so more often in adults than children.

How common is NET?

Carcinoid tumor is rare in children and more common in adults. Experts think that carcinoid tumor affects 4 in 100,000 adults. Carcinoid tumor in children and young adults is so rare that there is little data on how many young people have it.

How is NET diagnosed?

Some people with carcinoid tumors have symptoms, but others don't. The symptoms of carcinoid tumor depend on where the tumor is inside the body.

Patients with carcinoid tumor of the appendix usually have symptoms of appendicitis, such as pain in the abdomen. They may be diagnosed later with carcinoid tumor if the doctor removes the appendix and finds a tumor. Patients with carcinoid tumor in other parts of the digestive tract may have symptoms such as:

- Pain in the abdomen
- Nausea or vomiting
- Diarrhea

Patients with carcinoid tumor in the lungs may have symptoms such as:

- Trouble breathing
- Chest pain
- Wheezing
- Coughing up blood

Sometimes these symptoms are diagnosed as pneumonia by mistake.

In rare cases, patients with carcinoid tumor may develop carcinoid syndrome. Carcinoid syndrome is a problem that develops from the tumors making hormones. Symptoms include:

- Feeling flushed
- Nausea and vomiting
- Diarrhea

Lab Tests: If you have symptoms of carcinoid tumor, your doctor will order lab tests of your urine or blood to check your hormone levels.

Imaging: Your doctor will use scans such as CT and MRI to see where the tumor is and how big it is. Different types of PET scans can also help find more fast-growing neuroendocrine cancer cells.

Biopsy: To check if the tumor is carcinoid tumor your doctor will do a biopsy, taking a small sample from the tumor with a needle. An expert, called a pathologist, will study cells from the sample under the microscope and run other tests to see what kind of tumor it is.

How is NET treated?

Treatment for each person will be unique. You should go to an expert in neuroendocrine tumor treatment to decide the best approach for your tumor.

Surgery: If you have a carcinoid tumor, you may have surgery to remove the tumor and some surrounding tissue. Surgery is the best option for treating carcinoid tumor and preventing it from spreading.

When the carcinoid tumor is large or the cancer cells have spread to other parts of the body other treatments may include:

Somatostatin analogs: Somatostatin analogs are a type of treatment that may stop your body from making too many hormones. This may slow down the growth of the tumor when cancer cells have spread to other part of the body

Targeted therapy: Targeted therapy is a type of treatment that uses drugs that target certain genes or proteins to kill cancer cells. Neuroendocrine tumor cells have receptors on the surface of the cells called somatostatin. A type of targeted therapy called peptide receptor radionuclide therapy (PRRT) can target these cells.

Chemotherapy: is a type of treatment that uses stronger drugs to kill fasting growing cells.

Does NET run in families?

NET does not seem to run in families. But people with a genetic condition that can run in families called multiple endocrine neoplasia type 1 (MEN1) do have a higher risk of getting carcinoid tumor.

How does NET form?

We do not know what causes NET to form. Scientists are always working to understand how cancer starts, but it can be hard to prove. We know that patients with a condition called multiple endocrine neoplasia type 1 (MEN1) with changes in the gene called *MEN1* have a higher chance of developing bronchial and intestinal carcinoid tumor. So this gene may play a role in NETs of the lung and digestive tract.

What is the prognosis for someone with carcinoid tumor?

The estimate of how a disease will affect you long-term is called prognosis. Each person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate NET survival rates by how groups of people with NET have done in the past. In children, because there are so few cases of NET, these rates may not be very accurate.

The prognosis for children and young adults who have surgery to remove the tumor have a very good prognosis. Some studies show the 5-year survival rate for children and young adults with bronchial NET that has been removed is over 90%. Prognosis for people whose carcinoid tumor has spread to other parts of the body may be lower.

3. Paraganglioma

What is paraganglioma?

Paraganglioma is a type of neuroendocrine tumor that forms near certain blood vessels and nerves outside of the adrenal glands. The adrenal glands are important for making hormones that control many functions in the body and are located on top of the kidneys. The nerve cells involved in paraganglioma are part of the peripheral nervous system, meaning the part of the nervous system outside of the brain and spinal cord. These tumors can also be called extra-adrenal pheochromocytomas. Approximately 35-50% of paragangliomas may spread to other parts of the body.

How common is paraganglioma?

Paraganglioma is rare and it is estimated that only 2 people out of every 1 million people have paraganglioma. It is most often found in people aged 30 to 50 years old.

How is paraganglioma diagnosed?

Some people with paraganglioma have symptoms, but others don't. Symptoms can include:

- High blood pressure
- Fast heartbeat
- Sweating
- Headache
- Shaking or tremors

Lab Tests: If you have symptoms of paraganglioma, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging scans such as MRI, CT, and PET to look at where the tumor is and how big it is. They will also check for signs that the tumor has spread to other parts of the body.

How is paraganglioma treated?

Treatment of paraganglioma may involve many different doctors, including doctors who specialize in hormone disorders and doctors who diagnose and treat neuroendocrine tumors. Treatment options to discuss with your doctor include:

Medications: Your doctor may give you medications to control your symptoms, such as alpha blockers and may be followed by beta blockers, which are drugs to control high blood pressure.

Watch and wait: In some cases, the tumor grows very slowly. In this case it may be safest for your doctor to check your tumor regularly without treating it.

Surgery: Once paraganglioma is diagnosed, you may have surgery to remove the tumor. Sometimes surgery is not an option, in which case, your doctor will discuss other options with you.

Radiation therapy: Radiation therapy can be used to slow the tumors from growing and to help relieve symptoms.

It is important to talk with a team of specialists to decide what the right treatment is for you.

Does paraganglioma run in families?

Yes, paraganglioma can run in families, but not always. Some of these inherited cases may be associated with a genetic condition, such as Multiple Endocrine Neoplasia Types 2a and 2b, Von Hippel-Lindau Syndrome, and Neurofibromatosis type 1.

How does paraganglioma form?

Scientists have found mutations in approximately 20 different genes that they think may lead to pheochromocytoma and paraganglioma. Mutations in the genes *RET*, *VHL*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MDH2*, *IDH1*, *PHD1/PHD2*, *HIF2A/EPAS1/2*, *TMEM127*, *MAX*, *HRAS*, *MAML3* and *CSDE1* may play a role in forming pheochromocytoma and paraganglioma.

Scientists have found that some genetic conditions may be associated with having paraganglioma. **These genetic conditions include:**

- Multiple endocrine neoplasia 2 syndrome, types A and B (MEN2A and MEN2B)
- von Hippel-Lindau (VHL) syndrome
- Neurofibromatosis type 1 (NF1)
- Hereditary paraganglioma syndrome
- Carney-Stratakis dyad (paraganglioma and gastrointestinal stromal tumor [GIST])
- Carney triad (paraganglioma, GIST, and pulmonary chondroma)

What is the prognosis for someone with paraganglioma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Patients with a small paraganglioma that has not spread to other parts of the body have a five-year survival rate of about 95%. Patients with paraganglioma that has grown back (recurred) or spread to other parts of the body have a five-year survival rate between 34% and 60%.

4. Pheochromocytoma

What is pheochromocytoma?

Pheochromocytoma is a type of neuroendocrine tumor that grows from cells called chromaffin cells. These cells produce hormones needed for the body and are

found in the adrenal glands. The adrenal glands are small organs located in the upper region of the abdomen on top of the kidneys. About 80-85% of pheochromocytomas grow in the inner layer of the adrenal gland, called the adrenal medulla. About 15-20% of pheochromocytomas grow outside of this area and are called extra-adrenal pheochromocytomas or paragangliomas.

Most pheochromocytomas are benign, which means they are not cancer and do not spread to other parts of the body. Only about 10% of pheochromocytomas spread to other parts of the body.

How common is pheochromocytoma?

It is unknown how many people have pheochromocytoma because many people are never diagnosed. Most cases of pheochromocytoma occur in people aged 30 to 50 years old. One estimate suggests about only 8 people per 1 million people have pheochromocytoma, but this estimate may be low.

How is pheochromocytoma diagnosed?

Some people with pheochromocytoma have symptoms, but others don't. Symptoms may occur as often as several times a day to a couple of times per month. Some people may feel intense symptoms that last for a short period of time, called "paroxysmal attacks". These symptoms can include:

- High blood pressure
- Headaches
- Irregular heartbeat
- Sweating

Lab Tests: If you have symptoms of pheochromocytoma, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging scans such as CT, MRI, and PET to look at where the tumor is and how big it is. They will also check for signs that the tumor has spread to other parts of the body.

How is pheochromocytoma treated?

Treatment of pheochromocytoma may involve many different doctors, including doctors who specialize in hormone disorders and doctors who diagnose and treat cancer. Treatment options to discuss with your doctor include:

Medications: Your doctor may give you medications to control your symptoms, such as alpha blockers and beta blockers, which are drugs to control high blood pressure.

Surgery: Surgery is used to remove as much of the tumor as possible. In some cases, the entire adrenal gland may be removed.

Radiation therapy and chemotherapy: Radiation and chemotherapy treatments are used when pheochromocytoma has spread to other parts of the body.

Does pheochromocytoma run in families?

In some cases, pheochromocytoma can run in families. About 25-35% of cases of pheochromocytoma may be inherited. Some of these inherited cases may be associated with a genetic condition, such as Multiple Endocrine Neoplasia Types 2a and 2b, Von Hippel-Lindau Syndrome, and Neurofibromatosis.

How does pheochromocytoma form?

Scientists have found mutations in approximately 20 different genes that they think may lead to pheochromocytoma and paraganglioma. Mutations in the genes *RET*, *VHL*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MDH2*, *IDH1*, *PHD1/PHD2*, *HIF2A/EPAS1/2*, *TMEM127*, *MAX*, *HRAS*, *MAML3* and *CSDE1* may play a role in forming pheochromocytoma and paragangliomas. In many cases, it is not known what causes pheochromocytoma to form

If you have pheochromocytoma, you may have other genetic conditions that increased your chance of getting pheochromocytoma. These genetic conditions include:

- Multiple endocrine neoplasia 2 syndrome, types A and B (MEN2A and MEN2B)
- Von Hippel-Lindau (VHL) syndrome
- Neurofibromatosis type 1 (NF1)
- Hereditary paraganglioma syndrome
- Carney-Stratakis dyad (paraganglioma and gastrointestinal stromal tumor [GIST])
- Carney triad (paraganglioma, GIST, and pulmonary chondroma)

What is the prognosis for someone with pheochromocytoma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate pheochromocytoma survival rates by how groups of people with pheochromocytoma have done in the past. Patients with a small pheochromocytoma that has not spread to other parts of the body have a five-year survival rate of about 95%. Patients with pheochromocytoma that has grown back (recurred) or spread to other parts of the body have a five-year survival rate between 34% and 60%.

