SELECTED TOPICS IN BIOLOGY FOR DEGREE STUDENTS

Prof. Ibrahim, Baba Usman

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TERTIARY EDUCATION TRUST FUND

Book Development Project

The Tertiary Education Trust Fund (TETFUND) has the mandate to establish and nurture the Higher Education Book Development Project in Nigeria. Book scarcity has reached a crisis proportion in the country as evident not only in the quantity of books available but also in the quality of locally produced books. Given the seriousness of the paucity of reading and learning materials in Nigeria's higher educational institutions, TETFUND Book Development Project is designed to reactivate and nurture research and the publication of academic books and journals in hard and e-forms in Nigerian higher educational institutions, thereby empowering tertiary institutions in Nigeria to benefit from and contribute to knowledge production and nationally and globally. Advancement in science and technology, especially ICT and the influence of globalization have profoundly transformed the context, from and the scope of knowledge production that Nigerian higher educational institutions should be assisted to fully participate in and contribute to the global system of generating and disseminating knowledge. The uniqueness of the present intervention lies in the fact that through it, TETFund will assist Nigerian higher educational institutions restore and sustain the capacity for academic publishing.

The promotion of indigenous authorship and the resuscitation of local publishing of books are critical instruments in addressing the dearth of textbooks, including basic test and specialized textbooks in various disciplines in Nigeria's higher educational institutions. Restoring the culture of indigenous authorship and the production of indigenous books would ensure the availability of books that address local need and reflect familiar realities and experiences.

The book production component is one of the three areas of intervention of the TETFund Book project. The others are the revitalization of academic publishing and the support of academic journals. This first phase of the book production intervention is directed at the production of peer-reviewed basic textbooks written by Nigerian academics for universities, polytechnics and colleges of education and specialized books in various subject areas as well as the publication of books of high-quality PhD theses from Nigerian Universities that have successfully gone through a rigorous assessment process. This would contribute to solving the problem of paucity of books in Nigeria's higher educational institutions.

Tertiary Education Trust Fund,
6, Zambezi Crescent, Off Aguiyi Ironsi Street,
Maitama, Abuja, Nigeria.

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Dedicated to

THE STUDENTS AND STAFF OF THE DEPARTMENT

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FOREWORD

I have watched keenly over the years the aspirations and the keen interest of the Department of Biology in propagating and advancing the science course at this University, Ibrahim Badamasi Babangida University, Lapai. It is, therefore, a prime honour and respect to be asked to write the foreword of this book "Selected Topics in Biology for Degree Students" which the Department of Biological Sciences has compiled together for the benefit of students, as well as to propagate the scope of research in Biology and upgrade the University to the status and level of high performing academic institution.

The topics covered in the advent of this book were in-depth treated, comprehended and constituted important additions to the literature in the subject area. In this regard, I hereby recommend the book to biology degree students in universities.

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PREFACE

This book, "Selected Topics in Biology for Degree Students" covers a wide range of topics in Biology for the use of the degree students both in their academic studies and in pursuance of research studies. The range and scope of the book published by the Department of Biological Sciences covered aspects of Botany, Fisheries, Zoology, Statistics and, scope of scientific write up and orientations. The book shall be of immense importance for the degree undergraduate students as well as post graduate students in the course of their studies and research.

In conclusion, we recognized the gaps that exist in this volume, as the topics can still be explored more in-depth. This is an indication of more exploration in knowledge that can still be done, to widen more in the future, to propagate and advance the course of science.

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The department was established, right from the commencement of academic activities in the University in the year 2006. The department since then has enjoyed the good wishes, blessing and assistance of many personalities and organizations, who contributed immensely to the advancement of the department, TETFund Abuja inclusive. It is therefore our prestige and honour to acknowledge the followings personalities for their contributions to the advancement of the departments, the present Vice – Chancellor Prof. Adamu Kasim Abu, the immediate past Vice – Chancellors Prof. Muhammad Nasiru Maiturare, Late Prof. Ibrahim, Adamu Kolo, Late Prof. M. A. Daniyan and Late Prof. M. A. Chado. The contribution and assistance of TETFund, Abuja is also well appreciated and hereby acknowledged.

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We finally acknowledge the contribution of our visiting external examiners right from the inception of the department Prof. Olatunde, A. A. of the Department of Biology, University of Abuja, Prof. Fatoba of the department of Plant Science, University of Ilorin, Kwara State, and Prof. Jehu Auta of the department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Thank you and GOD bless you, Amen

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CHAPTER ONE: INTRODUCTORY ANIMAL HISTOLOGY

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1.1 Introduction

Animals are multicellular heterotroph that grow and develop through a series of stages and actively move about during all or part of their life. Histology is the scientific study of the fine detail of biological cells and tissues that have been carefully prepared using histological techniques. It is derived from the Greek word: *Histos* and *logia* means the study of tissues of living organisms. Therefore, Animal Histology is the study of the microscopic structures of cells and tissues of animals. Animal tissues are divided into four groups;

1. Epithelia Tissues,
2. Connective Tissue,
3. Muscular Tissue
4. Nervous Tissue.

1.2 Epithelia Tissue

Epithelium can be defined simply as "a layer of cells with a free surface". However, it is single or multiple layers of cells. In embryonic terms, they are derived from all the 3 germ layers: Ectoderm (mainly nervous system), Mesoderm (mainly muscle and the lining of body cavity) and Endoderm (mainly organs of digestive tract). Epithelial cells are exposed to toxic chemicals, pathogens and mechanical abrasion. - An epithelial cell of the small intestine may survive only a day or two before it is destroyed. - New epithelial cells are produced by division of stem cells (germinative cells) located near the basal lamina.

The Features/Characteristics (Fig 1) are;

a. they line the surface of the body: mainly located on the borders between the external and internal.

b. they have basal lamina: found where epithelium contacts the connective tissue.

c. they have basement membrane: a thin sheet of collagen and glycoprotein produced by epithelia and underlying connective tissue.

d. with few exceptions in stria vacularis of the cochlea, hypertrophied thyroid gland, it lacks blood vessels.

e. regeneration: a high rate of cell replacement by stem cells in the epithelium.

f. cellularity: tightly bound together by cell junction.

g. surface specializations of epithelia such as microvilli.

h. cilia/Flagella: movement of materials over epithelia. Can remove debris- e.g. tracheal cilia that direct mucus carrying particulates out of trachea.

a. they play role in homeostasis

Fig 1: Epithelial tissues showing features/characteristics.

The Functions of epithelial tissues are:

i. Protection: covering and lining surfaces, e.g. skin, epithelial cells (endothelium) lining blood vessels, body cavities = coeloms = peritoneal, pleural and pericardial coeloms). For example, the entire external body is covered by an epithelium.

ii. Absorption (tall columnar epithelium of intestine): by absorbing substances and preserving water and salts of the body.

iii. Secretion (epithelia of glands): by secreting fluids and chemicals substances necessary for digestion, lubrication, protection, excretion of waste products, reproduction and the regulation of metabolic processes of the body.

iv. Sensation (sensory cells, neuroepithelium - taste buds): by constituting parts of sense organs especially, of smell and taste.

v. Contractility (myoepithelium - often associated with glands such as sweat and mammary glands).

vi. Lubrication: by lining all the internal cavities of the body, including the peritoneum, pleura, pericardium, and the tunica vaginalis of the testis.

vii. Cleaning: Ciliated epithelium assists in removing dust particles and foreign bodies which have entered the air passages.

viii. Diffusion: Simple epithelium promotes the diffusion of gases, liquids and nutrients. Because they form such a thin lining, they are ideal for the diffusion of gases (e.g. walls of capillaries and lungs).

ix. Reduces Friction: The smooth, tightly-interlocking, epithelial cells that line the entire circulatory system reduce friction between the blood and the walls of the blood vessels.

x. Excretion: Epithelial tissues in the kidney excrete waste products from the body and reabsorb needed materials from the urine. Sweat is also excreted from the body by the epithelial cells in the sweat glands.

The features (Fig 2) of epithelial tissue classification are;

a. Simple (one cell thick): such as squamous (flat) - nucleus flattened with long axis parallel to basement; cuboidal (square) - nucleus is spherical; columnar (column) - nucleus oblong, long axis perpendicular to the basement membrane and pseudo - has a stratified appearance, however, is actually a single layer of cells with unaligned nuclei and some cells which do not reach the basement membrane.

b. Compound (more than one cell thick): such as stratified (more than one cell layer) and transitional.

Fig 2: Features of epithelium tissue classification

The different types of epithelial tissue are;

1. Simple epithelia:

a. Simple squamous epithelium: the cells are thin and flattened. It has little mechanical strength. It is found in absorptive surface e.g. air sacs of the lungs; lining ventral body cavity called mesothelium e.g. pleura, peritoneum, pericardium; lining heart and blood vessels called endothelium. Its thinness permits diffusion of materials through it. It also provides smooth linings to hollow structures such as blood vessels and the chambers of the heart. The characteristics are as presented in Fig 3.

Fig 3: Simple squamous epithelial tissue.

b. Simple cuboidal epithelium: they are found in glands and in lining of the kidney tubules (Fig 4). In glandular tissues e.g. salivary, thyroid glands. They constitute the germinal epithelium which produces egg in the female ovary and the sperm cells in male testes. This is the least specialized of all epithelia. The cells are roughly cub-shaped and possess a central spherical nucleus.

Fig 4: Simple cuboidal epithelium

c. Simple columnar epithelium: their cells are elongated and column-shaped. The nuclei are elongated and are usually located near the base of the cells. They are found lining the stomach and intestines (Fig 5), function in absorption and secretion; secretion of mucus.

Fig 5: Simple columnar epithelium

d. Simple ciliated columnar epithelium: they possess in addition to the above, fine hair-like outgrowths, called cilia on their free surfaces (Fig 6). They are capable of rapid, rhythmic, wave-like beating in a certain direction. This causes mucus secreted by goblet cells to move in specific direction. Found in air passages like the nose. They are also found in the uterus and fallopian tubes which helps propel the ovum to the uterus.

Fig 6: Simple ciliated columnar epithelium

e. Pseudostratified epithelium: these are found lining the male reproductive system. They can be ciliated e.g. trachea (Fig 10) or non-ciliated e.g. male urethra. They are columnar in shape.

Fig 10: Pseudostratified columnar epithelium

2. Compound epithelia:

a. Stratified squamous epithelium: this is the main protective tissue of the body. It is found lining the mouth, esophagus, anus and exposed surfaces. There are two forms;

i. Keratinized stratified squamous epithelium (Fig 7a): this is packed with fibrous protein. Found in apical layers of skin cells such as the epidermis. It is tough and water

resistant. It is made up of 3 layers: the top layer has no nuclei, are modified to form keratin – used to form hair, skin; middle layer has polymorphic cells – modified to form the epidermis; bottom layer, has basal cells, modified to form sole of feet, palms of hand.

ii. Non keratinized stratified epithelium (Fig 7b): they resist abrasion, dry out and must be lubricated. They are found to cover wet surfaces that have a lot of tears and wears e.g. oral cavity, pharynx, esophagus, anus, vagina

a-Keratinized b-Non-Keratinized

Fig 7: Stratified squamous epithelium

b. Stratified cuboidal epithelium (Fig 8): these are found lining the ducts of sweat glands, mammary gland, ovarian follicle, seminiferous tubule. They function in secretion and production.

Fig 8: Stratified cuboidal epithelium

c. Stratified columnar epithelium: this is similar to stratified cuboidal epithelium but its superficial cells are columnar and may be ciliated. They rarely line the larger ducts of some large glands, forms the conjunctiva, and occurs in small, isolated patches in some mucous membranes. They sometimes cover the respiratory surface of the epiglottis.

Fig 9: Stratified columnar epithelium

d. Transitional epithelium: they have the ability to accommodate stretching. They are found in urinary tract as fluid pressures varies thus in ureters, urethra and bladder (Fig 11).

Fig 11: Transitional epithelium

1.3 Connective Tissue

These are tissues in which cells are usually separated by greater amounts of intercellular substance. They serve to bind and hold body structures together. The study of connective tissue is called MESENCHYMOLOGY. They form the framework upon which epithelial tissue rests; Within which nerve and muscular tissues are embedded. They are developed from the mesenchyms, foetal supporting tissue. Connective tissue is the major supporting tissue of the body. It includes the skeletal tissues, bone and cartilage.

The functions of connective tissues are;

a. They are largely responsible for the cohesion of the body as an organism, of organs as functioning units and of tissues as structural systems. This cohesive function is achieved through their permeation of other tissues of the body.

b. They are essential for the protection of the body both in the elaborate defense mechanisms against infection and in repair from chemical and physical injuries.

c. Nutrition of nearly all cells of the body and the removal of their waste products in both medial is through the connective tissues.

d. They are also important in the development and growth of many structures.

e. Constitutes the major contributor to the homeostatic mechanism of the body so far as salts and water are concerned.

f. They act as the great storehouse for the body salts and minerals, as well as of fat.

g. They determine in most cases the pigmentation of the body.

h. The skeletal system (cartilage and bones) plus other kinds of connective tissue (tendons, ligaments, fasciae and others) make motion possible.

The components of connective tissues are;

a. Cellular Components such as;

1. Fibroblast: they are elongated, spindled shaped cells with many cell processes. They active cells. The more mature form with slow activeness is called FIBROCYTE.
2. Macrophages (Histiocytes): Shows pronounced phagocytotic activity. Originates from monocytes which are precursor cells in bone marrow. They function in phagocytosis of microorganism such as bacteria.
3. Mesenchymal cells: They are stem cells. They response to injury or infection by differentiating into fibroblast, macrophages etc.
4. Mast cells: They are large amoeboid mesenchymal cells. They have central nucleus and often packed within granules. The granules are made up of histamine & heparin which are released in inflammatory responses.
5. Plasma cells: Ovoid, weakly amoeboid with eccentric nucleus. Responsible for antibody production. Found in sites of high risk of invasion.
6. Melanocytes: Cytoplasm filled with minute granules. There granules may either be yellow or brown. They synthesize and store melanin.
7. Yellow fat cells: Yellowish in colour. Generally spherical with a thin shell of protoplasm enclosing a single enlarged fat drople
8. Brown fat cells: Moderately large and spherical. Have small droplets of variable size scattered in the cytoplasm.

b. Extracellular Components such as;

i. Fibrillar components:

a. Reticular fiber: these have affinity to silver thus ARGYROPHILIA. Abundant in lymphatic organs (spleen & lymph node), stomach muscle, endoneurium, liver, endocrine gland.

b. Collagenous fiber: they have longitudinally striated appearance. Synthesized with cells such as fibroblast, osteoblast, chondroblast, odontoblast, reticular cells, epithelial cells etc. They are the most abundant protein in the body.

c. Elastic fiber: Highly refractile and appear slightly yellowish. Prominent in elastic tissue such as elastic ligament.

ii. The non-fibrillar component of connective tissues appears amorphous with the light microscope and is the matrix in which cells and fibres are embedded. It consists of two groups of substance:

a) Those probably derived from secretory activity of connective tissues cells including mucoproteins, protein-polysaccharide complexes, tropocollagen and antibodies, and

b) Those probably derived from the blood plasma, including albumin, globulins, inorganic and organic anions and cations and water. In addition, the ground substance contains metabolites derived from or destined for blood.

Connective tissues can be classified as;

1. Connective Tissue Proper: They have many protein fibers. Have syrupy ground substance. It is divided into two categories based on above;

a) Loose Connective Tissue Proper: they are very common; they fill the space between muscle fibers. It has more ground substance and less fiber. There are three types;

i. Areolar loose connective tissue proper (they have little space; it is the least specialized with open framework distort without damage; underlying all epithelia; surrounding nerves, blood vessels, esophagus, trachea; it is the least specialized with open framework distort without damage; they have viscous ground substance to absorb shocks; they have elastic fiber that returns to original shape; they hold blood vessels and capillary beds (Fig 12)).

Fig 12: Areolar loose connective tissue proper

ii. Adipose loose connective tissue proper (this is similar to areolar but contains adipocytes for storing fats (Fig 13). They also absorb shocks and slow heat loss. There are two types: white fat tissue (the most common adipose tissue) and brown fat tissue (adipocytes contain many mitochondria).

Fig 13: Adipose loose connective tissue proper

iii. Reticular loose connective tissue proper (this has 3-dimensional network of supportive fiber called STROMA. The stroma supports functional cells – PARENCHYMA. They are found in the spleen, liver, lymph nodes and bone marrow (Fig 14)).

Fig 14: Reticular loose connective tissue proper

b) Dense Connective Tissue (Collagenous Tissue): They have more fibers, less ground substances. They are dense because they have high numbers of collagen fibers. They are divided into three categories;

i. Dense regular connective tissue (they have tightly packed, parallel collagen fibers; they attach muscle to bone (Tendon); they connect bone

to another bone or stabilize organ (Ligament)).

ii. Dense irregular connective tissue (they have interwoven networks of strengthening fibers; found in skin around the cartilage (Perichondrium), around bones (Periosteum) and forms capsules around liver and kidney).

iii. Elastic tissue (though both dense regular and dense irregular connective tissues contain elastic fibers, elastic tissue is mostly elastic fibers. e.g. elastic ligaments of the spinal column).

2. Supportive connective tissue: these are soft tissues that provide the weight of the body. There are 2 types of supportive connective tissues thus:

a) Cartilage: they are composed of chondrocytes. Chondrocytes are located in lacunae surrounded by an intercellular matrix. It is an avascular tissue. It has low metabolic activity & turnover except the embryo. Receive nutrients from perichondrium. Chondrogenesis is the process of cartilage development. There are different types of cartilage thus:

· Hyaline/joint cartilage (they lack bundles of fibers. Has collagen fibrils; Derived from Greekword 'hyalos' = glass; fresh sample is milky-white. they are flexible and resilient to mechanical forces. found in the respiratory tract (nose, larynx, trachea, bronchi); in embryo it plays role in long bone development).

· Fibrocartilage/Patella (found in areas subjected to high mechanical stress; found in intervertebral disk, pubic symphysis, temporo-mandibular joints; it is characterized by large numbers and concentrations of collagen fibers in the matrix with relatively little amorphous matrix).

· Elastic cartilage (found in areas subjected to high flexibility & elasticity; they provide the yellowish color in the fresh tissue).

b) Bone: osteogenesis is the development of bone. The first bone to develop is spongy bone called woven, immature or primary bone. Woven bone lacks order of lacunae (osteocytes). Primary bone is found in developing embryo. Composed of cells and extracellular matrix in which fibers are embedded. Extracellular matrix is calcified. They provide protection. The hematopoietic bone marrow is protected by the surrounding bony tissue. There are two main categories;

· Spongy/trabecular/cancellous bone (composed of lattice or network of branching bone spicules or trabeculae which contains bone marrow) and

· Compact/corticol bone (lack spaces visible to unaided eyes).

Bones have different cells that includes;

a) Osteoprogenitor cells: derived from mesenchyme cells.

b) Osteoblast: first cell to develop from osteoprogenitor cells. It is involved in formation of bone. Found on the boundaries of developing and growing bones.

c) Osteocytes: mature bone cells that developed from osteoblast: Located in the lacunae within bony matrix. Have cytoplasmic process located in canaliculi.

d) Osteoclasts: highest bone cells. It is involved in bone resorption. Found in eroding surfaces often in cavities called Howship's lacunae.

3. Fluid connective tissue: they have watery matrix of dissolved proteins. There are two types:

a. Blood: this consist of variety of cells suspended in a fluid medium called PLASMA. It has different components that include;

· Blood cells:

Ø Erythrocytes (circular biconcave discs; non-nucleated in mammals; primarily involved in respiration).

Ø Leucocytes (nucleated amoeboid cells; constitute important part of defense and immune system). Leucocytes are differentiated into;

§ Neutrophil: accounts for about 55-70%; segmented nucleus, cell diameter about 12μm; cytoplasm packed with small specific granules; they are phagocytic cells; they are important in inflammation and at sites of injury or wound; does in site of infections are mostly dead neutrophil.

§ Eosinophil: they represent about 1-4% of the blood cell; have bilobed nuclei with acidophilic granules; involve in selective phagocytosis; increase in the cell, its associated with allergic reaction or helminth parasitic infection.

§ Thrombocytes: they are small and colourless disc; they play role in blood clotting; they occur only in mammals.

§ Basophil: they represent about 1%; the nucleus is bilobed or s-shaped; there are large irregular basophilic granules; the granules are similar to those of the mast cells.

§ Lymphocytes: they represent about 25-30%; they are involved in immune response; they have little cytoplasm with rounded nucleus; classified as small or large depending on size of the cytoplasm.

§ Monocytes: they represent about 5%; have oval or kidney shaped eccentric nuclei; they are non-terminal cells i.e. can differentiate into phagocytic cells such as macrophages.

· Blood Platelets (they are derived from cytoplasm of megakaryocytes of the bone marrow. They initiate the process of blood clotting. They initiate the plugging up and sealing of damaged blood vessels).

b. Lymph: They are transparent intercellular or lymphatic vessel fluid. They include; lymphatic vessel fluids (lymphates). Important proteins such as immunoglobulin, from which protective antibodies are derived.

Fig 15: General characteristics of connective tissues

Table 1: Summarized characteristics of connective tissues

Tissue Type	Cells Present	Fibers Present	Matrix Characteristics
Connective Tissue proper			
Loose Connective Tissue:			
Areolar	fibroblasts macrophages adipocytes mast cells plasma cells	collagen elastic reticular	loosely arranged fibers in gelatinous ground substance
Adipose	adipocytes	reticular collagen	closely packed cells with a small amount of gelatinous ground substance; stores fat
Reticular	reticular cells	Reticular	loosely arranged fibers in gelatinous ground substance
Dense Connective Tissue:			
dense regular	fibroblasts	collagen (some elastic)	parallel-arranged bundles of fibers with few cells and little ground substance; great tensile strength
dense irregular	fibroblasts	collagen (some elastic)	Irregularly arranged bundles of fibers with few cells and little ground substance; high tensile strength
Supportive Tissue:			
Cartilage:			
hyaline (gristle)	chondrocytes	collagen (some elastic)	limited ground substance; dense, semisolid matrix
Fibrocartilage	chondrocytes	collagen (some elastic)	limited ground intermediate between hyaline cartilage and dense connective tissue
Elastic	chondrocytes	Elastic	limited ground substance; flexible but firm matrix

Bone (osseous tissue):

compact (dense)	osteoblasts osteocytes	Collagen	rigid, calcified ground substance with (canal systems)
spongy (cancellous)	osteoblasts osteocytes	Collagen	rigid, calcified ground substance (no osteons)

Fluid Connective tissue

Blood	erythrocytes leukocytes thrombocytes	"fibers" are soluble proteins that form during clotting	"matrix" is liquid blood plasma
Lymph	leukocytes	"fibers" are soluble liquid proteins that form during clotting	"matrix" is blood plasma

1.4 Muscular Tissue

Muscle (from *Latin musculus*, diminutive of *mus* "mouse") is a *contractile tissue* of animals. They are primarily concerned with contractility. They are responsible for movement of body parts. It is developed from embryonic mesoderm (except myoepithelium). The cells contain contractile filaments that moves pass each other thereby changes the size of the cell. The contractile filaments are bound by connective tissue. The cytoplasm is called sarcoplasm. The endoplasmic reticulum is called sarcolemma. Whilst muscle cells are called myocytes.

Muscular tissue is classified into three based on morphological and physiological functions thus;

a) Smooth: this is known as involuntary muscle (Fig 16). They lack cross striation. Have ability to undergo hyperplasia and hypertrophy as in the uterus of pregnant woman. They can regenerate. Found in the walls of hollow internal organs such as blood vessels, dermis, iris diaphragm, uterus

Fig 16. Smooth muscle

b) Skeletal: Make up about 40-50% of body mass (Fig 17). Myofibers are bound together in bundles or fascicles. Connective tissue in the muscle serves to bind and integrate the action of the various contractile units. The end of the muscles is attached to bone, cartilage or ligaments by tendons. The muscle functions in; skeletal movement; maintenance of body position; support soft tissues; guard body openings and maintenance of body temperature.

Fig 17. Skeletal muscle

c) Cardiac: It is a striated muscle (Fig 18). It has fiber branches (bifurcate). Each myocyte has one or two central nuclei. The fibers have more sarcoplasm. They possess a system of T-tubules.

Fig 18. Cardiac muscle

Table 2: The characteristics of the three types of muscles.

Character	**Skeletal**	**Smooth**	**Cardiac**
Location	Attached to skeleton	Walls of stomach, intestine etc	Walls of heart
Types of control	Voluntary	Involuntary	Involuntary
Shape of fibres	Elongated, cylindrical blunt ends	Elongated spindle – shape pointed ends	Elongated, cylindrical fibres that branch and fuse
Striations	Present	Absent	Present
No of nuclei per fibre	Many	One	One or two
Position of nuclei	Peripheral	Central	Central
Speed of contraction	Most rapid	Slowest	Intermediate
Ability of remain contracted	Least	Greatest	Intermediate

1.5 Nervous Tissue

Nervous tissue components are concerned primarily with rapid conduction of impulses; their cells are specialized for conduction. They therefore serve as the complex telecommunications network of the body. These tissues act in a sensory capacity, to receive, disseminate, and store information collected from receptors. In a motor capacity, nervous tissues provide response potential by controlling effectors such as muscles or glands. Nervous tissue carries electrical signals from one part of the body to another. Thus, the functions include;

i. allows an organism to sense stimuli in both the internal and external environment.

ii. analyzed and integrated to provide appropriate, coordinated responses in various organs.

iii. the afferent or sensory neurons conduct nerve impulses from the sense organs and receptors to the central nervous system.

iv. internuncial or connector neurons supply the connection between the afferent and efferent neurons as well as different parts of the central nervous system.

v. efferent or somatic motor neurons transmit the impulse from the central nervous system to a muscle (the effector organ) which then react to the initial stimulus.

vi. autonomic motor or efferent neurons transmit impulses to the involuntary muscles and glands.

vii. autonomic nervous system (ANS): Is formed from two chains of ganglia found along the spinal cord and scattered among body tissue, concerned chiefly with regulation of visceral activity.

Nervous tissue contains densely packed nerve cells called neurons. Neurons are cells that are specialized for irritability and conduct. The neuron consists of a cell body called soma or perikaryon and few - many attenuated cytoplasmic processes which radiate out of the cell body. Depending upon their functional role, these processes are termed axons or dendrites. Depending on the length, a fibre which carries impulse towards a cell body is called a dendron (long) or dendrite (short). An axon carries impulses away from cell body. Neurons can be classified based on its morphological and physiological properties.

Morphologically, they are classified into three (Fig 19);

a. Unipolar/pseudo unipolar neurons have a single process (axon); they have one process that emerges from the cell body, T-fashion, into two processes; these are found in sensory ganglia of dorsal roots of spinal nerves.

b. Bipolar neurons have two processes (one dendrite and one axon); these are very rare and have a limited distribution in the body; they are present in special sensory structures including the retina, olfactory epithelium, and vestibular and cochlear nerves).

c. Multipolar neurons possess several processes (several dendrites and a single axon); most neurons belong to this category.

Fig 18: Morphologically classified neurons

Physiologically, neurons can be classified into four thus;

a. Sensory neurons these receive sensory stimuli from the environment (from receptors) and from within the body (e.g. unipolar neurons).

b. Motor neurons (Fig 19) these control the effector organs (muscles, exocrine glands, endocrine glands); a motor neuron has many processes (cytoplasmic extensions) called dendrites, which enter a large, grey cell body at one end; a single process, the axon, leaves at the other end, extending towards the dendrites of the next neuron or to form a motor endplate in a muscle; dendrites are usually short and divided while the axons are very long and does not branch freely; the axon is surrounded by the myelin sheath, which forms a whitish, non-cellular, fatty layer around the axon; outside the myelin sheath is a cellular layer called the neurilemma or sheath of Schwann cells; the

myelin sheath together with the neurilemma is also known as the medullary sheath; this medullary sheath is interrupted at intervals by the nodes of Ranvier.

c. Interneurons/intermediate neurons these are typically found in the CNS and connect between other neurons (often between sensory and motor neurons).

d. Neurosecretory neurons these are specialized neurons that synthesize and secrete hormones.

Fig 19: motor neuron

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CHAPTER TWO: SOME ASPECTS OF FISH PRESERVATION AND PROCESSING TECHNIQUES

2.1 Fish Preservation

Fish preservation is the method of extending the shelf life of fish and other fishery products. Preservation method maintains the quality of fish for a longer period of time. A well processed fish will store for a long period.

2.2 Fish Processing

When fish are caught or harvested for commercial purpose, they need some processing, so that they can be delivered to the consumers, or next part of marketing chain in a fresh and undamaged condition.

2.3 Preservation Techniques

Many different techniques have been used to preserve fish quality and to increase their shelf life. They are designed to inhibit or reduce the metabolic changes that lead to fish spoilage, by controlling specific parameters of fish and, or that of its environment. The techniques are classified as follows:-

(a) Techniques based on temperature control:-

This involves the use of different types of technologies to decrease the fish temperature to levels, where metabolic activities, catalyzed by autolysis or microbial enzymes are reduced or completely stopped. This is possible by refrigeration or freezing, where the fish temperature is reduced, respectively to approximately 0^0C for refrigeration, -0^0C for Freezing and -156^0C for Cryo - Preservation.

(b) Techniques based on the control of water activity:-

Water activity (aw) is a parameter that measure the availability of water in fish flash. It is express as a ratio of water vapour pressure in fish / vapour pressure of pure water at the same temperature and pressure. Water activity (aw) varies from 0-1. Water is necessary for microbial and enzymatic reactions, and several preservation techniques have been developed to tie up this water and thus reduce the water activity (aw). These include drying, salting, smoking, freezing, freeze-drying, the use of water binding humectants and combination of

these techniques. Some of these techniques have been in use for thousands of years and can be implemented very simple e.g. salting.

(c) Techniques based on the control of oxydo-reduction (Redox) potential:-

Some spoilage bacteria and lipid oxidation require oxygen. Therefore, reducing the oxygen around the fish will increase its shelf life. This is possible by vacuum packaging, or by controlling or by modifying the atmosphere around the fish. Specific combination

of $C0_2$, 0_2 and N_2 characterized control (CA) or modified atmosphere (MA), vacuum package (CA) storages are often combine with refrigeration for fish preservation.

(d) Techniques based on the physical control of microbial fish loads, its chemical and enzymatic activity:-

The physical method, use heat (cooking, blanching, pasteurizing, sterilizing), ionizing irradiation (for pasteurization and sterilization) or micro wave heating. Cooking or pasteurizing are processes that do not allow complete inactivation of microorganisms and thus often require to be combined with refrigeration to preserve fish products and increase, their shelf life. This is not the case with sterilized products, which are stable at ambient temperature ($<40^0$c). These require packaging in metal cans or retort able pouches before the heat treatment "canning".

(e) Combination of several preservation techniques:-

Two or more of the already described, techniques can be combined to improve preservation efficiency, while reducing undesirable effects such as the denaturation of nutrient by severeheat treatment. Combinations already in use include pasteurisation – refrigeration characterized control (CA) or modified Atmosphere (MA) – refrigeration, salting – drying, salting – smoking and salting – marinating.

2.4 Fish Preservation and Fish Processing Methods

There are different methods used to preserve and processing fish. The methods include:-

- ü Freezing method
- ü Salting method
- ü Smoking method
- ü Drying method
- ü Curing method
- ü Dehydration method
- ü Pickling method
- ü Cooking method
- ü Canning method
- ü Fermentation method

2.4.1 Fish Preservation Methods

(a) **Salting method**

Salting is a traditional method of preserving fish in many countries of the world. It is often used in combination with drying and smoking. Drying the fish removes water and lowers the water activities (water available for the support of microbial growth which causes spoilage).Some water is removed from the fish during salting.

Salting also lowers the moisture or water content of fish and other fishery products to a point where micro - organisms cannot live and grow. Salt partially dehydrates the

fish and kills the bacteria. Salt (Nacl) also improves the fish texture because it firms up the fish.

2.4.2 Basic methods of applying salt to preserve fish

Salt is applied by the following basic methods: -

(a) kench salting in which granular salt is rubbed into the surface of the split fish, the fish are stacked with sprinkling of salt between each layer and the liquid (pickle) which form is allow to drain away.

(b) Pickle salting: - cover the fish with salt and pack them in layers in water tight containers. This forms the pickle that serves as the saturated brine solution that covers the fish completely.

(c) Brine salting: - immerse the fish in a saturated solution made up of twenty five (25) parts of salts and hundred (100) parts of water. Brine salting is done only as a temporary way to preserve fish before they are dried, smoked or processed.

(d) Dry salting: - involve running granular salt on the fish. The proportion of salt to fish varies from ten percent (10%) to thirty five (35%) of the fish weight.

Steps in salting method

(i) Place the fish either in crushed ice or frozen brine.

(ii) Remove the fins.

(iii) Remove the head (optional).

(iv) Split the fish along the dorsal section spread it open.

(v) Take out the black membrane of the fish.

(vi) Wash the fish thoroughly and drain it a little.

(vii) Rub the fish with salt.

(viii) Arrange the fish in a container; place the container inside a refrigerator.

(b) Salting - Smoking method

This method combines with salting, precooking and drying. The final process is smoking which dehydrates the fish further. The smokes give colour and flavour to the fish.

Steps in salting - smoking method

(i) Clean the fish by removing the gill and make half inch (1/2) slit in the belly. Wash the fish thoroughly with clean water.

(ii) Soak the fish in brine solution (one part of salt to ten part of water), for twenty (20) or more minutes, depending on the size of the fish.

(iii) Place the fish in the immersion basket made up of woven bamboo strips or wire netting. The basket will be suspended during the immersion in boiling brine. Cooked for two (2) to four (4) minutes, or more, depending on the size of the fish.