5. Adrenocortical Carcinoma (ACC)

What is adrenocortical carcinoma?

Adrenocortical carcinoma, or ACC, is a cancer of the adrenal glands, which are two small triangular-shaped glands that sit on top of each kidney. The outside of these glands is called the adrenal cortex. The adrenal cortex makes important hormones that help your body control water balance, blood pressure, stress response, and cause the body to have male or female traits. ACCs form in the adrenal cortex.

An ACC may be functioning, which means it makes more hormone than normal, or non-functioning, which means it has no effect on hormone production. A functioning ACC tumor often makes too much of the hormones cortisol, aldosterone, testosterone, or estrogen.

How common is adrenocortical carcinoma?

ACC is very rare, affecting around one case diagnosed in one million people in the US. It is more common in females than males.

How is adrenocortical carcinoma diagnosed?

ACC can cause pain in the abdomen, high blood pressure, acne, overgrowth of hair, and voice deepening. Other symptoms of ACC are different for females and males, since it can change hormone levels. Females may have an overgrowth in female genitalia and facial hair. Males may have abnormal penis growth or early puberty changes like increased muscle growth and body hair.

Lab Tests: If you have symptoms of ACC, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging tests such as ultrasound, CT, X-ray, PET, and MRI scans to look at the size of the tumor and whether it has spread to other parts of your body.

Biopsy: To check if the tumor is ACC, your doctor may perform a biopsy, taking a small sample from the tumor with a needle. A pathologist will study cells from the sample under the microscope to see what kind of tumor it is.

ACC is rare but, another type of tumor in the adrenal glands, adrenocortical adenoma, is quite common. Adrenocortical adenoma is not as dangerous as ACC. It can be difficult to tell the difference between them because they are both found in the adrenal glands and the cells can look similar. Getting the correct diagnosis is very important to determine the best treatment.

How is adrenocortical carcinoma treated?

Surgery: Surgery is used to remove as much of the ACC as possible. Small ACCs are often cured with surgery.

Chemotherapy: When the ACC tumors are large, or the cancer cells have spread to other parts of the body, chemotherapy is used with surgery.

Does adrenocortical carcinoma run in families?

ACC runs in families 50% of the time. Genetic testing is recommended for all close relatives of people with ACC.

When you have ACC, you may have other conditions that increase your chance of getting cancer. Genetic testing helps determine if you or your family members are at risk of developing ACC and other diseases. Genetic counseling is often recommended to help you understand the risks to you and your family members.

How does adrenocortical carcinoma form?

Scientists are always working to understand how cancer forms but it can be hard to prove. Because ACC can run in families, we know that changes in genes linked to Li-Fraumeni Syndrome, Beckwith-Wiedemann Syndrome, and Carney complex are important in causing ACC.

What is the prognosis for someone with adrenocortical carcinoma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

If you want information on your prognosis, it is important to talk to your doctor. NCI also has resources to help you understand cancer prognosis.

Doctors estimate ACC survival rates by how groups of people with ACC have done in the past. Because there are so few people with ACC, these rates may not be very accurate. They also don't consider newer treatments being developed.

If the ACC is small when it is found, prognosis is good and cure is likely. If the ACC is already large or has spread to other parts of the body, treatment is more difficult and the five-year survival rate is 36% to 46%.

6. Medullary Thyroid Cancer (MTC)

What is medullary thyroid cancer?

Medullary thyroid cancer, or MTC, is a cancer that forms in the thyroid. The thyroid is a gland located in the front of your neck, just below the Adam's apple. It is responsible for sending out hormones to the rest of your body. The inside of the thyroid is called the medulla. The medulla contains special cells called parafollicular C cells that produce and release hormones. MTC happens when the C cells become cancerous and grow out of control. MTC may also be called medullary thyroid carcinoma.

How common is medullary thyroid cancer?

Thyroid cancer is fairly common. There are four different types of thyroid cancers and MTC is the rarest type making up 3% to 4% of all thyroid cancers. About 1,000 people are diagnosed with MTC each year in the U.S.

How is medullary thyroid cancer diagnosed?

MTC can start as a lump in the throat. The tumor growing in the thyroid can make your voice hoarse by blocking your vocal chords or it can make it hard to breathe by blocking your windpipe. Sometimes people can have MTC for a long time without symptoms because the tumor remains small. MTC can spread to other organs, such as lung, liver, bones, and brain.

Imaging: MTC is diagnosed by your doctor first feeling your throat to check for a lump, followed by imaging scans of the thyroid. Imaging scans might include ultrasound, CT, or MRI.

Biopsy: The doctor will also want to take out a small amount of tissue, called a biopsy, from the thyroid using a very thin needle. A pathologist will look at the tissue under the microscope to see if there are cancer cells and, if so, what type of thyroid cancer it is.

How is medullary thyroid cancer treated?

MTC is usually treated by removing the thyroid. This surgery is called a thyroidectomy. In certain people with a high risk for MTC, such as people carrying certain gene changes, a thyroidectomy may be performed to prevent cancer.Besides surgery, sometimes other treatments are also required, including radiation therapy or chemotherapy. Also, targeted therapies are available that act on changes in DNA found in some cases of MTC.After treatment, your doctor will monitor levels of a tumor marker called CEA and the hormones produced by C cells to keep track of how well the treatment is working or if cancer has come back. CEA is a type of tumor marker found in the blood of those with MTC.

Does medullary thyroid cancer run in families?

Twenty-five percent of MTC cases run in families. MTC may be passed down when families carry a change in the *RET* gene that causes a condition called multiple endocrine neoplasia type 2, or MEN2. There are two types of MEN2: MEN2A and MEN2B.

MEN2A: If you have MEN2A, you have a high chance (90%) of getting MTC. You are also at risk (30% to 50%) for getting pheochromocytoma, a cancer of the adrenal glands. MEN2A is rare, affecting 1 in 40,000 people. MEN2A may also be called Sipple syndrome or PTC syndrome.

MEN2B: MEN2B can sometimes be passed from parent to child but most of the time, it isn't. If you have MEN2B, you have a 100% chance of getting MTC at a very young age. You also have a 50% chance of getting pheochromocytoma at some point in your life. MEN2B is also called Wagenmann–Froboese syndrome or MEN3.

How does medullary thyroid cancer form?

Scientists are always working to understand how cancer forms but it can be hard to prove. We know that some MTC cases have changes in the *RET* gene. MTC is also more common in females than males. This information gives scientists clues about how MTC forms and can lead to new treatments.

What is the prognosis for someone with medullary thyroid cancer?

The estimate of how a disease will affect you long term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- If the cancer has spread to other parts of your body
- If the cancer responds to chemotherapy
- How much of the tumor was taken out during surgery

Doctors estimate MTC survival rates by how groups of people with MTC patients have done in the past. Given that there are so few MTC patients, survival rates may not be very accurate. They also don't consider newer treatments being developed. We know that people can live with MTC for many years, even though there is no cure.

Probable Questions:

1. Discuss different types of endocrine cancer.
2. Discuss causes of endocrine cancer.
3. Discuss risk of endocrine cancers.
4. How diagnosis can be done of endocrine cancers?

5. Discuss treatment of endocrine cancers.
6. Discuss symptoms, risk factors and treatment of adrenal cancer.
7. Discuss symptoms, risk factors and treatment of parathyroid cancer.
8. Discuss symptoms, risk factors and treatment of neuroendocrine cancer.
9. Discuss symptoms, risk factors and treatment of pituitary cancer.
10. Discuss rare endocrine cancers.

Suggested Readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7^{th} Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics. Verma and Agarwal.

UNIT-XX

Hormone signaling pathways (G-protein coupled receptors, Receptor Tyrosine Kinases, and steroid hormone signalling)

Objective: In this unit we Will discuss different kinds of Hormone signalling pathways including G-protein coupled receptors, Receptor Tyrosine Kinases, and steroid hormone signaling.

Introduction:

Eukaryotic cells and bacteria release a large number of signals and establish communication. The method of action is binding the signals with the protein receptors present on surface of large cell and triggering a series of intracellular reactions called intracellular signaling or signal transduction.

Besides, many signals (steroids and bacterial autoinducer) enter the cell, interact with signaling system and establish signal transduction. For establishing intracellular signaling, one must fully understand the operation of any cell from their origin to death.

The first signaling molecule (cyclic adenosine monophosphate or cAMP) was known during early 1960s. However, importance of intracellular signaling could be realized after the discovery of changes made by mutagenesis in signaling pathway which results in cellular transformation which is now called as cancer. One could understand their function by mutagenizing their cellularfunction.

It is now known that bacteria can change the eukaryotic cell signaling and invade the cells. The bacterial toxins can hijack the control of host cells. Similarly complexity of bacterial signaling is also known. Now an enormous amount of information is available on cell signaling and signal transduction pathway both in prokaryotes and eukaryotes.

The Signaling System:

Signaling system is very complex which may be compared to electronic circuits. You know that electronic system is such that can integrate, modulate and amplify inputs and generate output signals when switched on or switched off after getting suitable signals. The signaling systems include few basic type of modules. There are four main processes, but the signaling system uses the one or more processes.

The types of signaling modules used in intracellular signaling are:

(a) Receptor kinases (e.g. tyrosine kinase, serine kinase, histidinekinase),

(b) Receptor non-kinases (e.g. serpentine, cytokine, His-Aspphosphorelay),

(c) Protein kinase (intracellular enzymes e.g. cyclin families, Aspkinase),

(d) Lipid modifying intracellular enzymes (e.g. p13K, p15K,PLC),

(e) Cyclic nucleotides (e.g. cAMP, cGMP),and

(f) Metal ions (e.g.Ca^{++})

The four main processes are as follows:

(a) Protein phosphorylation bykinases,

(b) Small molecule and protein interaction, often involvingphosphates,

(c) Protein-protein interaction, often mediated by common motifs (specific protein sequences) which frequently results in membrane recruitment when one component is tethered to a membrane,and

(d) Protein and DNA interaction that promotes gene expression or geneinhibition.