(iv) Drain the fish, allow them to cool after being cooked in brine solution place it in a layer of wire screen (rattan or bamboo) and have it dried in a cool and shady place.

(v) Smoke the fish in tin cans for one (1) to two (2) hours until, it gets golden brown. The length of smoking actually differs, depending on the size of the fish. And the smoke produced.

(vi) Packed the smoked fish in coarsely woven cane / bamboo basket. Line the size newspapers. Cool the fish completely before packing them to allow moisture to escape and prevent the attack of mould and bacteria.

(C) Drying method:-

This method is also known as natural dehydration. Like the salting method, it lowers the water content of the fish to a point where microorganisms, bacteria, enzymes and yeast cannot grow and multiply. The most popular fish preservation method is solar dehydration. It is done in combination with salting. Fish dried under the sun look and taste better. The method of drying involves the removal of the water content of the fish until such time, that the fish moisture is completely extracted. This method prevent the possible growth of the microorganisms, that causes food spoilage. It can be done using the heat of the sun, and it is called solar drying. In the dehydration, an artificially heating, air is used in the dehydration of the fish. This heated air comes from the mechanical driers.

Steps in drying method

The followings are the steps involved in the drying method of fish preservation:-

(i) wash the fish thoroughly

(ii) soak the fish in ten percent (10%) brine solution for half (1/2) hour to draw out the blood.

(iii) Squeeze or open the belly cavity, remove the visceral or internal organs.

(iv) Soak the fish for three (3) to six (6) minute in a concentrated brine solution to partially draw out the moisture or water content of the fish.

(v) Place the salted fish in drying trays and dry it under the sun.

(vi) When the fish are thoroughly dried, pack them and store them in a clean, dry place.

(D) Curing method

This method uses chemical preservatives (including vinegar and salt), smoke and other physical factors, to reduce the moisture or water content of the fish cured, fish or fishery products possess flavour and texture completely different from those of the fresh fish.

(E) Dehydration / Drying method

Dehydration is an artificial process of drying because it is done with the used of mechanical devices such as an oven, that produces artificial heat for drying.

(F) Pickling method

Pickling is a method of preserving fish in brine or vinegar. It can be done with or without bacterial fermentation; the method of pickling involves using vinegar as well as other spices and ingredients as preservatives. The acetic acid of the vinegar presents bacterial and other microorganisms from growing, which result in the preservation of the fish. This method improves the fish's taste.

(G) Fermentation method

Fermentation is a fish preservation method in which fish in brines solution, undergo chemical reaction. Fermentation is a process in which the musculature in the fish is broken through the enzymatic reaction. In this method, the flesh of the fish to be preserved, is allow to get "ripe". And with this, the flesh will start to disintegrate, until such time that the flesh will undergo "aging". Fermentation could take up to eight months in the normal process. However, this only took place in less than a month, when pure salt was used, and the temperature in the process increase from 37^0C to about 45^0C.

Steps in fermentation method

(i) clean the fresh fish well, remove scales, shells, seaweeds and other material.

(ii) Wash the fish in a weak brine solution (1 part of salt to 9 parts of water drain it well, cover the container water draining the al among to keep flies

(iii) Mix fish thoroughly with salt (1 part to 3 parts of alamang)

(iv) Place the fish-salt mixture in a clean container.

(v) Stored the preserved fish in a clean and warm place.

(H) Freezing method

This is a method of keeping fish in a condition close to that of a fresh fish using ice or chilling systems for a few week or for a longer period, depending on the need and situation of preservation. There are circumstances however, when fish need to be kept for long period, to enable fish to be distributed and sold in distant markets, and to stock pile products for lean season. If fish are properly frozen and stored at correct temperature, it is possible to provide a product which closely resembles fresh fish, in many cases, the consumer will be unable to distinguish between pieces of frozen fish and fresh fish. Fresh fish contains approximately eighty percent (80%) water. At normal atmospheric pressure, pure water will change from liquid to solid (ice) at 0^0c i.e. it will freeze. However, the water in fish fluid contains dissolve salts and chemicals which have the effect of lowering the temperature at which the water begins to freeze. The exact temperature of freezing varies between species and size of fish, but is usually between -1^0c and -2^0c. As the temperature drop below this critical temperature water tend to be frozen out of solution.

Steps to preserving fish by freezing

(i) Remove all the guts and clean the fish as soon as possible after catching.

(ii) Cut large fish into strips, and freeze small fish whole.

(iii) To preserve fish, first wrap them in a manner (polythene bag) that is air tight, and to prevent freezer burn. This will also aid in reducing the changers of

unwanted flavours that are not natural to the fish.

(iv) Make sure the freezer temperature is at or below zero degree °C. This will ensure that the preserving process is not disturbed by a lapse in temperature.

(v) Date the fish as its been froze, so as to know when they will no longer be safe to eat.

(I) Cooking method

Cooking is a way to prevent wastage or spoilage of fish. Cooking fish with vinegar prolonged the period of preservation.

2.4.3 Fish Processing Methods

(A) Canning method:-

Canning is the packing of fish in airtight containers such as tin, cans and glass, jar which prevent air and micro organisms from entering. Through the heat processing, microbial inside the can are destroyed, thus preventing spoilage under normal condition and allowing the fish to be stored for longer periods. Sardines and salmon are the most commonly canned fish in the market.

Steps in canning method

The followings are steps involved in the canning method of fish preservation:-

(i) Remove the scales of the fish.

(ii) Remove the internal organs, the head and the tail of the fish.

(iii) Cut the cleaned fish to fit the size of the can to be used. For thirty minutes (30Mins) soak the fish in twenty percent (20%) brine solution.

(iv) Half – fry the fish in oil.

(v) Fill each can with half – fried fish leave about ¼ inch space. Add a table spoon of corn oil and tomato sauce. Do not add salt because the fish has been brined.

(vi) Sealed the filleted cans temporarily. Use the first roll operation of the can sealer.

(vii) For ten minutes (10mins), stem the clinches cans without pressure to exhaust the air inside the cans. Then, seal the can, using the can sealer.

(viii) For forty five minutes (45minuts), process sealed cans at 15 Ib pressure, using the can sealer.

(ix) Immediately, cool the pressed can in running water.

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CHAPTER THREE: FISH FEED FORMULATION

3.1 GENERAL INTRODUCTIONS

The quality of fish feed used in fish production have high effect on the quality of fish product. How cheap one is able to produce the fish feed will give both the feed producer and fish producer a better maximum economic return (MER), which may be passed on to the consumers (users) or added to the 'personal effort' when calculating the economic return. The nutritionists have been concerned with development of feed that result in better growth and food conversion factor. Now the attention is on least cost and the next target would be how to improve the organoleptic properties of the product.

Feed formulation is the calculation for the combination ratio of feed ingredient to meet the specification of targeted feed to be consumed. The ingredients and their composition are represented by two letter e.g. rice bran (RB), maize germ (MG), fish meal (FM), soybean meal (SM), wheat middling (WM), CP for crude protein etc. and the content of nutrients are represented as percentage or gram per weight (i.e. numeric symbols). From the calculation (i.e. formulation) the ratio of combination is known to meet the constraints. This may include crude protein, energy, percentage amino acids, lipid content, percentage fatty acid, percentage mineral and vitamin, total fiber content etc. Thus **feed formulation can be defined as a mathematical modeling where letters represent names and numerical figures represent content of feed ingredients and feed to be composed.** There has been evolution of method used in feed formulation due to the variation in the interest of the feed user. Pearson's square, is an arithmetic method first used which was developed into Trial and Error and to Equational method to take care of more than two specifications (Sadiku, 2001). To meet the multi constraint feed requirement and give cost a place, many constraints are considered, giving rise to many equations and many ingredients are required giving rise to many variables (unknowns). These two factors makes the equation generation too many and so lengthy thereby consuming much time when the equation method is to be used.

3.2 TECNIQUES OF FEED FORMULATION

There are four conventional methods used for feed formulation, (1) Pearson (2) Trial and Error (3) Equational methods. (Algebraic method) (4)Linear programming (Computer software)

3.2.1 PEARSON SQUARE FORMULATION

Pearson square uses Arithmetic 'trick' to formulate feed. This trick is equivalent to quantitative aptitude. For example to formulate 30% crude protein using maize germ and

cottonseed is as follow: maize germ meal 20% crude protein and cottonseed meal 54% crude protein are available to formulate a 30% crude protein supplement feed.

Example 1:

Cottonseed 54% 30-20=10/(10+24) x 100%=29.41

Maize germ 20% 54-30=24/(10+24) x 100%=70.59

That is 29.41% cottonseed + 70.59% maize germ would give 30% crude protein diet.

For more than two protein sources, Pearson Square cannot give the actual amount of each ingredient required because the ingredients have to be grouped into two, those with crude protein greater than 30% and those with crude less than 30%. For example: using soybean meal 48%crude protein, cottonseed meal 54% crude protein, maize meal 20% crude protein and rice polishing 14% crude protein to formulate 30% crude diet. The ingredients have to be premixed so as to have just two components before they can be used in Pearson method. At the end, the percentage required for each group is known. The other problem of this Square method is that it cannot solve for more than one specification likes protein, lipid and energy.

Example 2:

Formulation of 30% crude diet using soybean meal 48%crude protein, cottonseed meal 54% crude protein, maize meal 20% crude protein and rice polishing 14% crude protein.

Premix of ingredients 50/50, w/w

Soybean meal and Cottonseed meal 51% crude protein

Maize meal and rice polishing 17% crude protein

Soybean+Cottonseed 51% 30-17=13/(13+21) x 100%=38.24%

Maize meal+rice polishing 17% 51-30=21/(13+21) x 100%=61.76%

Thus, 38.24% Soybean and Cottonseed + 61.76% Maize meal+Rice polishing would give 30% crude protein diet.

3.2.2 THE TRIAL AND ERROR METHOD

New (1987) developed the Pearson Square method into Trial-and-error method e.g. to formulate a diet 26% crude protein and 7%lipid from fish meal, groundnut, maize and soybean using table 3.1 as ingredient file.

Table 3.1 Ingredient file to formulate 26% crude protein and 7% lipid.

Ingredients name	Lipid content	Protein	Cost (N)
Fish Meal	6.0	55.0	600
Groundnut	13.7	34.5	350
Soybean Meal	1.3	46.8	490

Rice Bran	2.4	13.3	150
Maize Germ	4.5	9.8	180

Source: Modified from New (1987)

If we assumed that experiment set a minimum fish meal level of 10% supplying 0.6% lipid, 5.5% protein and costing N60 per t. Thus, we need two other feed stuff, one high in protein and one low in protein level. Since square can solve for one specification, Square is used to solve protein and lipid levels are calculated for all possible combination of the remaining ingredients.

As fish meal contributes 5.5% protein, we will have 26% -5.5% to find from 90% of the diet. Therefore what we are looking for now is 90% diet having 20.5%X (100/90) = 22.78% protein.

Square for each possible 2 ingredient combination are:

a. Soybean 46.8 13/(13+24) x 100=35.1x90/100=31.62%

22.8

Maize germ 9.8 24/(13+24) x 100=64.9 x90/100=58.38%

b. Soybean 46.8 9.5/(9.5+24) x 100=28.36x90/100=25.52%

22.8

Rice 13.3 24/(9.5+24) x 100=71.64 x90/100=64.48%

c. Groundnut 34.5 13/(13+11.7) x 100=52.6x90/100=47.37%

22.8

Maize germ 9.8 11.7/(11.7+13) x 100=47.4x90/100=42.63%

d. Groundnut 34.5 9.5/(9.5+11.7) x 100=44.8 x90/100=40.3%

22.8

Rice 13.3 11.7/(9.5+11.7) x 100=55.2 x90/100=49.7%

Each of these protein base calculations matches up the desired lipid level and their effect on prize can be checked in the diet formulation worksheet in table 3.2 below

Table 3.2 : Diet formulation worksheet

	Ingredient	Inclusion rate	Inclusion cost (Nt^{-1})	Lipid contribution	Protein contribution
	Fish meal	10.00	60.00	0.60	5.50
A	Maize meal	58.40	105.12	2.63	5.72
	Soybean				

meal	31.60	154.84	0.41	14.79	
		100.00	**319.96**	**03.64**	**26.01**
	Fish meal	10.00	60.00	0.60	5.50
B	Rice meal	64.50	96.75	1.55	8.58
	Soybean meal	25.50	124.95	0.61	11.93
		100.00	**281.70**	**2.76**	**26.01**
	Fish meal	10.00	60.00	0.60	5.50
C	Maize meal	42.60	76.68	1.92	4.17
	Groundnut meal	47.40	165.90	6.49	16.35
		100.00	**302.58**	**9.01**	**26.03**
	Fish meal	10.00	60.00	0.60	5.50
D	Rice meal	49.70	74.55	1.19	6.61
	Groundnut meal	40.30	141.05	5.52	13.90
		100.00	**275.60**	**7.31**	**26.01**

The Trial and Error is a technique in which all the possible formulations are worked out and the one that is most preferred is selected. In this case, the requirements 7% lipid, 26% protein and cost of ingredients were considered. The last formulation is closest to the requirements and accidentally the cheapest, costing N275.60, having 7.31% lipid and 26.01% protein.

The problems of this method include choice of ingredient to be used, the requirements are not accurately met, all possible combinations must be worked out one after the other and least cost may be arrived at, accidentally.

Formulation by Trial and Error is time consuming and less precise which does not fit into the current situation in which formulation is a frequent exercise because of factors that affect fish feed requirements, which does not give room for resource wasting as a result of Trial and Error technique.

3.2.3 EQUATIONAL METHOD

It has not been understood that there is a linear relationship between mixing of ingredients to meet the targeted requirement. Thus, the limited reliability of Trial and Error method while formulating for more than one specification has led to the search for a definite method with 100 percent degree of precision, where all the specification are met. In addition, all the possible and impossible combination of ingredients that ordinarily are not visible as in Pearson Square and cannot be conjured by mere mental arithmetic can be arrive at through algebraic methods. Sadiku (2000) concluded that this is a great advancement in feed formulation-a major break through such that formulation becomes a matter of mere imagination.

The number of equations generated is equal to the number of specifications and the number of unknown equals number of ingredients. For two specifications using two ingredient, a simple simultaneous equation with two unknown is desired. For three specifications with three ingredients, a set of three simultaneous equations with three unknown is desired and so on. For example: To formulate a 100g diet of 30% crude protein and 300kcalg^{-1} energy from soybean meal, wheat middling and fish oil using table 3.3 as ingredient file.

Table 3.3 Ingredient file for formulating 30% crude protein and 300kcalg^{-1} energy diet.

Ingredient	Percent crude protein	Energy kcalg^{-1}
Soybean meal	48.00	3.22
Wheat middling	17.00	1.67
Fish oil	0.00	8.0

Source: Sadiku (2000)

Computation file for formulating 30% protein and 300kcalg^{-1} energy diet

SM + WM + FO = 100……..equation 1

0.48SM + 0.1WM + 0FO = 30……..equation 2

3.22SM + 1.6WM + 8.00FO = 300……..equation 3

Solve equation1 and equation3

SM+WM+FO=100……..equation1

3.22SM + 1.67WM + 8.00FO=300……..equation.3

To eliminate FO then multiply 8.00 by equation1

8.00SM + 8.00WM + 8.00FO=800……..equation4

3.22SM + 1.67WM + 8.00FO=300……..equation3

4.78SM + 6.33WM + 0=500

Subtract equation3 from equation4

4.78SM + 6.33WM + 0=500……..equation5

Solve quation2 and equation 5

0.48SM + 0.17WM=30……equation 2

4.78SM + 6.33WM=500……equation 5

To eliminate SM multiply equation2 by 4.78 and equation5 by 0.48

2.29SM + 0.81WM = 143 ……..equation6

2.29SM + 3.04WM = 240 ……..equation7

Subtract equation 7fro equation6

-2.23WM= -97 ……..equation 8

Divide the both side by -2.23

WM = -2.23/-2.23 -97/-2.23=

WM =43.5

Substitute value of WM in equation2

0.48SM + 0.17(43.5) + 0FO=30

0.48SM + 7.4 = 30

0.48SM = 30-7.4 = 22.6

0.48SM = 22.6

SM = 22.6/0.48 = 47.08

SM = 47.08

Insert value of SM & WM in equation3

3.22(47.08) + 1.67(43.5) + 8FO = 300

151.6 + 72.65 + 8FO = 300

224.25 + 8FO = 300

8FO = 300 - 224.25

8FO = 75.75

FO = 75.75/8 = 9.47

Table: 4 production file for formulation of 30% crude protein and 300kcal energy diet.

Ingredient	*Including rate	%Crude protein	**Crude protein contribution	Energy (Kcal/g)	**Energy Contribution (kcal/g)
Soybean	47.08	48.8	23	3.22	151.6
Wheat	43.50	17.0	7	1.67	72.6
Fish	9.47	0.0	0	8.00	75.8
	100		30		300.00

Key *Calculated **Checked

The problem with this technique is that it does not optimize and cannot take more than 3 equation, 3 unknowns.