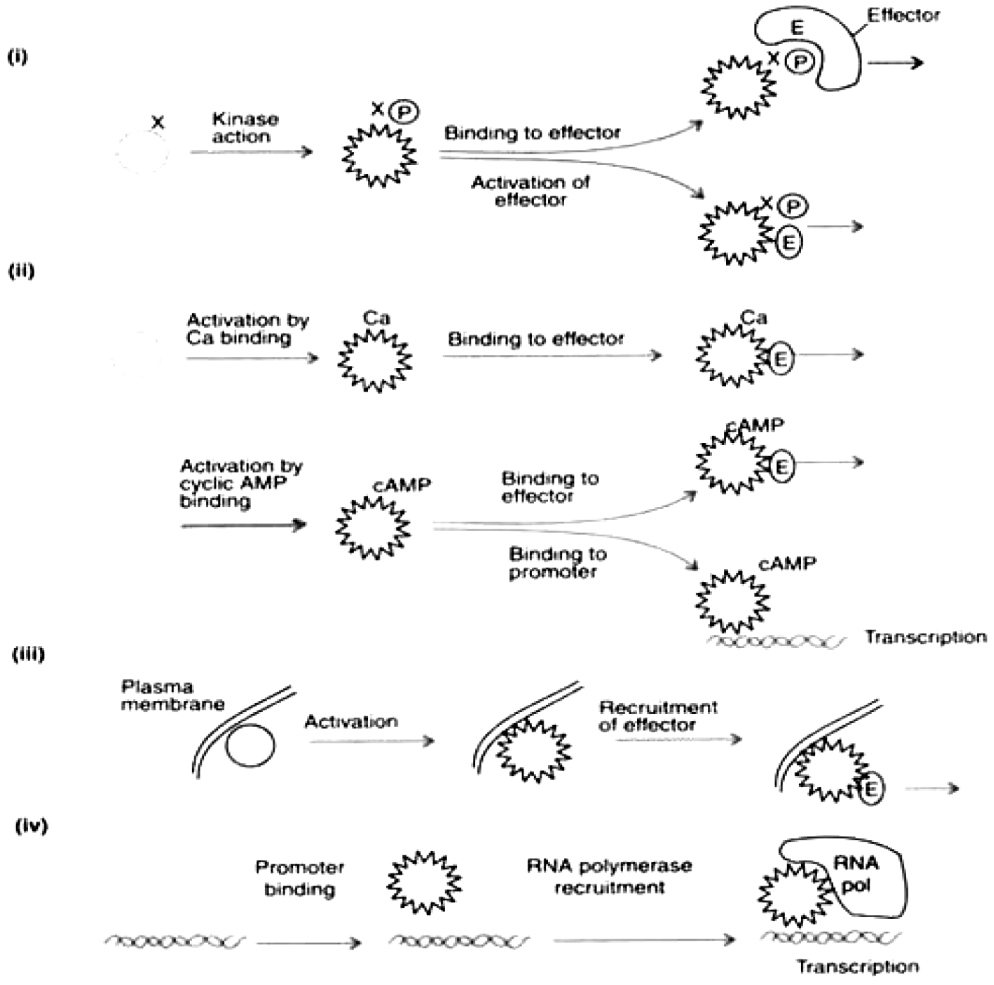


Fig. 27.8: Signalling molecules that use different types of interactions. *(i)* protein phosphorylation, where X=Tyr, Ser, Thr, His or Asp. *(ii)* interaction between small molecules and proteins, e.g. Ca^{++}, or cAMP. *(iii)* interaction between protein and protein, and *(iv)* interaction between protein and DNA which regulates transcription.

The Basic Building Blocks used in Signalling:

(a) Protein Phosphorylation: Protein phosphorylation is closely linked to cellular signaling. It exits in all signaling modules. The terminal y-phosphate is directly transferred from ARP (in some cases) to an acceptor protein by a protein kinase. The activity of the acceptor is modified example mitogen-activated protein (MAP) kinases in eukaryotes and histidyl-aspartyl phosphorelay in bacteria.

In some cases, indirect phosphorylation of protein also occurs (e.g. in G protein when binding of GTP activates their function, while GDP binding inactivates). There are secondary messengers which are used in intracellular signaling such as phosphorylated inositol's or cyclic nucleotides (cAMP, cGMP).

Kinases are regulated by any of a number of mechanisms: threonine and/or tyrosine phosphorylation, legend occupancy resulting in autophosphorylation or interaction with small molecules (e.g. cAMP or Ca^{++})

i. Histidine Kinases:

These are found in bacteria, lower eukaryotes and plants as trans membrane protein. They are stimulated to undergo self-phosphorylation by ligand occupancy.

ii. Protein Phosphatases:

Proteins which remove phosphate groups from proteins are called protein phosphatases. Protein kinases add phosphate group to proteins and play a key role in activation of signals.Specific phosphatases e.g.dephosphorylatephosphotyrosineandphosphoserine/ phosphothreonme play a key role in control of proliferation, differentiation and cell cycle Phosphoproteins take part in signaling. They moderate the phosphorylation status by regulating the balance of phosphatases and kinases.

(b) Nucleotide-BindingProteins:

The three nucleotides (GTP, cGMP and cAMP) play a major role in the intracellular signaling.

iii. GTP-BindingProteins:

There is a set of eukaryotic proteins (G proteins) that show GTPase activity. They bind to GTP and remove the terminal phosphate of GTP and produce GDP bound to G protein. This cycle operates similar to ATP and ADP cycles.

When GDP dissociates from the G protein and GTP binds again, the cycle is repeated (Fig. 27. 9). G proteins are of two type: the heterotrimeric G proteins (the dominating proteins), and the small G proteins or membrane of Ras super family (the intermediate member of the signaling pathways).

The heterotrimeric G proteins consist of three different subunits- α, β and y subunits The a- subunit has GTP-binding domain; hence Gβ has a role in signal transduction. The βy subunits transmit signals by non-covalent interaction with effector molecules. Activation of G proteins and Association of α-subunit from βy subunits are given in Fig. 27.9. GTPase activity results in after binding the Gá subunit with GDP and subsequent association with Gβy and down regulation

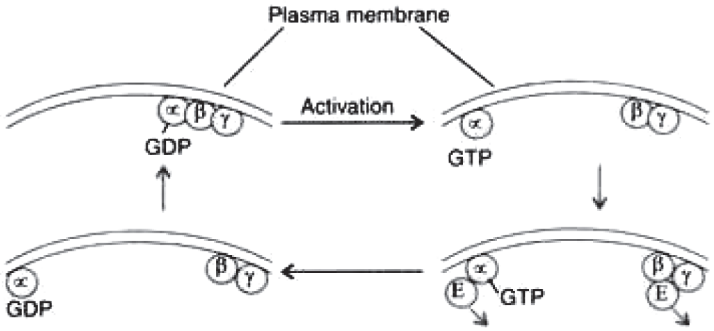


Fig. 27.9 : The function of membrane-bound heterotrimeric G proteins having α, β and γ subunits.

The small G proteins (Ras super family or p21 family) play a key role in many cellular functions such as proliferation and differentiation (Ras family), cytoskeletal organization (Rho) and nuclear membrane transport (Ran). The activity of small G proteins is modulated after interaction with several classes of proteins (Fig.27.10).

GDP-dissociation inhibitors (GDI) inhibit the loss of bound GDP and keep the G proteins in an inactive form to attenuate signaling from the activated G proteins. GTPase activity IS stimulated by GTPase-activating proteins (GAP). The removal of the bound GDP IS helped by guaninenucleotide exchange factors (GEF) which enable the GTP to bind and activate G proteins. Some of these factors have shown to be proto-oncogenes.

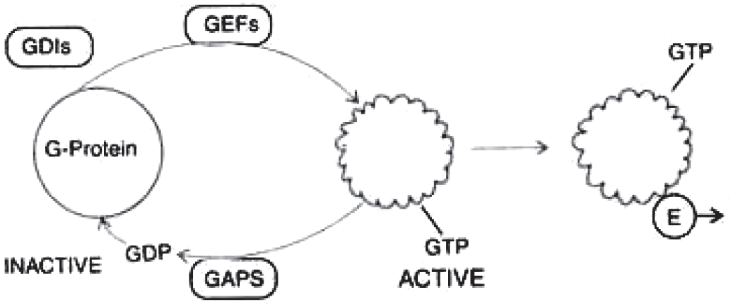


Fig. 27.10 : Functioning of small G proteins.

iv. Cyclin Nucleotide-BindingProteins:

In 1950s, cAMP was identified as the first intracellular signaling molecules. It mediates hormone action and acts as molecules transmitting the primary signal that

has been received at the cell membrane). The cAMP mediates the response to chemo-attractants. The adenylate cyclase and guanylate cyclase regulate the concentration of cAMP and cGMP,respectively.

The soluble bacterial adenylate cyclases produce cAMP which binds to c AMP receptor protein (CRP) and activate them. CRP is a transcription factor. The cAMP influences the expression of many of genes. Consequently bacteria become able to express metabolic enzymes which are required during growth. The cAMP also regulates the expression of the other genes which can cause pathogenesis.In eukaryotes heterotrimeric G proteins regulate the membrane-bound adenylate cyclases which produce cAMP. G proteins are coupled to transmembrane receptors. The cAMP-dependent protein kinases (protein kinase A) are the main effects of the cAMP signals.

While in the inactive form, protein kinase A consists of a dimer of regulatory (A) subunits and two catalytic (C) sub- units. The molecules of cAMP binds to reach R subunits and induceconformational changes. Consequently activated C subunits are released. This activated protein kinase A phosphorylates many substrates on serine or threonine (Fig. 27.11).

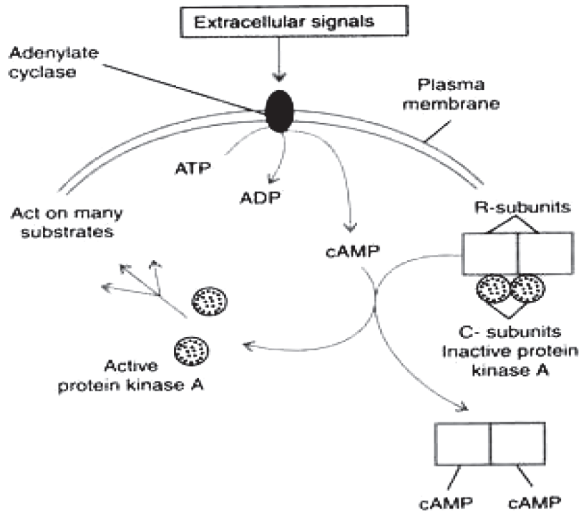


Fig. 27.11 : Production of cAMP and activation of protein kinase A.

Both the cycles work in eukaryotes by direct binding to proteins which form cation channels. Binding events result in opening of the channel.The G-protein-linked cell surface receptor generates small intracellular mediators thought cAMP pathways (Fig. 27.12).