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CHAPTER FOUR:

HYDROBIOLOGY (WATER QUALITY)

Dadi -Mamud Naomi John and Hamzat Aliyu

4.1 HYDROBIOLOGY

Hydrobiology is primarily an ecological science. The living conditions in water are determined by the physicochemical and characteristics of the body of water. Many of these features—for example, the chemical composition of the water (in particular, the composition and amount of biogenic elements and dissolved gases, the nature of bottom sediments, and the transparency of the water) are strongly influenced by aquatic organisms and are often determined by their life processes. Hydrobiology studies the role of living phenomena in the context of the aggregate of interdependent processes of the aquatic medium. Modern hydrobiology can be viewed as a sub-discipline of ecology but the sphere of hydrobiology includes taxonomy, economic biology, industrial biology, and physiology etc. Limnology and is divided into lotic system ecology (flowing waters) and lentic system ecology (still waters). One of the significant areas of current research is eutrophication. Special attention is paid to biotic interactions in plankton assemblage including the microbial loop, the mechanism of influencing water blooms, phosphorus load and lake turnover. Another subject of research is the acidification of mountain lakes. Long-term studies are called for changes in the ionic composition of the water of rivers, lakes and reservoirs in connection with acid rain and fertilization. Much of the early work of hydrobiologists concentrated on the biological processes utilizes in sewage treatment and water purification especially slow sand filters. Other historically important work sought to provide biotic indices for classifying waters according to the biotic communities that they supported. This work continues to this day in Europe in the development of classification tools for assessing waterbodies for the EU water framework directive Field of research interests. Hydrobiology also involved Marine biology and biological oceanography which is the study of marine organisms their behaviors and interactions with the environment and the associated fields of chemical, physical, and geological oceanography to understand marine organisms. As growing global population stresses the ability of our society to produce food, water, and shelter, there was to continue to look to the oceans to help sustain our basic needs. And advances in technology, combined with demand, to improve our ability to derive food, drinking water, energy sources, waste disposal, and transportation from the ocean.

It is important for the future generations to build upon our existing knowledge of the ocean and its potential to help meet the needs of the world and its inhabitants since hydrobiology is a very broad area, so most researchers can select a particular area of

interest and specialize in it. Specializations can be based on a particular species, group, behavior, technique, or ecosystem.

4.2 Introduction

Hydrobiology is greatly concerned with establishing a scientific basis for the rational exploitation of biological resources of the waters. This is bound up in many ways with the requirements of the marine and freshwater fishing industries, farm pond fishery, and the use of aquatic invertebrates and mammals (food-fish hydrobiology). Other practical applications of hydrobiology and stimuli to its development are the biological questions related to the use of the continental surface freshwater for drinking purposes and industrial supply, the protection of natural water against pollution, the self-purification of polluted water, and the biological methods of treating waste waters (sanitary hydrobiology). Hydro-biological methods are used to evaluate the extent of water pollution through the presence of certain indicator organisms (the so-called biological analysis of water quality). Hydrobiology studies the role of aquatic organisms as agents of self-purification. The concerns of technical hydrobiology are the related problems pertaining mainly to biological interference with the water supply and operation of ships apparatus and hydraulic structures, and the pipes and water supply lines of electrothermal power plants; the overgrowth of aquatic plants in reservoirs, and the damage to ships and port. New problems continue to arise, such as the need to determine the effect of plankton on the absorption and scattering of sound—information indispensable to specialists in underwater acoustics. Navigational hydrobiology is the study of biological interference (for example, bioluminescence) with naval activities; agricultural hydrobiology includes the study of the role of hydrobionts in the fertilization of rice paddies and the possibilities of fish breeding in these waters. The field of sanitary hydrobiology studies the effect upon aquatic organisms and their communities of the toxic substances in industrial effluent, the mechanism of the biological self-purification of water, and other matters relating to the pressing problem of supplying man's growing need for pure water.

Life on Earth depends upon water. Water comprises 70% of earth surface. It serves different functions ranging from its transport function through serving as solvent for most chemicals to serving habitat to many organisms. Many' organisms also depend on water for certain stages of their life. For instance, some insects and amphibians use water as their breeding sites while it serves as an agent of dispersal for many plant seeds and fruits. The biological diversity of aquatic areas is neglected world- wide, even in coral reefs that rival tropical rain forests in their extraordinary diversity of life Water is a critical issue for the survival of all living organisms. Some can use salt water but many organisms including the great majority of higher plants and most mammals must have access to freshwater to live. Some terrestrial mammals, especially desert rodents appear

to survive without drinking but they do generate water through the metabolism of cereal seeds and they also have mechanisms to conserve water to the maximum degree.

4.3 Water Quality Parameters

Water quality analysis is important to preserve and protect the natural ecosystem. A number of physico-chemical and biological methods have been carried out in water quality management. A study of different water parameters is very important for understanding of the metabolic events in aquatic ecosystem. The quality of surface water such as rivers, lakes and reservoir depend on their physical, chemical and biological prosperities. Water quality parameters were assessed in the determination of the water quality of Reservoirs. These parameters include:

Nitrate

Inorganic nitrate (NO_3-) and nitrite (NO_2-) are water soluble (as a result of their interaction with the positively charged portions of polar water molecules) and commonly exist as salts of nitric acid and nitrous acid, respectively. Nitrates are a form of nitrogen, which is found in several different forms in terrestrial and aquatic ecosystems. These forms of nitrogen include ammonia (NH3), nitrates (NO3), and nitrites (NO2). Nitrates are essential plant nutrients, but

in excess amounts they can cause significant water quality problems. Together with phosphorus, nitrates in excess amounts can accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in the types of plants and animals that live in the stream. This, in turn, affects dissolved oxygen, temperature, and other indicators. Excess nitrates can cause hypoxia (low levels of dissolved oxygen) and can become toxic to warm-blooded animals at higher concentrations (10 mg/L) or higher) under certain conditions. The natural level of ammonia or nitrate in surface water is typically low (less than 1 mg/L)

Nitrate generally, as a compound occurs naturally and also has many human-made sources. They are found in some lakes, rivers, and groundwater. You cannot taste, smell, or see nitrate in water. Consuming too much nitrate can be harmful. Nitrate is the most highly oxidized form of nitrogen compounds commonly present in natural waters. Other significant sources of nitrate are chemical fertilizers, decayed vegetable and animal matter, domestic effluents, sewage sludge disposal to land, industrial discharge and leachates from mines can contaminate stream, river, lake, and ground water. Unpolluted natural water contains minute amount of nitrate. Health effect of nitrate is can affect how blood carries oxygen and cause methemoglobinemia (also known as a blue baby syndrome). Bottle-fed babies under six months old are at the highest risk of getting methemoglobinemia. This illness can cause the skin to turn a bluish color and result in serious illness or death. (APHA, 2013).

pH

The pH of a solution is measured as negative logarithm of hydrogen ion concentration. At a given temperature, the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration. pH values from 0 to 7 are diminish acidic, 7 to 14 increasingly alkaline and 7 is neutral. Measurement of pH is one of the most important and frequently used tests, as every phase of water and wastewater treatment and waste quality management is pH dependent. The pH of natural water usually lies in the range of 4 to 9 and mostly, it is slightly basic because of the presence of bicarbonates and carbonates of alkali and alkaline earth metals. pH value is governed largely by the carbon dioxide/ bicarbonate/ carbonate equilibrium. It may be affected by humic substances, by changes in the carbonate equilibrium due to the bioactivity of plants and, in some cases, by hydrolysable salts, (WHO, 2004). The effect of pH on the chemical and biological properties of liquid makes its

etermination very important. It is used in several calculations in analytical work and its adjustment to an appropriate value is absolutely necessary in many of the analytical procedures. In dilute solution, the hydrogen ion activity is approximately equal to the concentration of hydrogen ion (Matos *et al.*, 2010).

Dissolved Oxygen (DO)

All living organisms are dependent upon oxygen in one form or the other to maintain the metabolic processes that produce energy for growth and reproduction. Aerobic processes which need free oxygen for wastewater treatment are of great interest. In a study titled Variation in concentration of dissolved oxygen and hydrogen concentration at the surface of tropical reservoir: a case study of lower Usuma reservoir in Bwari, Abuja Dissolved oxygen is required for respiration by most aquatic animals and apart from this, dissolved oxygen combined with other important elements such as Carbon, Sulphur, Nitrogen and Phosphorous that could have been toxicants in the absence of oxygen in the waterbodies to form carbonate, sulphate, nitrate and phosphate respectively that constitute the required compounds for aquatic organisms for survival (Araoye, 2008). Sensitivity to low levels of dissolved oxygen is species specific; however, most species of fish are distressed when DO falls to 2 mg/l. Mortality usually occurs at concentrations less than 2 mg/l and usually larger fishes are affected by low DO than smaller fishes. The depletion of oxygen in aquatic environment has many effects on biota particularly fishes which include mortality, reduced growth rate, impaired reproductive activity and also fish become more susceptible to diseases (Dankishiya *et al.*,2013).

Dissolved Oxygen (DO) is also important in precipitation and dissolution of inorganic substances in water. DO levels in natural waters and wastewaters depend on physical, chemical and biological activities in waterbody. The solubility of atmospheric oxygen in freshwater ranges from 14.6mg/L at 0°C to about 7.0mg/L at 35°C under normal atmospheric pressure (WHO, 2015). Since it is poorly soluble gas, its solubility directly varies with the atmospheric pressure at any given temperature. Analysis of DO

is a key test in water pollution control and wastewater treatment processes. The following illustrations reveal the importance of DO as a parameter:

i. It is necessary to know the DO level to assess quality of raw water and to keep a check on stream pollution.

ii. In wastewaters, dissolved oxygen is the factor that determines whether the biological changes are brought about by aerobic or anaerobic organisms.

iii. DO test is the basis of BOD test which is an important parameter to evaluate pollution potential of wastes

iv. DO is necessary for all aerobic biological wastewater treatment processes

v. Oxygen is an important factor in corrosion. DO test is used to control the amount of oxygen in boiler feed waters either by chemical or physical methods (APHA, 2013).

Hardness

Water hardness is a traditional measure of the capacity of water to precipitate soap. Hardness of water is not a specific constituent but is a variable and complex mixture of cations and anions. Total hardness is defined as the sum of the calcium and magnesium concentration, both expressed as $CaCO_3$, in mg/l. The degree of hardness of drinking water has been classified in terms of the equivalent $CaCO_3$ concentration as follows:

Soft	0-60 mg/L
Medium	60-120mg/L
Hard	120-180mg/L
Very hard	> 180mg/L

When total hardness is numerically greater than that of total alkalinity expressed as $CaCO_3$, the amount of hardness equivalent to alkalinity is called carbonate hardness. The amount of hardness in excess of total alkalinity expressed as $CaCO_3$ is non-carbonate hardness. Non-carbonate hardness is caused by the association of the hardness-causing cations with sulphate, chloride or nitrate and is referred to as "permanent hardness". This type of hardness, however, cannot be removed by boiling (APHA, 2014).

Biochemical Oxygen Demand (BOD)

The Biochemical Oxygen Demand (BOD) is an empirical standardized laboratory test which measures oxygen requirement for aerobic oxidation of decomposable organic matter and certain inorganic materials in water under controlled conditions of temperature and incubation period. The quantity of oxygen required for above oxidation processes is a measure of the test. The test is applied for fresh water sources (rivers, lakes), wastewater (domestic, industrial), polluted receiving water bodies, marine water (estuaries, coastal water)

and also for finding out the level of pollution, assimilative capacity of water as well as performance of waste treatment plant (APHA, 2014).

Therefore, biological oxygen demand represented the amount of biodegradable organic matter present in water. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic

life. The consequences of high BOD are the same as those for low dissolved oxygen: aquatic organisms become stressed, suffocate, and die. World Health Organization recommended a limit of less than 5.0 mg/l as ideal for water bodies.

Conductivity

Conductivity is the capacity of water to carry electrical current and it varies both with number and types of ions in the solutions which, in turn, is related to the concentration of ionized substances in the water. Most dissolved inorganic substances in water are in the ionized form and hence contribute to conductance (NIS, 2007). The higher the salinity and conductivity levels, the lower the DO levels in the water, which can cause issues for some aquatic plants and animals. Some aquatic life can tolerate salinity changes, however, most cannot, and will either die or become seriously sick.

Conductivity of water is important because **it tell how much dissolved substances, chemicals, and minerals are present in the water**. Higher amounts of these impurities will lead to a higher conductivity. Even a small amount of dissolved salts and chemicals can heighten the conductivity of water. Conductivity of water might indicate that a pollutant has entered the water. For drinking water, the electrical conductivity should be less than 1 mS/cm.

Phosphate (PO_4)

Phosphorous occurs in natural waters and in wastewater almost solely in the form of various types of phosphates. These forms are commonly classified into orthophosphates and total phosphates. These may occur in soluble form, in particles of detritus or in the bodies of aquatic organisms. Various forms of phosphate find their way into wastewater, effluents and polluted water from a variety of sources. Larger quantities of the same compounds may be added when the water is used or other cleaning, since these materials are major constituents of many commercial cleaning preparations. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm run-off and

to a lesser extent with melting snow. On the other hand, Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues. An analysis to determine the presence of phosphates in water and wastewater analysis has great significance. Phosphate in small concentration are used in water supplies to reduce scale formation; to increase carrying capacity of mains; to avoid corrosion in water mains; to remove iron and manganese in micro quantities and in coagulation especially in acid conditions. The presence of phosphate in large quantities in freshwaters, however, indicates pollution through sewage and industrial wastes. It promotes growth of nuisance causing micro-organisms. Though phosphate possesses problems in surface waters, its presence is necessary for biological degradation of wastewaters. Phosphorus is an essential nutrient for the growth of organisms and it helps for the primary productivity of a body of water (NIS, 2007).

Sodium

Sodium ranks sixth among the elements in order of abundance and is present in most natural water. The levels may vary from less than 1 mg Na/L to more than 500 mg Na/L. Relatively high concentrations may be found in brines and hard water softened by the sodium exchange process.

The ratio of sodium to total cations is important in agriculture and human pathology. Soil permeability can be harmed by a high sodium ratio. Persons afflicted with certain diseases require water with low sodium concentration. A limiting concentration of 2 to3 mg/L is recommended in feed water destined for high-pressure boilers. When necessary, sodium can be removed by the hydrogen-exchange process or by distillation. Sodium compounds are used in many applications including caustic soda, fertilizers and water treatment chemical (WHO, 2015).

Although it is generally agreed that sodium is essential to human life, there is no agreement on the minimum daily requirement. However, it has been estimated that a total daily intake of 120–400 mg will meet the daily needs of growing infants and young children, and 500 mg those of adults. . On the basis of existing data, no firm conclusions can be drawn concerning the possible association between sodium in drinking-water and the occurrence of hypertension. No health-based guideline value is therefore proposed. However, sodium may affect the taste of drinking-water at levels above about 200 mg/litre.

Potassium (K)

Potassium ranks seventh among the elements in order of abundance, yet its concentrations in most drinking water seldom reaches 100mg/L. Potassium is an essential element in both plant and human nutrition and it occurs in groundwater as a result of mineral dissolution (APHA, 2013).

Carbon dioxide (CO_2)

Aquatic vegetation and phytoplankton requires carbon dioxide for photosynthesis. It is produced as a result of respiration and decomposition. It gets dissolved in water and forms carbonic acid that affects water pH. Photosynthesis is the major cause for drain of carbon dioxide.

Heavy Metals

Studies on heavy metals in rivers, lakes, fish and sediments have constituted a major environmental focus especially during the last decade and heavy metals contamination of freshwater and sediment have been identified as a serious pollution resulting from industrialization. Protecting sediment quality is an important part of restoring and monitoring the biological integrity of our nation's water as well as protecting aquatic life, wildlife and human health. Heavy metals are produced from a variety of natural and anthropogenic sources. In fluvial environments, however, metal pollution can result from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products.

Cadmium (Cd^{2+})

Cadmium occurs in sulphide minerals that also contain zinc, lead or copper. The metal is used in electroplating, batteries, paint pigments and in alloys with various other metals. Cadmium is usually associated with zinc. It is highly toxic and has been implicated in some cases of poisoning through food. Minute quantities of cadmium are suspected of being responsible for adverse changes in arteries of human kidneys. It also generally causes cancers in laboratory animals and has been linked epidemiologically with certain human cancers. A cadmium concentration of 200μg/L is toxic to certain fishes. It may enter water as a result of

industrial discharges or the deterioration of galvanized pipe. The FAO-recommended maximum level for cadmium for irrigation water is 10μg/L/ (USEPA2012) drinking water standard for Cadmium is 0.005mg/L. BIS desirable limit is 1mg/L (WHO, 2004).

Chromium (Cr^{3+})

Chromium is found chiefly in chrome-iron ore. Chromium is used in alloys, in electroplating and in pigment. Chromium salts are used extensively in industrial processes and may enter a water supply through discharge of wastes. Chromate compounds frequently are added to cooling water for corrosion control. Chromium may exist in water supplies in both the hexavalent and the trivalents state although the trivalent form rarely occurs in potable water. The USEPA regulates total chromium in drinking water and has set a Maximum Contaminant Level of 0.1 mg/L. The World Health Organization (WHO) guideline is 0.05 mg/L for total chromium.

Copper (Cu^{2+})

Copper occurs in nature in its native state, the most important of which are those containing sulphide and those with oxides or carbonates. Copper salts are used in water supply systems to control biological growth of reservoirs and distribution pipe sand to catalyze in oxidation of manganese. Corrosion of copper-containing alloys in pipe fitting may introduce measurable amounts of copper into the water in a pipe system. FAO recommends maximum level for irrigation water is 200μg/L. USEPA drinking water standard for chromium and copper is 1 mg/L. permissible limit for copper is 0.05 mg/L (APHA, 2014).

Iron (Fe^{2+})

Iron occurs in minerals as hematite, taconite and pyrite. It is widely used in steel and other alloys. Elevated iron levels in water can cause stains in plumbing, laundry and cooking utensils and can impart objectionable taste and colour in foods.

Iron is an essential element for photosynthesis, DNA synthesis, and many other cellular functions for plants. With regard to fish, it is an integral component of proteins involved in cellular respiration and oxygen transfer. Aquaponic systems are often iron deficient due to low amounts of iron in commercial fish feeds Iron in water has many effects on aquatic life

both good and bad. Iron (Fe) occurs naturally in water at a rate of roughly 1-3 parts per billion (ppb) in ocean water, about 1 part per million (ppm) in river water and 100ppm in groundwater. Iron comes from various minerals in the soil, which is why groundwater contains the highest iron concentrations. Iron levels in water vary depending on several factors and can affect aquatic populations, behavior and health.

Iron is vital to the life of all aquatic creatures, especially mollusks and green plants. Iron promotes enzyme growth and gives blood its red color. Iron binds to oxygen and travels with it in the blood, transporting the carbon dioxide out. Green plants use iron for nitrogen binding. Phytoplankton, some of the smallest ocean creatures, depend so heavily on iron that the amount of iron present in water limits the amount of phytoplankton that can survive. At normal levels, iron is not deadly to any aquatic animals, but at higher levels when iron does not dissolve in water, fish and other creatures cannot process all the iron they take in from water or their food. The iron can build up in animals' internal organs, eventually killing them. Higher levels of iron in fish and aquatic plants also has negative effects on the people or creatures consuming them.

To protect freshwater aquatic life, the short-term maximum guideline for total iron **is** 1 mg/l and for dissolved iron is 0.35 mg/L.

Lead (Pb^{2+})

Lead in a water supply may come from industrial smelter discharges and mine or from dissolution of plumbing and plumbing fixture. Tap water that is not suitably treated may contain lead resulting from an attack on lead service pipes, lead interior plumbing, brass fixtures and fittings on solder pipe joints chiefly from galena (Pb). It is used in batteries, ammunition, solder, piping, pigments, insecticides and alloys. Lead also was used in gasoline for many years as an anti-knock agent in the form of tetraethyl lead.

Lead is a highly toxic metal in aquatic environment and its accumulation in fish tissues causes oxidative stress, neurotoxicity, and immune alterations (Lee *et al.* 2019). The WHO-recommended limit for lead levels in surface water is 0.01mg l.

Zinc (Zn^{2+})

Zinc is an essential and beneficial element for human growth. Its concentration above 5mg/L can cause a bitter astringent taste in water. The zinc concentration in water varies from 0.06 to 7.0 mg/L with a mean concentration of 1.33 mg/L. Zinc most commonly enters domestic water supply from deterioration of galvanized iron and dezincification of brass. In such cases lead and cadmium also may be present because they are impurities of the zinc used in galvanizing. Zinc in water also may result from industrial waste pollution.

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12

CHAPTER FIVE: ANIMAL MICRO-TECHNIQUES

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5.1 Animal Cell Culture

Animal cell culture is the processes by animal cells are grown in a favorable artificial environment. These cells are derived from multi-cellular eukaryotes with already established cell lines or established cell strains. In the mid-1900s, animal cell culture became a common laboratory technique, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century. Animal cell culture is now one of the major tools used in the life sciences in areas of research that have a potential for economic value and commercialization.

The development of basic culture media has enabled scientists to work with a wide variety of cells under controlled conditions; this has played an important role in advancing our understanding of cell growth and differentiation, identification of growth factors, and understanding of mechanisms underlying the normal functions of various cell types. New technologies have also been applied to investigate high cell density bioreactor and culture conditions. Many products of biotechnology (such as viral vaccines) are fundamentally dependent on mass culturing of animal cell lines.

At present, cell culture research is aimed at investigating the influence of culture conditions on viability, productivity, and the constancy of posttranslational modifications such as glycosylation, which are important for the biological activity of recombinant proteins. Biological produced by recombinant DNA (rDNA) technology in animal cell cultures include anticancer agents, enzymes, immunobiological [interleukins, lymphokines, monoclonal antibodies (mABs)] and hormones.

Animal cell culture has found use in diverse areas, from basic to advanced research. It has provided a model system for various research efforts:

a. The study of basic cell biology, cell cycle mechanisms, specialized cell function, cell-cell and cell-matrix interactions.

b. Toxicity testing to study the effects of new drugs.

c. Gene therapy for replacing non-functional genes with functional gene-carrying cells.

d. The characterization of cancer cells, the role of various chemicals, viruses, and radiation in cancer cells.

e. Production of vaccines, mABs, and pharmaceutical drugs.

f. Production of viruses for use in vaccine production (e.g., chicken pox, polio, rabies, hepatitis B, and measles).

5.1.1 Methods of Obtaining Cell Culture

There are three methods commonly used to initiate a culture from animals thus;

5.1.1.1 Organ Culture

Organ culture refers to a three-dimensional culture of tissue retaining some or all of the histological features of the tissue *in vivo*. The whole organ or part of the organ is maintained in a way that allows differentiation and preservation of architecture, usually by culturing the tissue at the liquid-gas interface on a grid or gel. There are disadvantages to organ cultures. Organs cannot be propagated so each piece of tissue can only be used once, which makes it difficult to assess the reproducibility of a response. And, of course, the particular cells of interest may be very small in number in a given piece of tissue so the response produced may be difficult to detect and quantify. It may not be possible to supply adequate oxygen and nutrients throughout the tissue because of the absence of a functioning vascular system, so necrosis of some cells occurs fairly rapidly.

5.1.1.2 Explant/Organotypic Culture

In explant culture, small pieces of the tissue of interest are simply allowed to attach to an appropriate substrate, usually one that has been coated with collagen, and are cultured in a rich medium, usually one containing serum. Following attachment, cell migration is promoted in the plane of the solid substrate. Traditionally, explants have been maintained in Maximov chambers in which cells are grown on coverslips sealed over a depression in a thick glass slide, and this approach is still in use. More recently, it has become common to use regular culture dishes, which are much more convenient since they do not need to be disassembled and reassembled at each feeding. One of the principal advantages of this method is that some aspects of the tissue's architecture can be preserved within the explant.

5.1.1.3 Dissociated Cell Culture

Cell culture refers to cultures derived from dissociated cells taken from the original tissue ('primary cell culture'). Cells are dispersed (mechanically and/or enzymatically) into a cell suspension which may then be cultured as a monolayer on a solid substrate, or as a suspension in the culture medium. These cultures have lost their histotypic architecture and often some of the biochemical properties associated with it. Cell cultures can be characterized and a defined population can be preserved by freezing. The most obvious advantage of cell culture, and of dissociated cell culture in particular, is

that it makes individual living cells accessible. All in all, primary dissociated cell cultures are particularly amenable to study using morphological and physiological techniques, which can be applied on a cell by cell basis. They are obviously less well suited to traditional biochemical approaches because the quantity of material obtainable from these cultures is usually limited and they contain a heterogeneous population of cells. One final drawback of working with primary cell cultures is that success is not automatic. Finding the conditions that permit good cell growth and maturation, getting culture to grow reproducibly, and documenting that you have accomplished all of this entails plenty of hard work.

5.1.2 History of cell culture media

Dawn of cultivation experiments (1882–1907): In 1882, Sydney Ringer developed Ringer's solution, a balanced salt solution of a composition that is close to that of bodily fluids, and successfully kept frog hearts beating after dissection and removal from the body. This was said to be the first instance of in vitro cultivation of animal tissue. Balanced salt solutions were developed one after another in the wake of Ringer's report. After the success of Ringer's solution, researchers began to pay attention to cells in culture devices and tried to maintain the cells. Nonetheless, the cells usually did not survive and rarely showed mitotic. In 1907, however, Ross G. Harrison successfully monitored an apparent outgrowth of nerve fibers of a frog for several weeks in lymph fluid that had been freshly drawn from the lymph sacs of an adult frog. This experiment is considered to be the beginning of animal cell cultivation.

Use of natural media (1907-): Alexis Carrel was a French surgeon and biologist who received the Nobel Prize in Physiology and Medicine in 1912 for his research on the vascular suture and transplantation of blood vessels and organs. He contributed greatly to tissue culture technology by devising a prototype of the cell culture flask that is used widely today and by establishing the aseptic manipulation technique. The first success of animal cell culture by Harrison inspired Carrel to send Montrose T. Burrows to work under Harrison's supervision in 1909. In 1912, Carrel demonstrated that the long-term cultivation of the cells that have been obtained from the connective tissues of chick fetuses is possible (for several months) with a periodic exchange of the medium. In 1913, he discovered that adding embryonic extract to blood plasma can dramatically increase cellular proliferation and extend the culture period of fibroblasts from the chick embryo heart.

Endeavors toward synthetic media (1911-): Margaret R. Lewis and Warren H. Lewis (1911) demonstrated that the Locke–Lewis solution—which is modified Locke's solution that additionally contains amino acids, bouillon, and glucose (or maltose)—is more effective for chick embryo cell cultivation than simple balanced salt solutions. They reported that glucose is especially important: if the concentration of glucose is not

sufficient in the medium, the chick embryo cells show vacuolar degeneration and die within a few days. In contrast, in their search for the active ingredients in embryonic extract, it was ascertained that the active substance is in the protein fraction and that the partially hydrolyzed proteins effectively promote the cell growth of chick embryo fibroblasts. They also confirmed the growth-promoting activity of amino acids and glutathione for chick embryo fibroblasts. They hypothesized that glutathione is required for the control of the redox environment during cell cultivation. Carrel's medium was supplemented with several natural products, such as casein digests (thymus-derived) nucleic acids, liver ash, and hemoglobin.

Birth of established cell lines (1940-): It is rare for healthy somatic cells that are derived from animals to acquire unlimited proliferative capacity during cultivation. They typically stop growing after a certain number of divisions (i.e. the Hayflick limit). In 1940, Wilton R. Earle et al. used carcinogens to successfully create immortal mouse fibroblasts (L cells), revealing that proliferation from a single cell is possible. In 1951, George O. Gey and his coworkers created an infinitely proliferating human cell line from a tissue of a patient with uterine cervical cancer (HeLa cells). Due to the emergence of these established cell lines, the sampling of cells from the tissues of animals in each experiment became unnecessary, enabling researchers worldwide to perform assays by using the same homogenous population of cells. This state of affairs made it easier to examine and to precisely quantify the subtle differences in the effects of culture media on cells. Thus, the development of culture media advanced rapidly as a result.

Establishment of basal media and research into protein-free media (1946-): Baker's medium and the other media that had been developed up to this point contained naturally derived components of unknown composition, including plasma, serum, bouillon, peptone, and tissue extracts. In order to find the crucial components in those natural materials and to develop defined media that are comparably efficient in the cultivation of cells, relative to the media containing natural ingredients, two main strategies were undertaken. The first strategy was to use dialyzed serum for the support of cells at minimum levels and to add defined components to maximize the proliferation of cells. The second strategy was not dependent on serum, or even proteins at all, and involved the formulation of media exclusively from definitive components. Fischer was a pioneer of the first strategy. He dialyzed blood plasma to remove the low-molecular-weight fraction. Culture media that were supplemented with dialyzed blood plasma could sustain cells only for a short period, indicating that the low-molecular-weight fraction was essential for the survival of cells. Then, he discovered that the amino acids are the key substance in the low-molecular- weight fraction.

Identification of serum substitutes and the development of serum-free media tailored to a cell type (1970-): Insulin was discovered earlier by Frederick Banting and Charles Best (1921), but full-scale research into this peptide as a supplement for culture media began in the 1960s. Initially, the effectiveness of insulin alone was found to be inferior to that of serum, but the use of insulin in combination with low-concentration serum

yielded a higher level of efficacy of baby hamster kidney (BHK) cell growth. This finding led researchers to conclude that insulin acts in a coordinated manner with serum components. Growth factors were discovered one after another during this era: nerve growth factor, epidermal growth factor, insulin-like growth factor, fibroblast growth factor (FGF), platelet-derived growth factor, and transforming growth factor (TGF). The addition of these growth factors to a culture medium increased cellular proliferation. Nevertheless, their effect on cell proliferation, as with insulin, was found to be almost always inferior to the effect of serum. Under these circumstances, in 1976, three key reports were published that accelerated the development of serum-free media. Ham's group discovered that a trace element of selenite is necessary for the serum-free cultivation of human diploid cells. Larry J. Guilbert and Iscove showed that, besides selenite, a combination of transferring and albumin is a good serum substitute. Izumi Hayashi and Gordon H. Sato discovered that a combination of several hormones and growth factors is an effective serum substitute. Prompted by their discoveries, attempts at serum-free culture by using serum substitutes (e.g. several hormones and growth factors, transferrin, and selenite) grew in number and a variety of serum-free media was developed, with each medium tailored to researchers' cell type of interest.

Improvements to basal media (1970-): In addition to being a source of hormones, growth factors, carrier proteins, and lipids, serum increases the levels of various low-molecular- weight compounds in basal media. As a result, traditional basal media from which serum is excluded were sometimes unable to adequately support cell growth. The report that the performance of DMEM, MEM, and Ham's F-12 as basal media in serum-free cultures is inadequate, but that the DMEM/F-12 medium, in which Ham's F-12 and DMEM are combined in a 1:1 ratio, shows better performance occasionally when used for certain types of cells. The reason seems to be the large number of constituents in Ham's F-12 and the high concentration of several nutritional constituents in DMEM: mixing the two allows each to complement the weaknesses of the other. Moreover, Murakami et al. reported that the RDF medium - a 2:1:1 mixture of RPMI 1640, DMEM, and Ham's F-12— yields more effective cell growth of hybridomas than does the DMEM/F12 medium. Mixed media, however, do not always show a level of performance that is better than that of a single medium. For example, the ferrous sulfate that is contained in Ham's F-12 is toxic to nerve cells and nerve cells proliferate more readily at a reduced osmolarity. Thus, DMEM alone is more effective than DMEM/F-12 in this case. Naturally, the composition of a basal medium that is used for serum-free culture should be optimized for each cell type. In addition, it seems that the optimization also depends on the scale of the culture and its method.

Medical and industrial applications of animal-cell culture technology (1978-): Inspired by the 1982 clinical application of recombinant human insulin expressed in *Escherichia coli*, researchers actively proceeded to produce growth hormones, interferon α, and other substances by using *E. coli* or yeast as a host. With *E. coli* and yeast, however, it was impossible to produce proteins with glycosylation. Animal cells thus started to be

used for the production of recombinant proteins, like tissue plasminogen activator, erythropoietin, interferon β, and monoclonal antibodies. The host cells that have been used in the manufacture of biopharmaceutical products include CHO cells, mouse myeloma NS0 cells. BHK cells, human embryonic kidney 293 cells, and human retinal cells. Among these, the CHO and NS0 cells have become especially popular in the field of biopharmaceutical manufacturing for the following reasons:

ü technological advances in mass-culture methods for these two cell lines;

ü sufficient knowledge about the safety of viruses that these two cell lines contain; and

ü remarkable advances in high-expression sub-lines that were derived from these two cell lines.