(c) Role of Intracellular Concentration of Ca^{++} in Cell Signaling:

Calcium is found in Cytoplasm and maintained in a very low concentration(10-100 nM). But its concentration varies with cell cycle, exogenous source or release from the stores. It gets complexes in membrane bound vesicles acting as stores. A highly specific

protein calmodulin (CaM) binds to Ca^{++} and transmit the signal. Ca-binding to CaM brings about changes in conformation of CaM.

ConsequentlyCaM interacts with many effectors including CaM-modulated kinase. The most extensively studied CaM is the phosphatase calcineurin which is associated with several cellular activities such as NO synthesis, apoptosis, and induction of T lymphocytes. In eukaryotic cells Ca^{++} acts as a second messenger. Fig. 27.12 shows the two major pathways by which G-protein- linked cell surface receptors generate small intracellular mediators.

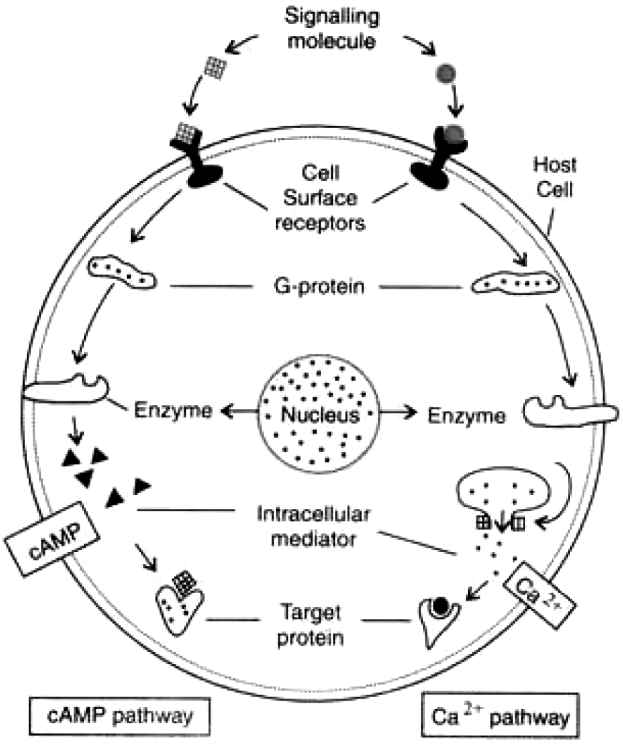


Fig. 27.12 : Generation of small intracellular mediators by G-protein-linked cell.

Role of Phosphorylated Lipids in Cell Signaling:

In eukaryotes lipids are involved in signaling process. Cellular phospholipases attack the lipid moieties of the membrane to produce different types of signaling molecules. For example, phosphatidylinositol lipids play a role in cellular stimulation. They have inositol as head, the six- membered carbon ring with a -OH group on each carbon.

On the basis of phosphorylation status of inositol head group, several phosphatidylinositols are found in the cells. The activity of three enzymes triggers their signaling role. These are: phosphoinositide 52 -kinase (P15p, phosphoinositide 32 -kinase (P13K), and phospholipase C (PLC). Extracellular signals regulate all these enzymes.

(d) Regulation ofTranscription:

Both types of cells are able to respond to any signal by changing their gene expression. In a signaling pathway the end point acts as signal. Regulators causing changes in expression of many genes in bacteria are called 'global regulators'. In prokaryotes post-transcriptional events regulate expression of many of the transcriptional factors for example cAMP-mediated CRP- DNA interactions.

In prokaryotes, phosphorylation or protein-protein interactions regulate the control of transcriptional factors and also select the other factors to the promotors. Besides, some other factors also get translocated from cytoplasm to the nucleus and regulate transcription.

(e) Role of Cell Membrane inSignaling:

Cell membrane acts as boundary of the cell through which extracellular signal has to enter. In bacteria histidine kinases act as receptor and directs signals across the membrane. Besides, there are many signal molecules which are associated with cell membrane because the end effect is membrane-associated.

The components can be well organized in three-dimensional way in cell membrane. The signaling components recruit the other molecules to the membrane where they interact with other factors. For example, GTP-bound Ras activates Raf kinase to recruit Raf to the membrane where the membrane-bound kinase activates it throughphosphorylation.

3. Prokaryotic Signalling Mechanisms:

Intracellular signaling is very complex like electronic circuit. Genome size of different bacteria varies and those organisms work according to genes present in them.In bacteria the generic mechanism of regulation is called signaling systems which includes:

a. The histidyl-aspartae phosphorylation systems (the main module of bacteria used to receive and process incoming signals such as chemotaxis, response to osmolarity, oxygen and phosphate, and virulencesystem),
b. The cAMP and CRP (involved in regulation of hundreds of genes. The cAMP is controlled at transcriptional and post-transcriptional levels. Binding of CRP- cAMP complex induces gene expression).

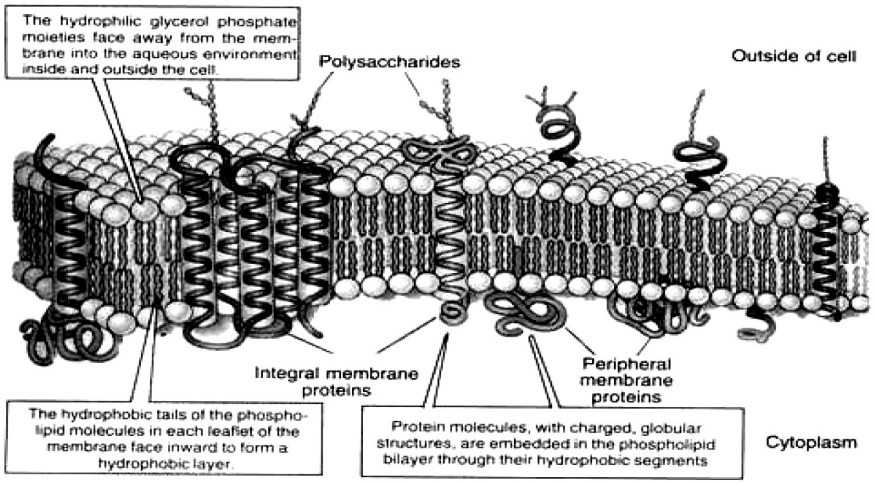
4. Eukaryotic Signalling Pathway:

Earlier it was thought that signaling process in eukaryotes was very complex to understand in molecular terms. Fragmented understanding about individual components could be known.The knowledge of signaling expanded with the development of new techniques such as genome sequencing, increasing number of reagents (isolated components, specific probes e.g. antibodies for individual components and selective inhibitors).

In spite of all these, no pathway has been fully elucidated. The best characterized pathway is the Ras activation and MAP kinases of which several details are unclarified. They are interconnected and cannot work without reference to others.

The Phospholipase C/Inositol Triphosphate Pathway:

The phospholipase C, beta or gamma is activated by membrane signaling events and cleaves PIP2 to produce diacylyglycerol (DAG) and inositol triphosphate (IP_3). These activates the release of Ca^{++} ions and results in activation of protein kinase C (PKC), which phosphorylates many additional protein substrates.



Bacterial cell membrane structure : Extracellular signal has to enter via cell membrane which acts as boundary of the cell.

The Adenylate Cyclase, cAMP and Protein Kinase A Pathway:

Adenylate cyclase is activated at the membrane by interaction with the activated heterotrimeric G protein G_5. The cAMP is generated and binds to and activates protein kinase A (PKA), which phosphorylates manysubstrates.

Integrin's, the Rho Family and Organization of Cytoskeletal:

The integrins are the signalling molecules that interact with theextracellular matrix on the outside of the cell and various proteins-linked to actin on the cells interior. The proteins involved include á-actin, lalin, tensin, vinculin andpavilin.

A local adhesion is formed upon activation that includes focal adhesion kinase (FAK). The Src kinase is recruited and several proteins in the complex are activated by phosphorylation by Src and FAC. These signals lead to the Ras/Raf, Rho signaling pathway and to cytoskeletal rearrangement.In eukaryotes, the central role of signaling pathway of a cell is to define its phenotype and function. The increasing novel knowledge about the components of signaling pathways and the types of genes which they interact are

already being applied in new strategy to combat the cancer. For example, genetically engineered viruses are attempted to grow in such cells that lack functional p53 and kill thesecells.

There are about 2000-5000 signal transduction proteins in mammalian cells. Bacteria have capacity to utilize eukaryotic signaling pathway during the process of infection. These findings make a line between the signaling pathways involved in infection and the other responsible for the pathology in diseases such as cancer and inflammation.

Eicosanoids (Gr. ecosn = 20):

These are derived from arachidonic acid, a C-20 fatty acid with 4 double bonds, e.g., prostaglandins, thrombaxanes and leukotriene's. These are called local hormones because they are short lived and have autocrine and paracrine effect.

Cytokine receptors:

Cytokine receptors are receptors that bind cytokines. In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics, and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleiotropy of cytokines are a consequence of their homologous receptors, many authorities are now of the opinion that a classification of cytokine receptors would be more clinically and experimentally useful.

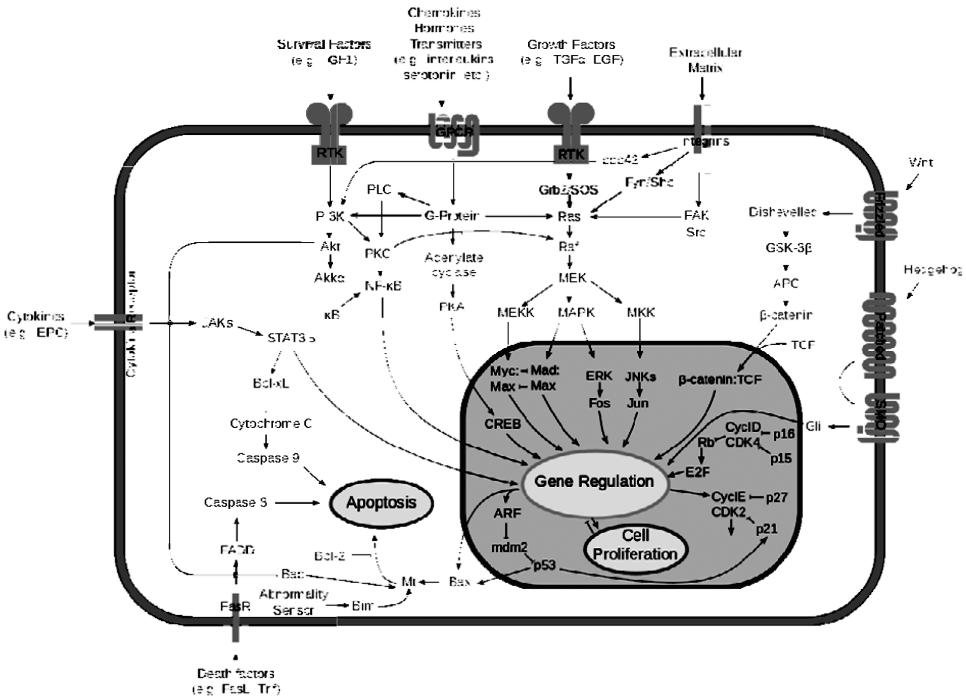


Fig: Signal transduction. (Cytokine receptor at center left.)

Classification of Cytokine Receptors

A classification of cytokine receptors based on their three- dimensional structure has been attempted.

(Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.)

Type I cytokine receptors whose members have certain conserved motifs in their extracellular amino-acid domain. The IL-2 receptor belongs to this chain, whose (γ) chain (commonto several other cytokines) deficiency is directly responsible for the X-linked form of Severe Combined Immunodeficiency (X-SCID).

Type II cytokine receptors, whose members are receptors mainly for interferons.

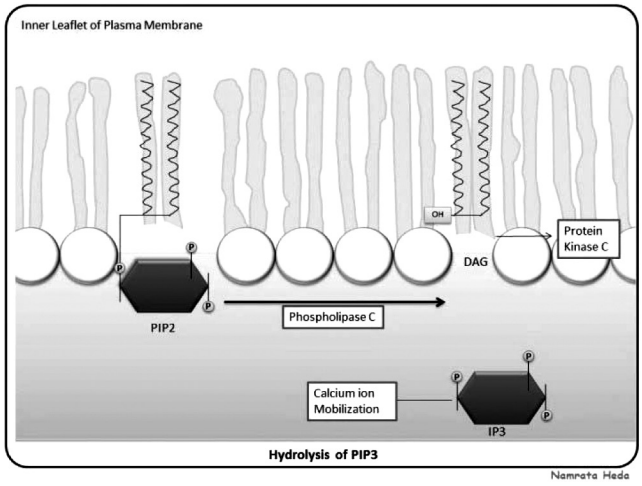
Immunoglobulin (Ig) superfamily, which are ubiquitously present throughout several cells and tissues of the vertebrate body

Tumor necrosis factor receptor family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like receptors, CD40, CD27 and CD30, besides the ligands on which the family is named (TNF).

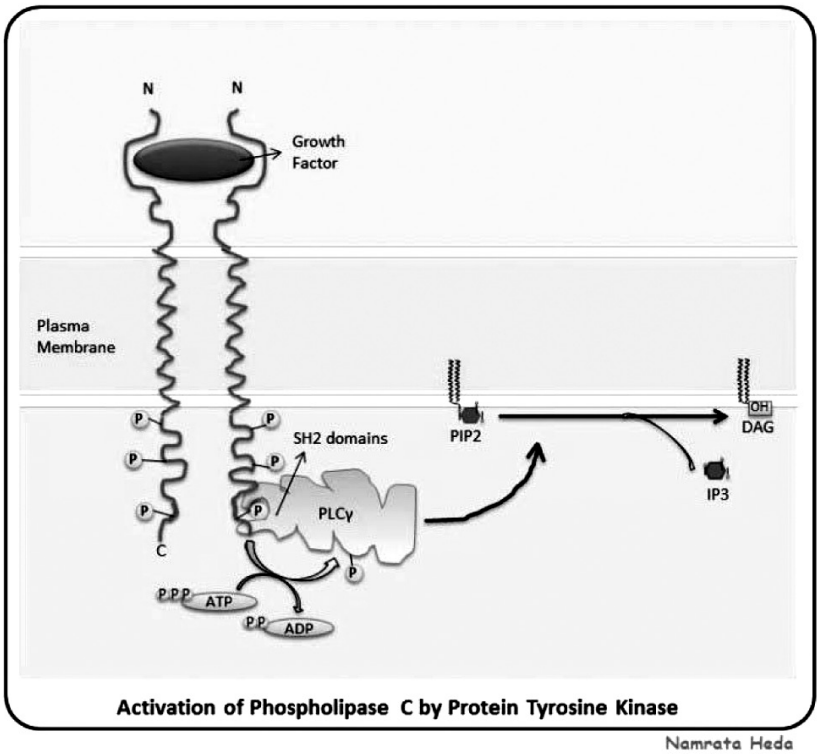
Chemokine receptors, two of which acting as binding proteins for HIV (CXCR4 and CCR5). They are G protein coupled receptors.

Phospholipids and Ca^{2+} ion mediated signaling:

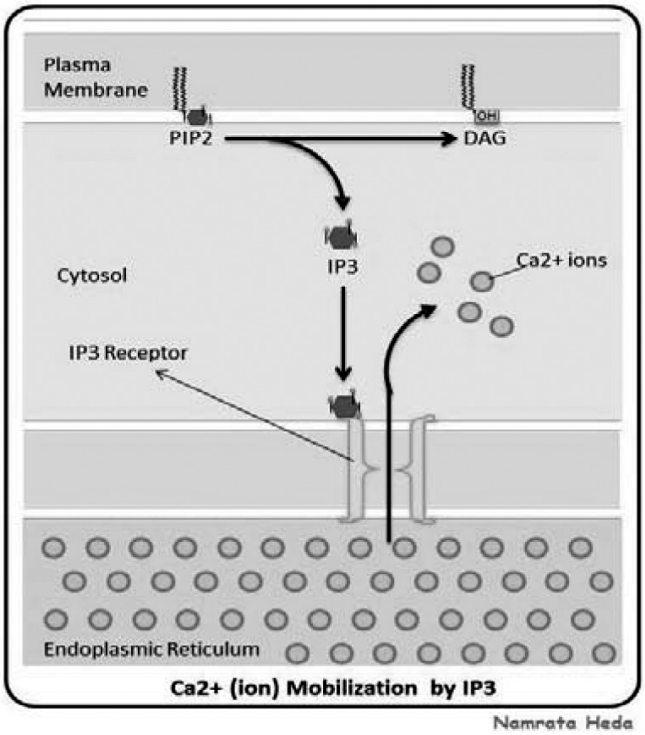
Phosphatidylinositol 4,5-bisphosphate abbreviated as PIP2 is a phospholipid present in the inner leaflet of the bilayer of the plasma membrane. The second messengers are derived from this small component (phospholipid) and the pathway is based on thesemessengers.



The hydrolysis of PIP2 takes place by the enzyme phospholipase C as can be seen in the adjacent figure. It is interesting to note that the enzyme phospholipase C is ultimately activated by G- protein coupled receptors (GPCRs) or protein tyrosine kinases. This is so because one form of phospholipase C (PLC-β) is stimulated by G proteins while another form of phospholipase C (PLC-γ) contains SH2 domains (as can be seen in the figure shown below) and hence it associates with activated receptor protein tyrosine kinases. This interaction helps PLC-γ tolocalize to plasma membrane and also leads to its phosphorylation. This tyrosine phosphorylation increases PLC-γ activity, which in turn stimulates hydrolysis ofPIP2.

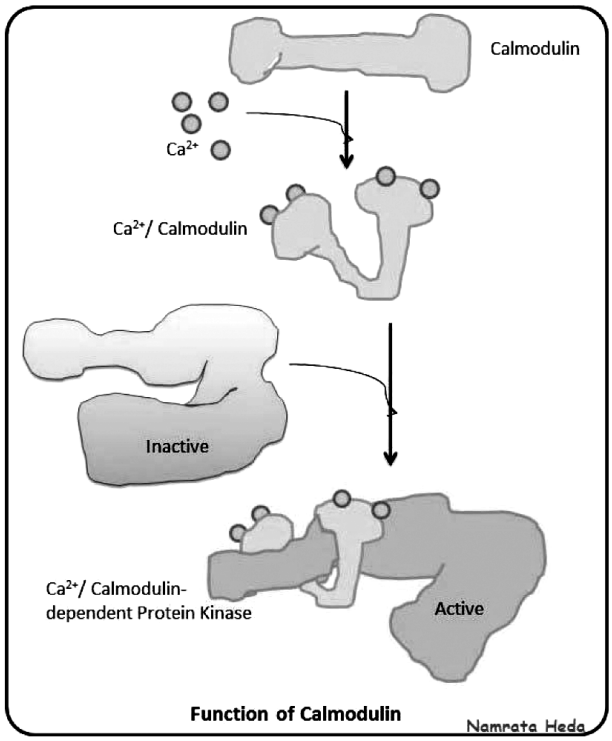


The hydrolysis of PIP2 produces two distinct second messengers as diacylglycerol and inositol 1,4,5-triphosphate which is abbreviated as IP3. Both these messengers stimulate different downstream signaling pathways thereby triggering two distinct cascades of intracellular signaling. Diacylglycerol stimulates protein kinase C mobilization while IP3 stimulates Ca^{2+}(ions) mobilization. The diacylglycerol as second messenger activates serine/threonine kinases which belongs to the protein kinase C family which play an important role in cell growth and differentiation. IP3, another second messenger is released into the cytosol and it acts to release the Ca^{2+}(ions) from intracellular stores. The level of the Ca^{2+}(ions) inside the cell is very low and is maintained by pumping through Ca^{2+}(ion) pumps across the plasma membrane.



The Ca^{2+}(ions) are pumped into the ER and hence ER is considered to be the store of intracellular Ca^{2+}(ions). Here, IP3 binds to the receptors in the ER membrane as can be seen in the adjacent diagram. These receptors are ligand-gated ion channels and hence, there is efflux of Ca^{2+}(ions) into thecytosol.This increase of Ca^{2+}(ions) in the cytosol has an effect on variety of proteins like protein kinases. For example, there are some members of protein kinase C (PKC) family that requires Ca^{2+}(ions) as well as diacylglycerol for their functioning. Hence, these PKC family members are regulated by both IP3 and diacylglycerol.