5.1.3 Applications of Animal Cell Culture

a) Model system: Cell cultures are used as model system to study the basic cell biology and biochemistry. To study the interaction between cell and disease-causing agents like bacteria. To study the effect of drugs. To study the process of aging and used to study triggers of aging. Nutritional study.

b) Cancer research: The difference between the normal and cancer cells can be studied using animal cell culture techniques. Normal cell can be converted into cancer cells by using radiation. Chemicals and viruses. The mechanisms and causes of cancer cells can be studied. Cell culture can be used to determine the effective drugs for selectively destroy only cancer cells.

c) Virology: Animal cell cultures are used to replicate the viruses instead of animals for vaccine production. Cell culture can be used to detect and isolate viruses. It can be used to study growth and development cycle of viruses. It can also be used to study the mode of infection.

d) Toxicity testing: Animal cell culture is used to study the effects of new drugs, cosmetics and chemicals on the survival and growth of a number of types of cell especially liver and kidney cells. Used to determine the maximum permissible dosage of new drugs.

e) Vaccine production: Used in the production of viruses and these viruses are used to produce vaccines. Vaccines such as polio, rabies, chicken pox, measles etc. are produced using animal cell culture.

f) Genetically engineered protein: Animal cell cultures are used to produce commercially important genetically engineered protein such as monoclonal antibodies, insulin, hormones etc.

g) Replacement tissue/organ: Animal cell culture can be used as replacement tissue or organs. E.g. artificial skin can be produced using this technique to treat patients with burns and ulcer.

h) Genetic counseling: Fetal cell culture from pregnant women can be used to study or examine the abnormalities of chromosomes, genes using karyotyping and these findings can be used in early detection of fetal disorders.

i) Genetic engineering: Culture animal cells can be used to introduce new genetic materials like DNA or RNA into the cell. It is used to study the expression of new genes and its effect on the health of the cell. Insect cells are used to produce commercially important proteins by infecting them with genetically altered baculoviruses.

j) Gene therapy: Culture animal cells can be genetically altered and used in gene therapy techniques or use of viral vector.

k) Drug screening and development: They are used to study cytotoxicity of new drugs. Used to find out effective and safe dosage of new drug. Cell –based assay plays an important role in pharmaceutical industry.

5.1.4 Basic Equipment and Facilities in Animal Cell Culture

1) **Sterile Work Area:** Where possible, a separate room is made available for clean cell culture work. The room should be free of through traffic and, if possible, equipped with an air flow cabinet which supplies filtered air around the work surface. A HEPA (High Efficiency Particle Air Filter) filtered air supply is desirable but not always affordable. Primary animal tissue and micro-organisms must not be cultured in or near the cell culture laboratory and the laboratory must be specifically designated for clean cell culture work. Clean laboratory coats should be kept at the entrance and should not be worn outside of this laboratory and brought back in. If strict sterility is needed, a laminar flow hood offers the best sterile protection available. If a hazardous chemical is to be handled a Class II Biohazard Cabinet which has a vertical laminar flow should be used. However, for primary cultures and also if no laminar flow hood or sterile room is available, an area for sterile work should be set aside, where there is no thoroughfare. If aseptic techniques are adhered to and the area kept clean and tidy, sterility can be easily maintained. All work surfaces, benches and shelves and the base of the airflow cabinets must be kept clean by frequent swabbing with 70% ethanol or an alternative disinfectant. If an airflow cabinet cannot be provided, the culture work may be done on a clean bench using a Bunsen burner to create a sterile 'umbrella' under which the work can be done.

2) **Incubation Facilities:** In addition to an airflow cabinet and benching which can be easily cleaned, the cell culture laboratory will need to be furnished with an incubator or hot room to maintain the cells at 30-40 °C. The incubation temperature will depend on the type of cells being cultivated. Insect cells will grow best at around 30 °C while mammalian cells require a temperature of 37 °C. It may be necessary to use an incubator which has been designed to allow CO_2 to be supplied from a main supply or gas cylinder so that an atmosphere of between 2-5% CO_2 is maintained in the incubator. In general, many cell lines can be maintained in an atmosphere of 5% CO_2:95% air at 99% relative humidity. The concentration of CO_2 is kept in equilibrium with sodium bicarbonate in

the medium. Different media have differing buffering capacity. If a CO_2 controlled incubator is not available, or cultures must be kept sealed in flasks (i.e., after treatment with some volatile substances), then cells may be maintained in flasks sealed after gassing with 5% CO_2:95% air, or vessels kept in boxes gassed and then sealed with pressure sensitive tape. In the case of boxes, the humidity must be maintained with a dish of water. Various media may be used so that a controlled CO_2 atmosphere is not required and in this case a CO_2 incubator is not necessary. Hepatocytes in primary culture are often maintained in Leibovitz L-15 medium which does not require a CO_2 atmosphere; however, flasks must not be sealed (as the hepatocytes require a high O_2 tension which is reduced with time in sealed ungassed vessels). Most cell lines are maintained at 36.5 °C, although some cultures, such as skin cultures may require lower temperatures. Cultured cells can generally survive lower temperatures, but rarely survive temperatures greater than 2 °C above normal, and therefore the incubator should be set to cut out at approximately 38.5 °C to prevent cell death. Incubators are designed to regulate an even temperature and this is more important than accuracy, i.e., temperature should be ±0.5°

3) **Refrigerators and Freezer (-20°C):** Both items are very important for storage of liquid media at 4°C and for enzymes (e.g., trypsin) and some media components (e.g., glutamine and serum) at -20°C. A refrigerator or cold room is required to store medium and buffers. A freezer will be needed for keeping pre-aliquoted stocks of serum, nutrients and antibiotics. Reagents may be stored at a temperature of -20°C but if cells are to be preserved it may be necessary to provide liquid nitrogen or a -70°C freezer.

4) **Microscopes:** A simple inverted microscope is essential so that cultures can be examined in flasks and dishes. It is vital to be able to recognize morphological changes in cultures since these may be the first indication of deterioration of a culture. A very simple light microscope with x100 magnification will suffice for routine cell counts with a hemocytometer, although a microscope of much better quality will be required for chromosome analysis or autoradiography work. A microscope with normal Kohler illumination will be needed for cell counting. An inverted microscope will also be needed for examining flasks and multi-well dishes from underneath. Both microscopes should be equipped with a x10 and a x20 objective and it may be useful to provide a x40 and a x100 objective for the normal microscope. Additional features such as a camera, CCD video camera, adapter and attachments and UV facility may also be required for some purposes.

5) **Tissue Culture Ware:** A variety of tissue culture plasticware is available, the most common being specially treated polystyrene. Although all tissue culture plasticware should support cell growth adequately, it is essential when using a new supplier or type of dish to ensure that cultures grow happily in it. The tests to ensure this, such as growth curves and time of reaching a confluent monolayer, are similar, to those used to ensure that serum batches are satisfactory. Cells can be maintained in Petri dishes or flasks (25 cm^2 or 75 cm^2) which have the added advantage that the flasks can be gassed and then

sealed so that a CO_2 incubator need not be used. This is particularly useful if incubators fail. Tissue culture ware is always chosen to match the procedure. Sometimes it may be necessary to condition a surface by pretreatment with 'spent' medium which has been used with another culture (conditioned medium). The choice of vessel depends on several factors: whether the culture is in suspension or grows as a monolayer; the cell yield; whether it needs CO2 or not; and what form of sampling is to be taken place. Cost can also be a limiting factor. Cell yield is proportional to available surface areas. It is important to ensure that an even monolayer can grow, especially in the currently popular multi-well dishes (24, 48 and 96 wells). For adherent cells to which histological stains may be applied, cover slips fitted into multi-well dishes which can be removed and treated with various organic solvent in staining are required. Commercially available multi-well slide-chamber dishes are also a convenient, but costly, alternative. Normal tissue culture ware is not resistant to organic solvents.

6) **Washing Up and Sterilizing Facilities:** Availability of a wide range of plastic tissue culture reduces the amount of necessary washing up. However, glassware such as pipettes should be soaked in a suitable detergent, then passed through a stringent washing procedure with thorough soaking in distilled water prior to drying and sterilizing. Pipettes are often plugged with nonabsorbent cotton wool before putting into containers for sterilizing. Glassware, such as pipettes, conical flasks, beakers (covered with aluminum foil) are sterilized in a hot air oven at 160 °C for one hour. All other equipment, such as automatic pipette tips and bottles (lids loosely attached) are autoclaved at 121 °C for 20 min. Sterilizing indicators such as sterile test strip is necessary for each sterilizing batch to ensure that the machine is operating effectively. Autoclave bags are available for loose items. Aluminum foil also makes good packaging material.

7) **Liquid N_2/Deep Freezer:** Invariably for continuous and finite cell lines, samples of cultures will need to be frozen down for storage. It is important to maintain continuity in cells to prevent genetic drift and to guard against loss of the cell line through contamination and other disasters. The procedure for freezing cells is general for all cells in culture. They should be frozen in exponential phase of growth with a suitable preservative, usually dimethylsulfoxide (DMSO). The cells are frozen slowly at 1 °C/min to -50°C and then kept either at -196°C immersed in liquid N_2 (in sealed glass ampoules) or above the liquid surface in the gas phase (screw top ampoules). Deterioration of frozen cells has been observed at -70°C, therefore, -196°C (liquid N_2) seems to be necessary. To achieve slow freezing rates a programmable freezer or an adjustable neck plug or freezing tray for use in a narrow-necked liquid nitrogen freezer can be used. Alternatively, ampoules may be frozen in a polystyrene box with 1" thick walls. This will insulate the ampoules to slow the freezing process to 1 °C/min in a -70°C freezer.

5.2 Preparation of Histological Sections

In order to prepare thin sections for examination by microscopy, it is necessary to preserve the tissues (fixation) and embed them in a supporting medium (such as paraffin wax or resins) prior to sectioning. Sections are usually stained in order to provide contrast.

5.2.1 Fixation

The process through which cell structure is preserved is called fixation. Since cells rapidly deteriorate after a tissue has been removed from the body, achieving adequate fixation is often the most difficult task confronting a histologist. "Artifacts" are changes to the original structure of cells and tissues that arise from tissue deterioration and from the fixation process itself. Thus, a skilled histologist employs techniques that minimize the formation of artifacts in different types of tissues, and has is the ability to distinguish artifacts from normal cell structures.

Fixation of tissue is done for several reasons. One reason is to kill the tissue so that postmortem decay (autolysis and putrefaction) is prevented. Fixation preserves a sample of biological material (*tissue* or *cells*) as close to its natural state as possible in the process of preparing tissue for examination. The fixative needs to preserve the tissues as close as possible to the living state. These fixatives have the ability to either stabilize or denature proteins.

The primary function of a fixative is to preserve the cellular structure of the tissue. Fixation is necessary to protect and harden the tissue against the deleterious effects of later procedures which otherwise would disrupt cellular structure beyond recognition. Furthermore, fixation minimizes a process called autolysis. Autolysis is the degradation of cellular structure which results from the release of degradative enzymes from the excised tissue itself.

There are varying types of fixatives such as chemical fixatives. A widely used example is formaldehyde, which has the advantage of being cheap and penetrates tissues rapidly. For better fixation, it is necessary to use pH buffers in the fixative. The most common fixative for light microscopy is 10% neutral buffered formalin (4% *formaldehyde* in *phosphate buffered saline*). For electron microscopy, the most commonly used fixative is *glutaraldehyde*, usually at a 2.5% solution in *phosphate buffered saline*. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of CH_2 (*methylene*) linkage, in the case of formaldehyde, or by a C_5H_{10} cross-links in the case of glutaraldehyde. Other fixatives often used for electron microscopy are *osmium tetr oxide* or *uranyl acetate*. Formalin fixation leads to degradation of mRNA, mRNA and DNA in tissues.

Frozen section fixation: this is a rapid way to fix and mount histology sections. It is used in surgical removal of *tumors*, and allow rapid determination of margin (that the tumor has been completely removed). It is done using a refrigeration device called a *cryo stat*. The frozen tissue is sliced using a *microtome*, and the frozen slices are mounted on a glass slide and stained the same way as other methods. It is a necessary way to fix tissue for certain stain such as antibody linked *immunofluorescence* staining. It can also be used to determine if a tumor is malignant when it is found incidentally during surgery on a patient.

5.2.1.1 Types of Fixations

There are generally three types of fixation process:

a. Heat fixation: After a smear has dried at room temperature, the slide is gripped by tongs or a clothespin and passed through the flame of a Bunsen burner several times to heat-kill and adhere the organism to the slide. This generally preserves overall morphology but not internal structures because it denatures the proteolytic enzyme and prevent autolysis.

b. Perfusion: forceful flooding of tissue. The fixative is injected into the heart with the injection volume matching cardiac output. The fixative spreads through the entire body, and the tissue doesn't die until it is fixed. This has the advantage of preserving perfect morphology, but the disadvantages that the subject dies and the cost is high (because of the volume of fixative needed for larger organisms)

c. Immersion: In this the sample of tissue is immersed in fixative of volume at a minimum of 20 times greater than the volume of the tissue to be fixed. The fixative must diffuse through the tissue to fix, so tissue size and density, as well as type of fixative must be considered. Using a larger sample means it takes longer for the fixative to reach the deeper tissue. Best in a slight vacuum.

5.2.2 Dehydration

Tissues fixed in aqueous solutions will maintain a high-water content, a condition that can be a hindrance to later processing. Except in special cases (freezing method, water-soluble waxes, and special cell contents), the tissue must be dehydrated (water removed) before certain steps in this processing can be successful. Dehydration is achieved using an ascending series of alcohols (70%, 95%, 100%).

5.2.3 Embedding

This is a process where dehydrated and cleared tissue are infiltrated with the embedding material. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatin, or wax) which is then hardened. The most

commonly used embedding or support medium is paraffin wax, with a melting point of about 56°C. The tissue is then transferred to molten paraffin wax (in an embedding oven) for a couple of hours. The tissue is then placed in a square or rectangular mold, and orientated in the required position, prior to adding hot wax to form a wax block. Embedding can also be accomplished using frozen, non-fixed tissue in a water-based medium. Pre-frozen tissues are placed into molds with the liquid embedding material, usually a water-based glycol, Cryogen, or resin, which is then frozen to form hardened blocks.

5.2.4 Microtomy

Sections of the tissue embedded in the wax block are cut on a machine, known as a microtome, using special knives (nowadays these are disposable). Typically, series or ribbons of sections are cut at a thickness of 6-8μm. The sections are transferred to the surface of a hot water-bath (where the sections flatten and lose any wrinkles). Sections are collected on glass microscope slides (standard dimensions of 3 x 1 inches). In order for the sections to adhere to the slides they are dried for up to 24 hours in a drying oven (at a temperature of about 40°C). This prevents sections falling off the slides in the later stages of preparation.

5.2.5 Staining

The most common staining technique is known as Hematoxylin and Eosin (H&E) staining. In order to stain the sections, the wax needs to be removed. This is done using a wax solvent such as xylene. The slide is then hydrated using a series of descending alcohols (100%, 95%, 70%) and then water. The slide is then immersed in Hematoxylin stain, rinsed in running water (preferably alkaline), followed by staining with Eosin, and rinsing in water.

Staining is an auxiliary technique used in *microscopy* to enhance contrast in the *micros copic* image. Stains and *dyes* are frequently used in *biology* and *medicine* to highlight structures in *biological tissues* for viewing, often with the aid of different *microscopes*. Stains may be used to define and examine bulk tissues (highlighting, for example, *muscle fibers* or *connective tissue*), *cell* populations (classifying different *blood cells*, for instance), or *organelles* within individual cells. Biological staining is also used to mark cells in *flow cyto metry*, and to flag *proteins* or *nucleic acids* in *gel electrophoresis*. *In vivo* staining is the process of dyeing living tissues—in vivo means "in life" (compare with *in vitro* staining). By causing certain cells or structures to take on contrasting colour(s), their form (morphology) or position within a cell or tissue can be readily seen and studied. The usual purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells or tissues.

In vitro staining involves colouring cells or structures that are no longer living. Certain stains are often combined to reveal more details and features than a single stain alone. Combined with specific protocols for *fixation* and sample preparation, scientists and physicians can use these standard techniques as consistent, repeatable diagnostic tools. A *counterstain* is stain that makes cells or structures more visible, when not completely visible with the principal stain.