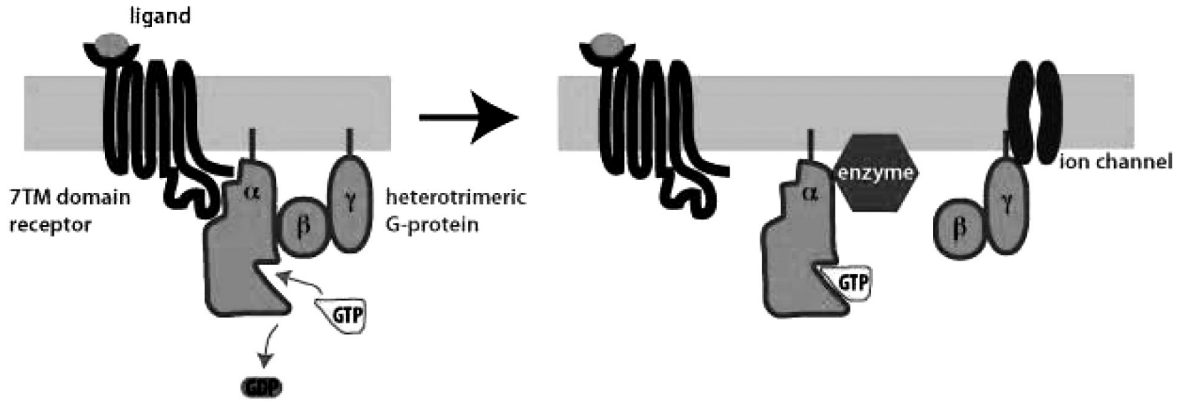
Calmodulin is another very important protein to mention while we are studying about Ca^{2+}(ions). The word 'calmodulin' means - cal(cium) + modul(ate) + in(g). Thus, calmodulin is 'calcium modulating' protein that mediates most of the activities of Ca^{2+}(ions). Calmodulin is dumbbell shaped protein which has four Ca^{2+}(ions) binding sites (figure is shown below). When the Ca^{2+}(ions) concentration in the cell increases, calmodulin is activated. This active Ca^{2+}/calmodulin complex then binds to a variety of target proteins, like Ca^{2+}ion/calmodulin - dependent protein kinases thereby rendering them active. The examples of Ca^{2+}ion/calmodulin dependent-protein kinases are: myosin light-chain kinase and members.



When there is a change in plasma membrane's potential i.e.; when there is membrane depolarization, the voltage-gated Ca^{2+} ion channels are opened in the plasma membrane. Because of the opening, there is influx of Ca^{2+}(ions) from the extracellular fluid into the cytosol of the cell. This increase in the levels of Ca^{2+}(ions) further triggers the opening of the another receptor called the ryanodine receptor in the plasma membrane which further releases the Ca^{2+}(ions) from the intracellular stores. This increase in the Ca^{2+}(ions) results in triggering the release of neurotransmitter. Hence, we can say that Ca^{2+} ion plays an important role in converting electric signals to chemical signals. In muscle cells, the ryanodine receptors on the sarcoplasmic reticulum. These receptors maybe opened directly when there is membrane depolarization.

G-protein coupled receptors:

The largest family of cell surface receptors are the G-protein coupled receptors (GPCRs). There are hundreds of different GPCR proteins, and nearly a third of all drugs target this type of receptor. A diverse set of ligands bind to this type of receptor, including peptide hormones, neurotransmitters, and odor molecules. These receptors all have a similar structure with seven transmembrane domains. On the basis of their seven transmembrane domain structure, many GPCRs have been identified in the human genome. Proteins that were identified by sequence homology, but whose ligands are not known, are termed orphan receptors.



GPCRs associate with heterotrimeric G-proteins (green), that is, G-proteins composed of three different subunits: alpha, beta, and gamma. The subunits are tethered at the membrane surface by covalently attached lipidmolecules.

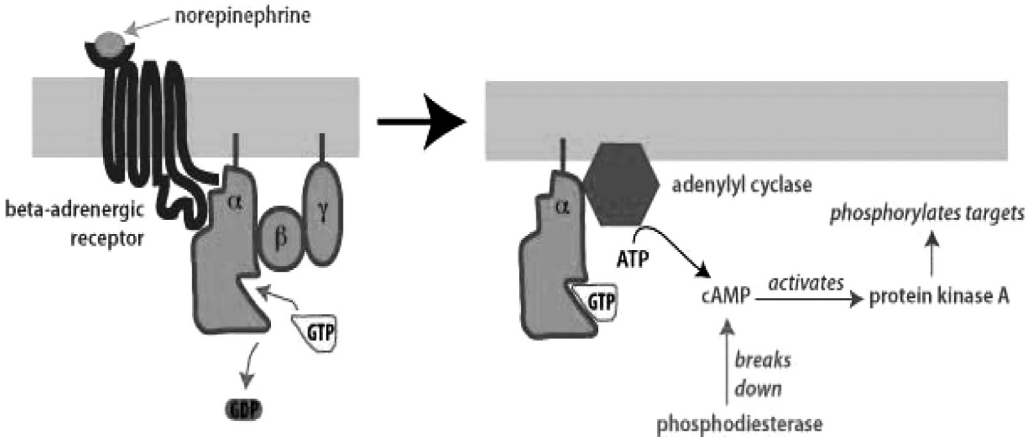
When a ligand binds, the receptor activates the attached G-protein by causing the exchange of GTP (yellow) for GDP (red). The activated G-protein then dissociates into an alpha (G-alpha) and a beta-gamma complex. G-alpha bound to GTP is active, and can diffuse along the membrane surface to activate (and sometimes inhibit) target proteins, often enzymes that generate second messengers. Likewise, the beta-gamma complex is also able to diffuse along the inner membrane surface and affect protein activity. Inactivation occurs because G-alpha has intrinsic GTPase activity. After GTP hydrolysis, G-alpha bound to GDP will reassociate with a beta-gamma complex to form an inactive G-protein that can again associate with areceptor.

The GTPase activity of the G-alpha can be made faster by other proteins—sometimes the target protein, sometimes a separate regulatory protein. Cholera toxin causes a chemical modification that *prevents* GTP hydrolysis and leads to unregulated signaling.

Different G-alpha proteins activate different second messenger pathways. There are several different classes of heterotrimeric G-proteins that are defined by their different G-alpha subunits. One type of G-alpha activates the enzyme adenylyl cyclase, which catalyzes the formation of the second messenger cyclic AMP (cAMP). Because an activated adenylyl cyclase can generate many molecules of cAMP, this is a means to amplify the signal. cAMP can have several effects, but a major effect is to bind to and activate protein kinase A (PKA; also known as cAMP- dependent kinase). PKA then phosphorylates target proteins in the cell. cAMP is rapidly broken down by phosphodiesterases, limiting the length of the signal.

A specific example of a receptor that couples to this type of G-protein is the beta-1 adrenergic receptor found in the heart. Beta 1 receptors are the principal type of adrenergic receptor found in the heart. The ligand for this receptor is norepinephrine,

the neurotransmitter that is released by sympathetic postganglionic neurons. (As well, the hormone epinephrine, released from the adrenal medulla, is also a ligand for these receptors.) Stimulation of beta-1 receptors causes increased cAMP and PKA activation. PKA phosphorylates various target proteins in cardiac cells to cause an increase in both the heart rate andthe strengthof cardiacmuscle contraction. Beta-1 receptors are the targets of drugs (beta blockers) that are used to treat heart failure and hypertension.



Another example involving GPCR signaling that stimulates adenylyl cyclase is the regulation of secretion in the small intestine. This regulation is disrupted by cholera toxin. The effect ofcholera toxin is to lead to persistent activation of adenylyl cyclase because it destroys the GTPase activity of G-alpha. There is over-production of cAMP, continuous activation of PKA, and continuous phosphorylation of CFTR, causing excessive fluidsecretion.

A different type of G-alpha activates the enzyme phospholipase C. This type of G-alpha couples to various GPCRs found on smooth muscle, such as the oxytocin receptor shown in the example below

Phospholipase C is an enzyme that cuts PIP2, a membrane phospholipid, to generate two second messengers, IP_3 and diacylglycerol (DAG). IP_3 is water soluble, diffusing through the cytosol to bind to and open a ligand-gated Ca^{++} channel in the endoplasmic reticulum (or sarcoplasmic reticulum in muscle cells). Thus, stimulation of a receptor linked to this G-alpha is a way to increase Ca^{++} inside the cytosol. Ca^{++} in the cytosol exerts its effects by binding to Ca^{++}-binding proteins such as calmodulin. In the uterus, the increase in intracellular Ca^{++} that results from oxytocin signaling causes the smooth muscle to contract.DAG is lipid soluble and stays in the membrane. It activates protein kinase C (PKC), which, like PKA phosphorylates particular target proteins.

Desensitization:

In the continuing p:resence of ligand, many GPCRs show desensitization. The mechanism is shown in the figure. A protein known as a G-protein Receptor Kinase

(GRK) phosphorylates the receptor on particular residues. This increases its affinity for a protein called beta-arrestin (red), that binds to the receptor. This reduces signaling by *preventing* the association with the G- protein.

There are several potential outcomes once beta-arrestin binds to the receptor. One possibility is that beta-arrestin targets the receptor for endocytosis, leading to receptor downregulation (a decreased number of receptors on the cell surface). Another possibility is the activation of beta- arrestin-dependent signaling pathways that are independent of G-protein signaling. Beta-arrestincan act as a scaffold that binds and brings together other intracellular signaling proteins. The physiological significance of beta-arrestin-dependent signaling is still being worked out.

Steroid Hormone superfamily mediated signal transduction:

As already noted, all signaling molecules act by binding to receptors expressed by their target cells. In many cases, these receptors are expressed on the target cell surface, but some receptors are intracellular proteins located in the cytosol or the nucleus. These intracellular receptors respond to small hydrophobicsignalling molecules that are able to diffuse across the plasma membrane. The steroid hormones are the classic examples of this group of signaling molecules, which also includes thyroid hormone, vitamin D_3, and retinoic acid.

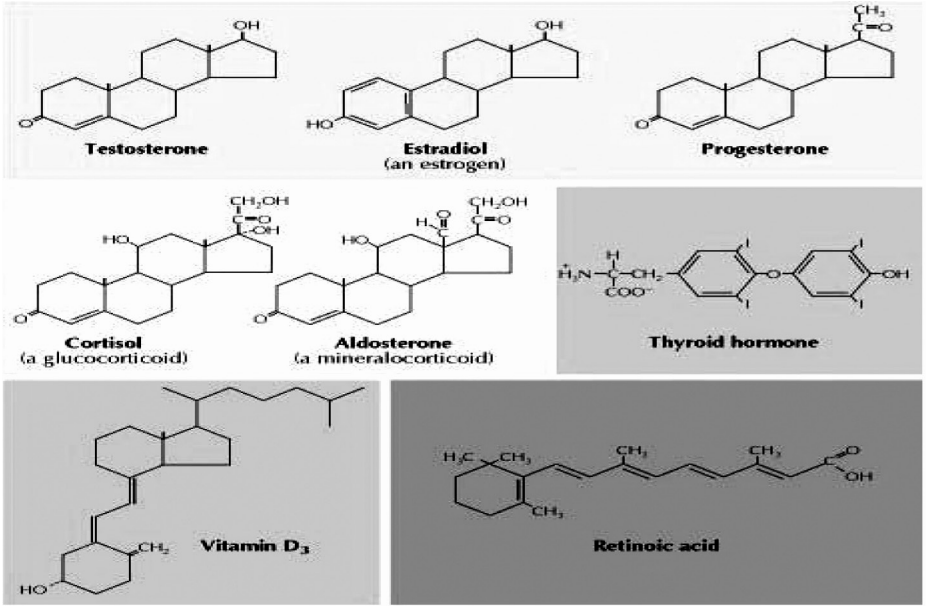


Figure: Structure of steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid

The steroid hormones (including testosterone, estrogen, progesterone, the corticosteroids, and ecdysone) are all synthesized from cholesterol. Testosterone, estrogen, and progesterone are the sex steroids, which are produced by the gonads. The

corticosteroids are produced by the adrenal gland. They include the glucocorticoids, which act on a variety of cells to stimulate production of glucose, and the mineralocorticoids, which act on the kidney to regulate salt and water balance. Ecdysone is an insect hormone that plays a key role in development by triggering the metamorphosis of larvae toadults.

Although thyroid hormone, vitamin D_3, and retinoic acid are both structurally and functionally distinct from the steroids, they share a common mechanism of action in their target cells. Thyroid hormone is synthesized from tyrosine in the thyroid gland; it plays important roles in development and regulation of metabolism. Vitamin D_3regulates Ca^{2+} metabolism and bone growth. Retinoic acidand related compounds (retinoids) synthesized from vitamin A play important roles in vertebratedevelopment.

Because of their hydrophobic character, the steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid are able to enter cells by diffusing across the plasma membrane. Once inside the cell, they bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors, which are members of a family of proteins known as the steroid receptor superfamily, are transcription factors that contain related domains for ligand binding, DNA binding, and transcriptional activation. Ligand binding regulates their function as activators or repressors of their target genes, so the steroid hormones and related molecules directly regulate gene expression.

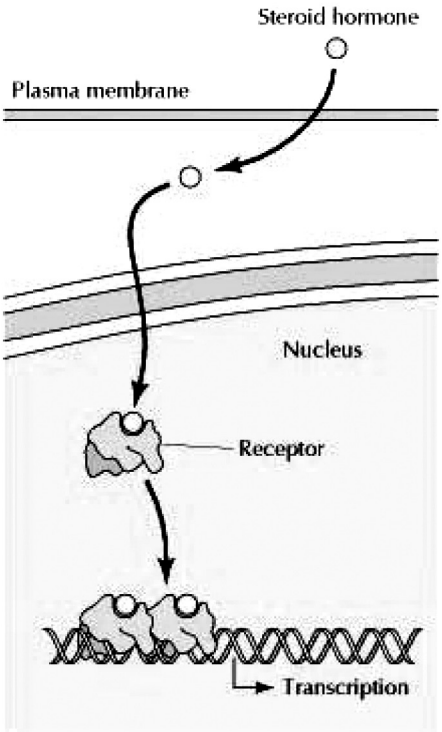


Figure: Action of steroid hormones. The steroid hormones diffuse across the plasma membrane and bind to nuclear receptors, which directly stimulate transcription of their target genes. The steroid hormone receptors bind DNA as dimers.

Ligand binding has distinct effects on different receptors. Some members of the steroid receptor superfamily, such as the estrogen and glucocorticoid receptors, are unable to bind to DNA in the absence of hormone. The binding of hormone induces a conformational change in the receptor, allowing it to bind to regulatory DNA sequences and activate transcription of target genes. In other cases, the receptor binds DNA in either the presence or absence of hormone, but hormone binding alters the activity of the receptor as a transcriptional regulatory molecule. For example, thyroid hormone receptor acts as a repressor in the absence of hormone, but hormone binding converts it to an activator that stimulates transcription of thyroid hormone-inducible genes

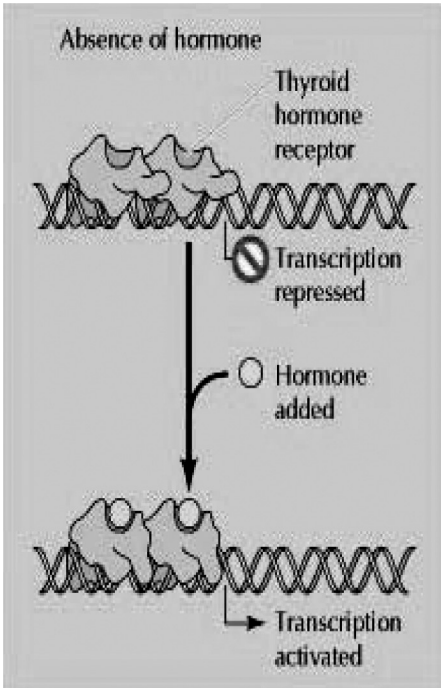


Figure: Gene regulation by the thyroid hormone receptor. Thyroid hormone receptor binds DNA in either the presence or absence of hormone. However, hormone binding changes the function of the receptor from a repressor to an activator of target genetranscription

Neurotransmitter Receptors in Cell Signaling Transduction

Chemical transmission is the major means by which nerves communicate with one another in the nervous system. Many different types of neurotransmitters play an important role in the process of chemical transdmittion. The neurotransmitters achieve cell signaling transduction through neurotransmitter receptors on the postsynaptic membrane. The neurotransmitter receptor perform large specificity and potency. Many receptors have been isolated and purified biochemically, and many have also been cloned

and sequenced. Neurotransmitter receptors can also be grouped according to the type of primary effector to which they couple. This classification leads to four major categories of receptors.

Four groups of Neurotransmitter Receptor in cell signaling transduction

Group I: Ligand-gated ion channels as neurotransmitter receptors

These ligand-gated ion channels include nAChR, GluN1, GluN2A-D, GluN3A,B, GluA1-4, GluK1-3, 4-5, GABAA, GlyR, IP3-R1, IP3-R2, IP3-R3, 5HT3, P2X1-7 and Nicotiniccholinergic (muscle [$\alpha\beta\gamma\delta\varepsilon$] and neuronal [$\alpha$ or $\alpha\beta$] subtypes).

Receptors in this category include those that are activated by synaptically released neurotransmitter and occur on the cell surface (mostly, the intracellular ligand-gated receptor for IP3 is present in the smooth endoplasmic reticulum). Upon the binding of an agonist to these ligand-gated ion channels, the receptors undergo a conformational change that facilitates opening of the intrinsic ion channel (some ligand gated ion channel receptors (e.g., NMDA and GABAA) are also found at extrasynaptic locations).

The permeability to specific ions is a characteristic of the receptor; for example, both the neuronal nicotinic cholinergic receptors (nAChR) and N-methyl D-aspartate (NMDA) receptors are selectively permeable to Na+ and Ca^{2+} ions, whereas GABAA andglycinereceptors are primarily permeable to Cl- ions. As a result of the changes in ion conductance, the membrane potential may become either depolarized, as occurs for nAChRs or NMDA receptors, or hyperpolarized, as observed for GABAA or glycine receptors.

Group II: Receptors with intrinsic guanylyl cyclase activity as neurotransmitter receptors

The representative receptor with intrinsic guanylyl cyclase activity is GC-B. Receptors in the this group possess intrinsic guanylyl cyclase activity and generate cyclic GMP (cGMP) upon activation of a receptor. These receptors consist of an extracellular binding domain, a single transmembrane-spanning domain (TMD), a protein kinase–like domain and a guanylyl cyclase catalytic domain. Ligand binding results in a conformational change in the receptor and activation of the guanylyl cyclase catalytic region. Receptors with intrinsic guanylyl cyclase activity are often very highly phosphorylated in the absence of agonist and rapidly undergo dephosphorylation uponactivation.

Group III: Receptors with intrinsic or associated tyrosine kinase activity as neurotransmitter receptors:

Receptors with intrinsic or associated tyrosine kinase activity include TrkB, EGFR, FGFR1- FGFR4, IGFR-1, Trk A, ErbB2, ErbB3, ErbB4, Trk C, PDGFR α and β, gp130 +

CNTFRα and LIFRβ, 2 × gp130 + IL6Rα and gp130 + LIFRβ. Receptors in the third group possess intrinsic receptor tyrosine kinase (RTK) activity themselves or are closely associated with cytoplasmic tyrosine kinases (RATK). Structurally, RTKs possess an extracellular ligandbinding domain, a single TMD and an intracellular catalytic kinase domain.

Three distinct events underlie signal transduction at RTKs: (1) Initially, upon ligand binding to an RTK, the receptor undergoes a dimerization that results in the juxtaposition of the two cytoplasmic domains. (2) Contact between these domains is thought to result in a stimulation of catalytic activity, (3) which in turn results in an intermolecular autophosphorylation of tyrosine residues both within and outside of the kinase domain. Once autophosphorylated, RTKs can recruit a number of cytoplasmic proteins and initiate a series of reactions involving protein– proteininteractions.RATKs, such as those for the neurotrophic cytokines (leukemia inhibitory factor, interleukin-6 or ciliary neurotrophic factor) do not possess intrinsic tyrosine kinase activity themselves, but upon activation, they undergo dimerization and are then able to recruit cytoplasmic tyrosine kinases (such as Janus kinase). The latter then phosphorylate the RATK on tyrosine residues (in addition to being tyrosine phosphorylated themselves) and facilitate protein–protein interactions, as observed forRTKs.

Group IV: G-protein-coupled receptors as neurotransmitter receptors

G-protein-coupled receptors within neurotransmitter receptors include Acetylcholine receptors, Adenosine receptors, ATP receptors, Dopamine receptors. This group of receptors involves G proteins. Numerically, more diverse types of receptors have been demonstrated to operate via an intervening G protein than by any other mechanism. These G protein–coupledreceptors(GPCRs) have a characteristic seven TMD structure. G-protein-coupled neurotransmitter receptors can be further divided into four functional categories: (1) Some GPCRs, such as GABAB, α2-adrenergic, D2-dopaminergic or M2 muscarinic (mAChR), regulate the changes in K+ conductance independently of second-messenger production. (2) A second group of GPCRs is linked to the modulation of adenylyl cyclase activity. This regulation may be either positive, as in the case of activation of the β2-adrenergic receptor, or negative, as occurs following activation of the α2-adrenergic receptor. Changes in the concentrations of cAMP regulate the activity of protein kinase A (PKA). (3) A third group of GPCRs is linked to the activation of phosphoinositide-specific phospholipase C (PLC) with the attendant breakdown of PIP2 and formation of IP3 and DAG. These receptors are linked to changes in Ca^{2+} homeostasis and protein phosphorylation via the action of protein kinase C (PKC). Other effector enzymes that may be regulated by IP3-linked GPCRs include phospholipases A2 and D. (3) A fourth, and unique, mechanism for the activation of a GPCR is that utilized by the visual pigment

rhodopsin, which structurally is a prototypical GPCR. However, in this case it is light, rather than a chemical stimulus, that triggers the activation of rhodopsin. Photoactivated rhodopsin activates transducin, a G-protein, which is coupled to cGMP phosphodiesterase with a concomitant increased rate in the hydrolysis of cGMP toGMP.