The different types of staining techniques are:

i. *Haematoxylin and eosin staining* protocol is used frequently in *histology* to examine thin sections of tissue. *Haematoxylin* stains cell nuclei blue, while *eosin* stains cytoplasm, connective tissue and other extracellular substances pink or red. Eosin is strongly absorbed by *red blood cells*, colouring them bright red. In a skillfully made H & E preparation the red blood cells are almost orange, and collagen and cytoplasm (especially muscle) acquire different shades of pink. When the staining is done by a machine, the subtle differences in eosinophilia are often lost. Hematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of hyaline cartilage) blue. In contrast, eosin stains the cytoplasm and collagen pink. The Hematoxylin is a basic dye that stains acidic components of cells a blue color. This characteristic is known as basophilia. Hematoxylin stains the nuclei of cells, and the RER of the cytoplasm. Eosin is an acidic dye that stains the basic components of the cells a reddish-pink color. This characteristic is known as acidophilia. Most of the cytoplasm of cells is stained by eosin. Bone matrix is also stained by eosin.

ii. *Papanicolaou staining*, or Pap staining, is a frequently used method for examining cell samples from various bodily secretions. It is frequently used to stain *Pap s mear* specimens. It uses a combination of *haematoxylin*, *Orange G*, *eosin Y*, *Light Green SF y ellowish*, and sometimes *Bismarck Brown Y*.

iii. PAS staining - *Periodic acid-Schiff* staining is used to mark *carbohydrates* (*glyc ogen*, *glycoprotein*, *proteoglycans*). It is used to distinguish different types of glycogen storage diseases. PAS is a widely used staining technique that stains the neutral sugars of glycosaminoglycans a pink color. Common components stained positively with PAS include mucus, the basal lamina and glycogen.

iv. *Masson's trichrome* is (as the name implies) a three-colour staining protocol. The recipe has evolved from Masson's original technique for different specific applications, but all are well-suited to distinguish cells from surrounding *connective tissue*. Most recipes will produce red *keratin* and muscle fibers, blue or green staining of *collagen* and *bone*, light red or pink staining of *cytoplasm*, and black *cell nuclei*.

v. The *Romanowsky stains* are all based on a combination of eosinate (chemically *reduced eosin*) and *methylene blue* (sometimes with its oxidation products *azure A* and *azure B*). Common variants include *Wright's stain*, *Jenner's stain*, *Leishman stain* and *Giemsa stain*. All are used to examine *blood* or *bone marrow* samples. They are preferred over H&E for inspection of blood cells because different types of *leukocytes* (white blood

cells) can be readily distinguished. All are also suited to examination of blood to detect blood-borne parasites like *malaria*.

vi. Silver staining is the use of *silver* to stain *histologic sections*. This kind of staining is important especially to show *proteins* (for example type III *collagen*) and *DNA*. It is used to show both substances inside and outside *cells*. Silver staining is also used in *te mperature gradient gel electrophoresis*. Some cells are argentaffin. These *reduce* silver solution to metallic silver after *formalin fixation*. This method was discovered by Italian *Camillo Golgi*, by using a reaction between *silver nitrate* and *potassium dichromate*, thus precipitating silver chromate in some cells (see *Golgi's method*). Other cells are argyrophilic. These reduce silver solution to metallic silver after being exposed to the stain that contains a *reductant*, for example *hydroquinone* or formalin.

vii. *Sudan staining* is the use of Sudan dyes to stain sudanophilic substances, usually *lipids*. *Sudan III*, *Sudan IV*, *Oil Red O*, and *Sudan Black B* are often used. Sudan staining is often used to determine the level of *fecal fat* to diagnose *steatorrhea*.

viii. Orcein staining is used to stain elastic fibers a dark brown-purple color. This is used, for example, to show the elastic components in the walls of arteries, or in the matrix of elastic cartilage.

ix. Oil Red O is used to stain lipids a red-orange color in unfixed frozen sections.

a. Toluidine blue is a so-called metachromatic stain. It is a blue stain that stains specific components of tissues a purple color. This change in staining color is known as metachromasia. Metachromasia is seen in the matrix of hyaline cartilage, or in the granules of mast cells.

b. Impregnation is a staining technique in which blocks of tissue are processed in solutions containing metals such as silver or gold, which attach to specific

c. components in tissues. The silver or gold are then further processed (reduced) and develop into dark metallic deposits. The stained blocks are only then sectioned. Silver impregnation is widely used in neurohistology to stain neurons and their processes. Silver impregnation techniques are also widely used to demonstrate reticular fibers.

5.2.6 Paraffin Method of Section Preparation

This method is most and widely employed. Although this technique is not universally applicable, e.g. it does not work well with hard tissues such as bones from animals, it does present many advantages over alternative methods. The necessary reagents are inexpensive, readily available, and much less toxic to humans than those used in most other techniques. The steps for the method are;

a. Tissue Resection & Fixation

This occurs when an animal is sacrificed to remove (resect) certain organs (such as lungs, kidneys and liver). The ways in which for instance rodents can be sacrificed

ethically includes by inhalation of carbon dioxide, methoxyflurane, or halothane. If exposure to chemicals contradicts the objectives of the investigation, cervical dislocation (rupturing of the spinal column in the neck) can be performed. Or the use of a rodent guillotine to decapitate sedated mice. This is instantaneous and painless, and allows bleeding out of the blood, which otherwise fills the body cavity during dissection. It also allows the organs to drain of blood that might interfere with an analysis of organ-specific proteins. The fixation process must start as soon as possible after resection of the sample by

1. Labeling the tissue cassettes in pencil as "lungs"
2. Fill a vial about 2/3 full with the fixative.
3. Remove the organ from the rodent (o.5g may be appropriate) by placing on a Petri plate chilled in ice bucket. Then transfer it into cryostorage vial and store on ice until transferring it to the -80^{O}C freezer.

b. Dehydration

After fixation, the water must be removed from the tissue block, a process called dehydration. Isopropyl alcohol (IPA) is a favored reagent because it is miscible in paraffin. The tissue must not be dehydrated rapidly because this will cause distortion of the tissue. Rather, dehydration is carried out in a slow, step-wise manner by passing the tissue block through a series of solutions of increasing IPA concentration. In this way the water is fully leached out and replaced with IPA.

a. 70% IPA for 1 hr
b. 70% IPA for 1 hr
c. 85% IPA for 1 hr
d. 95% IPA for 1 hr
e. 100% IPA for 1 hr
f. 100% IPA for 1 hr

c. Infiltration and Embedding in Paraffin

Prior to sectioning, the tissue block must be infiltrated with a material that acts as a support during the sectioning process. For the method described here, paraffin serves this purpose. During infiltration, the paraffin will equilibrate within the tissue block, eventually occupying all of the space in the tissue that originally held by IPA. After infiltration, the tissue is allowed to solidify in a mold, embedded within a small cube of paraffin. Infiltration involves;

a. Discarding the 100% IPA from the last dehydration step, and fill the vial about 3/4 full with melted paraffin.

b. Allow the tissue to equilibrate for 1 hour in an incubator set at 58^{O}C. Equilibration means to allow a solution to reach a stable concentration within a tissue. Thus, for example, after 1 hour the IPA will have reached 70% within the tissue block.

c. Pour the paraffin into the container labeled for paraffin disposal.

d. Repeat step 'a' using fresh melted paraffin.

Embedding involves;

a. Placing a base-pieces for two embedding molds in a plastic Petri plate – label the plate along the edge appropriately

b. Decant the paraffin from the second infiltration step into the waste container

c. Working quickly but carefully, use forceps to transfer the tissue blocks to the well of separate base mold, snap the base of tissue cassette into the base mold and then fill the mold with paraffin.

d. Allow the paraffin to solidify at room temperature (if the paraffin begins to solidify homogeneously around the tissue block, allow the paraffin in the base mold to melt in the incubator, and then allow it to solidify).

5.2.7 Sectioning with a Microtome

This is accomplished by using a cutting apparatus called a microtome. The microtome will drive a knife across the surface of the paraffin cube and produce a series of thin sections of very precise thickness. The objective is to produce a continuous "ribbon" of sections adhering to one another by their leading and trailing edges. The thickness of the sections can be preset, and a thickness between 5 - 10 μm is optimal for viewing with a light microscope. The sections can then be mounted on individual microscope slides. Preparation and mounting of the embedded tissue block on the microtome is very important to successful sectioning. The paraffin surrounding the tissue block must be first trimmed, and then secured to a holder which is then mounted on the microtome.

5.2.8 Mounting on Microscope Slides

This is permanently attaching the section to microscope slides. If "serial" sections are desired, (i.e., sections that reveal sequential layers of the tissue structure) then sectioning must be performed carefully and systematically. Label the microscope slides appropriately. Wash the microscope slides with soap and water, and rinse free of soap with tap water. Place the slides in a coplin jar and rinse several times with roH_2O. Handling the slides only by their edges, place the slides in your slide storage box, and allow to dry.

However, note that during sectioning the sections are not perfectly flat, but rather slightly crinkled. This is normal, and the sections will become flattened by floating them on water held at 45^{O}C. The solution also contains an adhesive, Surgipath/Leica, which causes the tissue section to bind to the slide. Carefully transfer the sections to a solution held in a 45^{O}C water bath. Within a few seconds sections flatten and the wrinkles disappear. Dip a clean microscope slide into the adhesive solution, and slowly pull it upward, out of the solution, allowing sections to adhere to the surface. Make sure that the slide is oriented with the label facing upward. Dry the bottom of the slide and carefully blot excess adhesive from around the sections (be careful not to touch the sections themselves). Allow the slides to dry overnight in the storage box.

5.2.9 Clearing and Staining

Before a section can be stained the paraffin must be removed, a process called clearing. After clearing, only the tissue remains adhering to the slide. Clearing is accomplished by passing the mounted sections through the solvent Clearene (Surgipath/Leica) that dissolves the paraffin. Staining of histological sections allows observation of features otherwise not

distinguishable. For routine histological work, it is customary to use two dyes, one that stains certain components a bright color and the other, called the counterstain that stains other cellular structures a contrasting color. While literally hundreds of staining techniques have been developed, the two stains most widely used for routine work are hematoxylin and Eosin Y. Hematoxylin stains negatively charged structures, such as DNA, a blue color. Eosin imparts a red color to most of the other cell components. To produce permanent staining with hematoxylin, the dye must be oxidized to "hematein", which is achieved by treating the tissue sections with Scott's solution. Clear and stain your slides with the following schedule of solutions held in Coplin jars:

a. Clearing and Rehydration: Clearing agent 1 for 3minutes; Clearing agent 2 for 2minutes and Clearing agent 3 for 1minute. 100% IPA for 30seconds; 85% IPA for 30seconds; 70% IPA for 30 seconds and Tap water for 30 seconds

b. Staining: Hematoxylin for 2 minutes; Tap water for 30 seconds; Scott's solution for 1 minute; Tap water for 30 seconds; Buffer for 1 minute; Tap water for 30 seconds; 70% IPA for 1 minute; 95% IPA for 1 minute; Eosin Y for 1 minute.

c. Rinsing, Rehydration & Mounting Prep 95% IPA for 2-3 minutes; 100% IPA for 2-3 minutes; Clearing Agent for 1 minute; Clearing Agent for 1 minute and Clearing Agent for 1 minute

5.2.10 Preparation of permanent mounts

This is the final step in this procedure where the mount section is under a coverslip. This is accomplished by covering the section in a medium that will harden and produce a clear binder between the slide and cover slip. The ideal mounting medium should not distort the stain color, or yellow and become brittle with age. Thus, the use of mounting resin called Permount (Fisher Scientific). The procedure is;

a. Place 2-3 drops of resin over the section.

b. To avoid entrapping air bubbles, lower the cover slip slowly from one side of the droplet.

c. Place the slide on the slide warmer and carefully place a lead weight on top of the cover slip. There should be enough mounting medium to completely cover the bottom of the cover slide, and budge slightly around the edges.

d. Leave slides on the warmer for at least 24 hours; excess medium can then be cut from edges of cover slip with a razor blade.

e. Do not allow slides to dry before mounting under cover slides.

5.3 Preparation of Histological Sections of Bone

Because bone tissue is hard and calcified, special histological techniques are used to prepare sections.

i. Decalcification: The most common techniques involve calcium removal from the tissue (decalcification) after fixation and prior to wax embedding. Acids, such as formic acid or nitric acid, can be used as decalcifying agents. After decalcification the tissue is soft and can be embedded and processed as in standard histology. It is also possible to use chelating agents, such as EDTA, which specifically bind calcium. These chelating agents are less damaging to the tissue than acids, but the decalcification process may be quite long (several weeks or more).

ii. Ground sections: It is possible to grind the bone until the sample is sufficiently thin for histological observation. The cells and organic tissue are destroyed in such preparations, though the canaliculi and cell lacunae are well seen. (Similar techniques are used by geologists to prepare thin sections of rock samples).

iii. Sections of non-decalcified bone: It is possible to embed bone tissue in a hard resin and section it with special knives (tungsten-carbide). Small samples for electron microscopy can be cut with diamond knives.

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CHAPTER SIX:

ETHICS AND INTELLECTUAL PROPERTY IN BIOTECHNOLOGY

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6.1 Biotechnology as an innovation

'Bio' refers to life and 'technology' refers to the application of information for practical use, i.e. the application of living organisms to create or improve a product. "Biotechnology" encompasses a variety of techniques, such as selecting natural strains of organisms that carry desirable traits, making hybrids by fusing cells from different parental sources, using chemicals and radiation to create mutant strains, or genetically engineering plants, animals, and micro-organisms to contain specific phenotypic characteristics. At its most general level, biotechnology concerns techniques for using the properties of living things to make products or services. Biotechnology consists of 'the controlled employment of biological agents, e.g. micro-organisms or cellular components, for favorable use'. Biotechnology has been defined as 'Janus-faced' implying that there are two sides to it. On one side, we know that the technology allows DNA to be modified so that genes can be moved from one organism to another. On the other, it also entails comparatively new techniques whose results are untested and should be met with care. Biotechnology is 'the integrated use of biology, microbiology, biochemistry and technology in production or as service operation'. Biotechnology is the commercial employment of micro-organisms and living plant and animal cells to create substances or effects beneficial to people. It includes the production of antibiotics, vitamins, vaccines, plastics, etc. It involves the industrial application of living organisms or their products, which entails the intentional manipulation of their DNA molecules. It may mean making a living cell execute a particular task in a predictable and controllable way. The term biotechnology is occasionally also applied to processes in which micro-organisms such as yeasts and bacteria are cultured under strictly controlled environmental conditions. For this reason, fermentation is occasionally called the oldest form of biotechnology. Genetic engineering techniques are frequently, but not always, used in biotechnology. The Universities Press Dictionary of Biology defines biotechnology as 'the application of technology to biological processes for industrial, agricultural and medical purposes. The Oxford Dictionary of Biology defines biotechnology as 'the

develop development of techniques for the application of biological processes to the production of materials of use in

medicine and industry.' The employment of cells and biological molecules to explain problems or make valuable products. These biological molecules include DNA, RNA and proteins. Biotechnology may be defined as 'the utilization of living organisms in systems or processes for the production of valuable products; it may involve algae, bacteria, fungi, yeast, cells of higher plants and animals or subsystems of any of these or isolated components from living matter'.

In summary, biotechnology is the 'application of the theory of technology and biological science to generate new products from raw materials of biological origin, e.g. vaccines or food', or, in other words, it can also be defined as 'the exploitation of living organism/s or their product/s to change or improve human health and its surroundings'.

An innovation is something that is new. It includes new products as well as new processes. There are two types of innovations; primary innovation (it is one that makes a major advance in a new area; it makes it possible to generate other innovation; an example is the ability to create organisms with new genetic information) and secondary innovation (is one that build on a primary innovation; having a variety of genetically altered plants; methods have been created to enhance the process, such as the development of the gene gun).

Innovations may be from; efforts of highly trained lab researchers; technicians striving to solve practical problems; agricultural producers experimenting with ways to improve their efficiency; businesses seeking new entrepreneurial opportunities; people who have experienced major losses and are seeking solution; consumers who want something better and government funded efforts to solve problem. Innovations lead to new technology.

6.2 Issues and Bioethics

Biotechnology has a wide array of applications. Because biotechnology often involves working with living organisms, issues and ethical questions may arise. Some people believe it is unsafe and unethical to change the genetics of an organism using biotechnology. Others believe that people have the ethical obligation to find ways to improve the quality of life for all people.

6.2.1 Identifying an Issue

An issue is a subject or problem that has more than one point of view. They are sometimes stated as questions. Areas where issues in biotechnology are likely include: changing the genetic material of an organism using artificial means; planting genetically modified crops; raising genetically modified animals; consuming foods from genetically modified plants and animals; using substances that alter or enhance the normal processes

of plants and animals; maintaining genetic diversity in wild plants and animals; taking drugs produced through genetic modification and injecting foreign genes into the human body to promote good health. The two most controversial areas in biotechnology are genetic engineering and cloning of organisms. A clone is grown from a single somatic cell of its parent. Somatic cells are the cells that compose tissues, organs, and parts of the body, other than germ cells, which make up the reproductive system in organisms.

6.2.2 Ethics and Biotechnology

Ethics is set of principles that guide human actions. Some people refer to ethics as the rule of appropriate human conduct. Ethics deals with moral principles and values. Our ethics focus on the rightness or wrongness of particular actions. It is difficult to set specific ethical rules. This is because ethics relates to personal judgment and how a person thinks in terms of morals, culture, and/or religion.

Bioethics is the area of ethics that deals with the life sciences. It also includes political and social areas. It is recognized as an important area in the acceptance of biotechnology. For instance, the human genome project has allocated 3 percent of its funds to bioethics and associated issues. Ethic in science can be divided into three areas: scientific misconduct, questionable research practices and other misconduct.

However, because biotechnology deals with genes in living organisms, the ethical implications go even further. Using the wrong protocol and making inaccurate observations violate science ethics. Purposefully reporting data that are false or fabricated is beyond ethics and is a criminal activity.

6.2.3 Specific Ethical Issues

The Human Genome Project: the human genome project involves the mapping of all genes in humans. The project has the following goals; identify all genes in human DNA (80,000 to 100,000); determine the sequences of the chemical base pairs; maintain database of the information; develop methods of data analysis and study ethical issues associated with human genome information and uses. One outcome of the project is the possibility that disease will be detected before it occurs. The disease could then be avoided or treated. The advantages linked to the project therefore, seems tremendous. The ethical issues surrounding the project is addressed by creating: ELSI. ELSI is the ethical, legal and social implication programme. It provides specific guidelines for those involved with the project.