Ras / Rafand MAP Kinase Pathway:

The gene family ras encodes small GTPases that are involved in cellular signal transduction. Ras the super-family of proteins regulates diverse cell behaviors such as cell growth, differentiation and survival. Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals. Because these signals result in cell growth and division, disregulated Ras signaling can ultimately lead to oncogenesis and cancer.

Ras proteins function as binary molecular switches that control intracellular signaling networks. Ras-regulated signal pathways control processes such as actin- cytoskeletal integrity, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. Ras and Ras-related proteins are often deregulated in cancers, leading to increased invasion and metastasis, and decreased apoptosis. Activated Ras activates the protein kinase activity of RAF kinase. RAFkinase phosphorylates and activates MEK. MEK phosphorylates and activates a mitogen- activated protein kinase (MAPK).

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis. MAPK pathways are activated within the protein kinase cascades called “MAPK cascade”. Each one consists of three enzymes, MAP kinase, MAP kinase kinase (MKK, MEKK, or MAP2K) and MAP kinase kinasekinase (MKKK or MAP3K) that are activated in series. A MAP3K that is activated by extracellular stimuli, which phosphorylates a MAP2K on its serine and threonine residues and this MAP2K activates a MAP kinase through phosphorylation on its serine and tyrosine residues.

The phosphorylation of tyrosine precedes to the phosphorylation of threonine, although phosphorylation of either residue can occur in the absence of the other. Because both tyrosine and threonine phosphorylations are required to activate the MAP kinases, phosphatases that remove phosphate from either sites will inactivate them. This MAP kinase signaling cascade has been evolutionary well-conserved from yeast to mammals. Cascades convey information to effectors, coordinates incoming information from other signaling pathways, amplify signals, and allow for a variety of responsepatterns.

Down-regulation of MAP kinase pathways may occur through dephosphorylation by serine/threonine phosphatases, tyrosine phosphatases, or dual-specificity phosphatases and through feedback inhibitory mechanisms that involve the phosphorylation of upstream kinases. Drugs that selectively down-regulate MAP kinase cascades could prove to be valuable as therapeutic agents in the control of malignant disease.

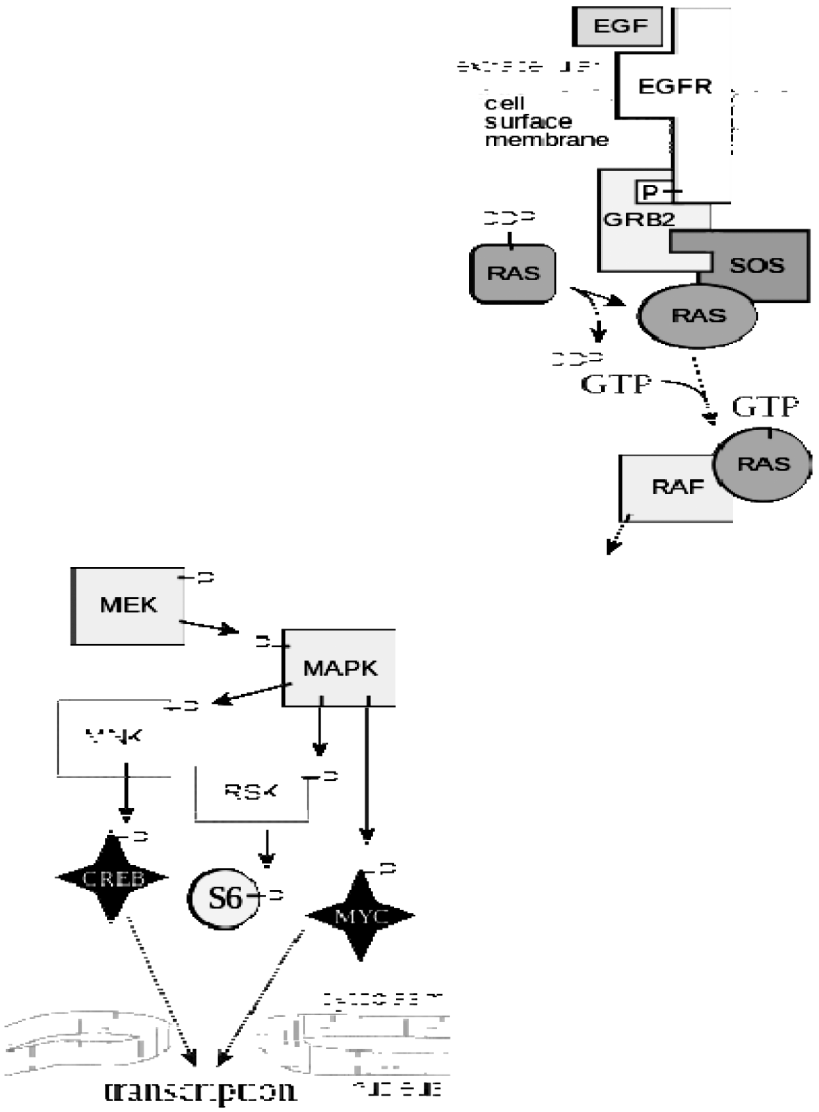


Figure: Key components of the MAPK/ERK pathway. "P" represents phosphate, which communicates the signal. Top, epidermal growth factor (EGF) binds to the EGF receptor (EGFR) in the cell membrane, starting the cascade of signals. Further downstream, phosphate signal activates MAPK (also known as ERK). Bottom, signal enters the cell nucleus and causes transcription of DNA, which is then expressed as protein.

JAK-STAT signaling pathway:

The JAK-STAT signaling pathway is a chain of interactions between proteins in a cell, and is involved in processes such as immunity, cell division, cell death and tumour formation. The pathway communicates information from chemical signals outside of a cell to the cell nucleus, resulting in the activation of genes through a process called transcription. There are three key parts of JAK-STAT signaling: Janus kinases (JAKs), Signal Transducer and Activator of Transcription proteins (STATs), and receptors (which bind the chemical signals). Disrupted JAK-STAT signaling may lead to a variety of diseases, such as skin conditions, cancers, and disorders affecting the immune system.

Here are 4 JAK proteins: JAK1, JAK2, JAK3 and TYK2. JAKs contains a FERM domain(approximately 400 residues), an SH2-related domain (approximately 100 residues), a kinase domain (approximately 250 residues) and a pseudokinase domain (approximately 300 residues). The kinase domain is vital for JAK activity, since it allows JAKs to phosphorylate (add phosphate molecules to) proteins.There are 7STAT proteins: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. STAT proteins contain many different domains, each with a different function, of which the most conserved region is the SH2 domain. The SH2 domain is formed of 2α-helices and a α-sheet and is formed approximately from residues 575-680.STATs alsohave transcriptional activation domains (TAD), which are less conserved and are located at the C-terminus. In addition, STATs also contain: tyrosine activation, amino-terminal, linker, coiled-coil and DNA-binding domains.

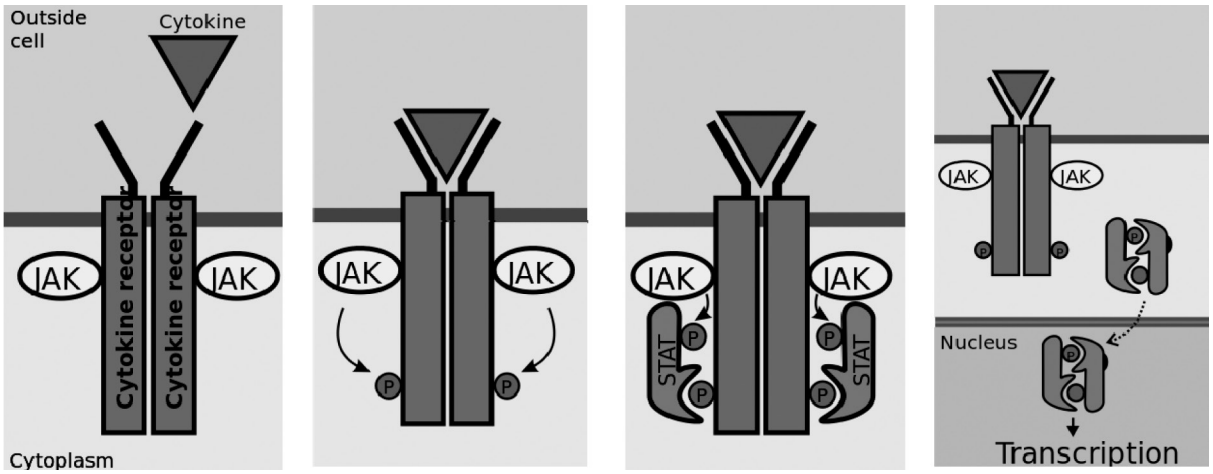


Fig: Key steps of the JAK-STAT pathway. JAK-STAT signalling is made of three major proteins: cell-surface receptors, Janus kinases (JAKs), and signal transducer and activator of transcription proteins (STATs). Once a ligand (red triangle) binds to the receptor, JAKs add phosphates (red circles) to the receptor. Two STAT proteins then bind to the phosphates, and then the STATs are phosphorylated by JAKs to form a dimer. The dimer enters the nucleus, binds to DNA, and causes transcription of targetgenes.

Probable Questions:

1. Describe the role of protein phosphorylation in signal transduction.
2. Describe role of Intracellular Concentration of Ca^{++} in CellSignaling.
3. Describe the role of Phosphorylated Lipids in Cell Signaling.
4. Classify cytokine receptors in details.
5. Write down the role of calmodulin in signal transduction.
6. Describe the basic components of G-protein coupled receptor.
7. What is desensitization?
8. How signal is transmitted by steroid hormones.
9. Describe the role of neurotransmitter receptors in signal transduction.
10. How signal is transmitted thorough Ligand-gated ion channels ?
11. Describe Ras / Raf and MAP Kinase Pathway with suitable diagrams.
12. Describe JAK-STAT signaling pathway with suitable diagram.

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7^{th} Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics. Verma and Agarwal.

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e-sources.