Cloning: the cloning of sheep or cattle is seen as a way to increase production or gain products more efficiently. Therefore, cattle producing an abundance quality of beef would be interesting to clone. It might have the advantage of testing pharmaceutical compounds. Testing drugs on clones would remove some interference. However, the

fear of cloned animal is that it can adversely affect the diversity of species. The main ethical issue associated with cloning is the possibility of cloning human beings. Besides, the morals and religious aspects, there is the fear of eugenics. Eugenics is the science of altering the hereditary qualities of a species or breed. Therefore, there is a general consensus among biotechnology scientists that human beings should not be cloned. Much debate focuses on the cloning of human embryo. An embryo is an animal in the early stage of growth and differentiation. The study of embryo could lead to a better understanding of diseases, such as cancer and Parkinson's. Some culture and religions see embryo as human beings, while others consider life to begin only after birth. Cloning embryo could therefore, be perceived as cloning human beings.

Genetic Engineering (Genetic Modification): it is the process that uses laboratory-based technologies to alter the DNA make-up of an organism. The barrier between species does not represent a problem with this technology. People could argue against genetic engineering on a moral or religious base. Crops genetically modified to carry herbicides or pest resistance are the products of long and costly years of development. Companies that develop these plants apply for a patent. A patent grants an inventor the exclusive right to make, use or sell the invention. Once a patent has been delivered for a specific genetically engineered crop, only the company that owns the patent can sell or license the crop. Anyone wanting to use the crop has to pay the company. Companies might genetically engineer crops so the seed are sterile. This means that the seed could not be saved for next growing season. The seed would have to be bought again the following year. In a biotechnology world, only those who could afford the modified crop seed would be able to grow the crop. This is a major issue as it relates to poor nations that might need a genetically modified crop for their survival, but cannot afford it.

6.2.4 Issues in Biosafety

Biosafety describes the principles, procedures and policies to be adopted to ensure the environmental and personal safety. Biosafety refers to containment principles, technologies and practices that are required to prevent unintentional exposure to pathogens and toxins, or their accidental release into the environment. Biosafety describes the principles, technologies and practices that are implemented to prevent the exposure of laboratory workers to microorganisms. A fundamental objective of any biosafety program is the containment of potentially harmful biological agents, toxins, chemicals or radiation etc. With the increasing emphasis on adoption of genetic engineering technique in different countries in their life science research and development activities, the biosafety issues are gaining importance to ensure safety of the public and the environment. Biosafety is not only a personal requirement but essential collective efforts to ensure biological safety for a clean and safe environment. This certainly requires awareness among the public along with rules, regulations, monitoring bodies etc. Awareness among the researchers is must so that biological safety

can be well taken care at the grass root level. Recognizing the need of biosafety in genetic engineering research and development activities, an international multilateral agreement on biosafety "the Cartagena Protocol on Biosafety (CPB)" has been adopted by 167 parties, including 165 United Nations countries, Niue, and the European Union. The Protocol entered into force on 11 September 2003, and its main objectives are to set up the procedures for safe trans-boundary movement of living modified organisms, and harmonize principles and methodology for risk assessment and establish a mechanism for information sharing through the Biosafety Clearing House (BCH).

6.2.5 Why Biosafety?

a. With the increasing number of countries adopting molecular tools and techniques in their life science research and development activities especially in the areas of agriculture and medicine, the biosafety issues are gaining importance to ensure biological safety for the public and the environment.

b. To conduct research in a safer manner is not only a personal requirement but essential collective efforts to ensure biological safety for a clean and safe environment.

c. This certainly requires rules, regulations, monitoring bodies and awareness among the public. Thus, biosafety *per se* is an integral part of the laboratory research,

requires awareness among the researchers so that biological safety can be well taken care at the grass root level.

d. GMOs have got commercial applications in agriculture and healthcare industry, often for a better value and quality of the products. However, there are key differences between these two sectors. Healthcare industry is a highly regulated and the products are generally life-saving drugs where certain minor risks can be easily compromised with the life-saving benefits. With the adoption of GM crops in 29 countries grown over 160 million hectares world over, the concerns associated with GMOs are widely discussed. However, the concerns differ greatly, depending on the particular gene-organism combination. Therefore, a case-by-case approach would be required for assessment of the associated biosafety concerns. The safety concerns in agriculture not necessarily associated with the characteristics of the products but the way it is produced, particularly in case of human food.

e. The key players of the healthcare industry (medical practitioner, drug industry and the regulatory authorities) are often well aware of the latest biosafety concerns.

f. The GMOs and the purified products are handled under contained environment. Thus, there is minimized public concern associated with the use of GMOs in healthcare industry.

g. On the other hand, agriculture deals with the crop and animal husbandry; protecting them from insect pests and diseases; improving their taste, quality and acceptability to the consumers, and studying their nutritional and associated effects.

6.2.6 Steps Forward

a) Though there has been increasing awareness about biosafety all over the globe, there is lot to be understood and followed in laboratory research, particularly at grass-root level in the developing countries.

b) Many countries have put into place effective regulatory procedures that are much more rigorous for GM than non-GM food.

c) However, formulation of basic guidelines with multilevel regulatory bodies is required for compliance of biosafety measures.

d) Though biosafety regulatory bodies may exist, their effective functioning is a big question in many of the countries using GMOs and their produc

e) The need of the day is to strengthen internal regulatory body and create awareness among the research mangers, scientists and students about the biosafety requirements.

f) The internal regulatory body at research institute level such as an Institutional Biosafety Committee (IBSC) or its equivalent body consisting of experts from different relevant disciplines.

6.3 Intellectual Property Right/Patent

Intellectual property rights refer to the legal rights given to the inventor or creator to protect his invention or creation for a certain period of time. These legal rights confer an exclusive right to the inventor/creator or his assignee to fully utilize his invention/creation for a given period of time. It is very well settled that Intellectual Property plays a vital role in the modern economy. It has also been conclusively established that the intellectual labor associated with the innovation should be given due importance so that public good emanates from it.

Intellectual Property Right is a strong tool, to protect investments, time, money, effort invested by the inventor/creator of an Intellectual Property, since it grants the inventor/creator an exclusive right for a certain period of time for use of his invention/creation. Intellectual Property Right enhances technology advancement in the following ways:

a. It provides a mechanism of handling infringement, piracy, and unauthorized use

b. It provides a pool of information to the general public since all forms of Intellectual Property are published except in case of trade secrets.

c. Intellectual property protection can be sought for a variety of intellectual efforts including:

a) Patents

b) Industrial designs relate to features of any shape, configuration, surface pattern, composition of lines and colors applied to an article whether 2-D, e.g., textile, or 3-D, e.g., toothbrush and

c) Trademarks relate to any mark, name, or logo under which trade is conducted for any product or service and by which the manufacturer or the service provider is identified; trademarks can be bought, sold, and licensed; trademark

has no existence apart from the goodwill of the product or service it symbolizes;

d) Copyright relates to expression of ideas in material form and includes literary, musical, dramatic, artistic, cinematography work, audio tapes, and computer software; geographical indications are indications, which identify as good as originating in the territory of a country or a region or locality in that territory where a given quality, reputation, or other characteristic of the goods is essentially attributable to its geographical origin.

6.3.1 History of Intellectual Property Right/Patent

The laws and administrative procedures relating to Intellectual Property Right have their roots in Europe. The trend of granting patents started in the fourteenth century. In comparison to other European countries, in some matters England was technologically advanced and used to attract artisans from elsewhere, on special terms. The first known copyrights appeared in Italy. Historically, an ambivalent patent practice existed in regard to inventions involving biological materials. In view of the emergence of modern biotechnology, the past decade has led to a firm practice in this regard.

6.3.2 Patent

A patent is a government-granted right for an individual or business to produce and sell a product for a number of years. A patent is awarded for an invention, which satisfies the criteria of global novelty, non-obviousness, and industrial or commercial application. Patents can be granted for products and processes. A patent grants the patentee the right to exclude others from commercially using the patented invention for a period of twenty years. The underlying assumption is that, through exclusivity, potential inventors are incited to devote their resources to Research and Development of new products and inventions, because of the prospect of acquiring patents and, therefore, exclusivity in the exploitation for a limited period of time. In view of the immensely high investments needed for the development of biotechnological products, this presumption would particularly apply to bio-industry.

6.3.2.1 Reason for Patenting

The basic reason for patenting an invention is to make money through exclusivity, i.e., the inventor or his assignee would have a monopoly if,

a. the inventor has made an important invention after taking into account the modifications that the customer, and

b. if the patent agent has described and claimed the invention correctly in the patent specification drafted, then the resultant patent would give the patent owner an exclusive market.

c. the patentee can exercise his exclusivity either by marketing the patented invention himself or by licensing it to a third party.

6.3.2.2 Things that are Not Patentable

An invention, which is frivolous or which claims anything obvious or contrary to the well-established natural law. An invention, the primary or intended use of which would be contrary to law or morality or injurious to public health. A discovery, scientific theory, or mathematical method. A mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine, or apparatus unless such known process results in a new product or employs at least one new reactant. A substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance. A mere arrangement or re-arrangement or duplication of a known device each functioning independently of one another in its own way. A method of agriculture or horticulture.

Any process for the medicinal, surgical, curative, prophylactic diagnostic, therapeutic or other treatment of human beings or any process for a similar treatment of animals to render them free of disease or to increase their economic value or that of their products. An invention relating to atomic energy. An invention, which is in effect, is traditional knowle

6.3.2.3 Types of Patents

a. Utility patents: any new and useful process, machine, article of manufacture, or composition of matter, or any new and useful improvement thereof.

b. Design patents: new, original, and ornamental design for an article of manufacture.

c. Plant patents: production of any distinct and new variety of plant.

6.3.2.4 Importance of Patent to Biotechnology

While we rely on the Intellectual Property system to stimulate innovation across the economy, the modern biotechnology sector is perhaps the most patent dependent by virtue of the enormous costs of discovery in such a long-lead and labor-intensive industry.

6.4 Ethical Considerations in Sperm Bank

A sperm bank (cryobank) is specially licensed enterprise that collects and stores sperm from volunteers. Sperm is used by women to achieve pregnancy with a person who is not her sexual partner. The process for introducing the sperm into the woman is called artificial insemination and it is one of the oldest and most common procedures in assisted reproduction. A sperm donor will be biological father of every child produced as a result of his donation. However, he is not intended to be a legal father. Sperm can be stored for as long as twenty years. However, only 50% of the sperm cells survive that time and have normal capability to fertilize the egg.

6.4.1 History of Sperm Bank

The first successful artificial insemination of a woman with her husband's sperm was recorded in 1790. However, it took centuries for human society to accept the idea to use the sperm of a man other than the woman's husband to achieve a pregnancy. Another problem was linked with the sperm preservation. The first successful human pregnancy with frozen sperm was reported in 1953 and this triggered interest in the possibility of sperm banks. The first one was established in early 70s in USA. However, the full worth of using frozen sperm, in contrast to fresh one, came to the daylight after identification of sexually transmitted disease - AIDS. The use of frozen sperm with a grace time of six months give time to retest the donor for the presence of HIV, hepatitis and other sexually transmitted diseases. The first sperm bank established for therapeutic purpose of infertility at Iowa USA and Tokyo Japan on 1964, which was possible by tremendous scientific advancement like human sperm cryopreservation with glycerol on 1949. The first pregnancy with thawed freezing sperm on 1953 following to the effect of cryopreservation on human sperm on 1776.

6.4.2 Ethical Consideration

a. Limiting the number of donor offspring. The most important issue is limiting the number of off-spring from a single donor. This would prevent accidental consanguinity and incest between donor offspring and spread of genetic

b. Emotional and psychological effects of being a donor offspring. This is a special problem. The majority of donor-conceived children never learn how they were born, while others may suffer of the knowledge that they have a large number of half-siblings.

c. Risk of infection and genetic diseases. The guidelines for selection of a donor are very strict. It is generally accepted that sperm donors as well as sperm should undergo screening that they cannot transmit sexually (hepatitis B and C and HIV) or hereditary disease on the offspring.

d. Age for sperm donors. Literature data on the impact of sperm donor age on outcome of insemination is deficient. It is generally accepted that sperm fertilization ability deteriorates with increasing men age.

e. Anonymous versus non-anonymous sperm donation. This is a very sensitive issue while parents, donors and offspring may have different considerations on anonymous versus non-anonymous sperm donation. The reasons recipients are choosing anonymous or non-anonymous donor, or the reasons donors want to be anonymous or non-anonymous, vary greatly from individual to individual and couple to couple.

f. Who is the father? A key question is whether donor -conceived children should be informed of their biological father and, if so, when, and how much information about donors should be revealed. Some children may react to such information by social and psychological disorders especially if they cannot get more information about the father and cannot meet him.

6.4.3 Advantages and Disadvantages of Sperm bank

a. The sperm bank can provide to their clients safe, disease-tested sperm from a wide selection of screened and tested anonymous donors, with comprehensive physical and intellectual information about them.

b. The bank cannot guarantee successful conception, as well as healthy pregnancy or child, and cannot determine the mental or physical characteristics of a child.

c. Failures in the operation of sperm banks. The most important are:

a) Many have no accurate records of babies born from donated sperm of single donor;

b) They do not have update about medical history on donors (their medical form is an image of one day in the life; and what if the donor is a healthy young fellow who do not report accurately about family history);

c) They do not share medical information amongst families (for instance, how many donor-conceived children have been diagnosed with serious diseases such as attention deficit disorder, attention deficit hyperactivity disorder, Autism, Tourette's, Von Recklinghausen disease etc.);

d) Some do not adequately counsel their donors.

6.5 Ethical Considerations in Design Baby

In 2004, the term “Designer Baby” could be found in the Oxford English Dictionary and it was explained as a baby whose genetic makeup has been artificially selected by genetic engineering combine with in vitro fertilization to ensure the presence and absence of particular gene and characteristics. Revolution in technology, fierce competition for survival and desire to achieve a superior human has led new generation scientists to

develop a disease free genetically modified offspring with desired traits. The term 'Designer baby' has gained much popularity amongst rich and western countries where an offspring is artificially selected by genetic engineering combined with In Vitro fertilization. Success in modifying other mammals through these methods led proposals to apply such methods on humans for biological improvement. The first designer baby was Adam Nash, produced by a team from

the Reproductive Genetics Institute in Chicago, led by Yuri Verlinski. Adam was created to obtain his hematopoietic tissue, which was necessary to treat Molly, his six-year-old sister, who suffered from Fanconi anemia. After four failed attempts, Adam was finally born on 29 August 2000 and the blood from his umbilical cord was able to be transplanted by Dr. John Wagner's team to his sister in the first week of October that year, in the University Hospital, Minneapolis, Minnesota.

6.5.1 Technique of Creating Designer Baby

The whole process is done artificially where first the ovaries are hyper stimulated by giving hormones and then when the follicles mature. Transvaginal ultrasound-guided oocyte retrieval is done and through ICSI technique (intra cytoplasmic sperm injection) ovum are fertilized in test tube. These fertilized ova are brought till embryo (eight cell) stage at which point cells are removed and tested using a technique known as Pre implantation Genetic Diagnosis (PGD). PGD is performed prior to implantation. Genetic diseases like Cystic fibrosis, Down's syndrome, muscular dystrophy, sickle cell anaemia etc. are tested by using the technique of bio technology that is polymerase chain reaction. Analysis of genetic material (DNA) from a single cell is performed using a technique called FISH (fluorescent in situ hybridization) or PCR. During the analysis on the single cell, the embryos are kept in culture and allowed to further divide. Many couples use this procedure if there are any inherited disorders in their genes to decrease the possibility that the disorder will passed to their child. If the genetic disorder appears the defected genes are replaced with healthy genes. Unaffected embryos are transferred into the uterus to achieve an outcome of a chromosomally normal baby. Some genetic disorders are specific to one gender or another, such as hemophilia, which usually affects boys. Doctors may examine the cells to determine the gender of the embryo. In a case where a family has a history of hemophilia, only female embryos are selected for placement in the uterus. This practice is at the center of a larger debate about whether parents should be able to choose embryos purely on the basis of gender. Some people worry that it could lead to an imbalance between genders in the general population, especially in societies that favor boys over girls, such as China.

6.5.2 Future/Bioethics Concern

a) Ethical, legal, social controversies loose grip on society as demands of creating such babies make its own market.

b) However, only the rich can afford such techniques as the IVF and Pre implantation genetic diagnosis costs high per cycle and a couple needs to repeat attempts for successful pregnancy as the success rate of IVF is only 10 -35%

c) The bioethicists are concerned about the future of humans. As such enhance humans artificially selected might become prejudiced against one another due to a feeling of lost common humanity with non-enhanced groups.

d) Also, the society will be bias towards the superior humans than the natural ones.

e) Moreover, the future of these designer babies is still not known.

f) There is a wide variety of biological risks with genetic modifications. New diseases may emerge which will be difficult to prevent.

g) One report from Children conceived through cytoplasmic transfer has been diagnosed with pervasive developmental disorder where symptoms range from mild delay in speech to autism.

h) The booming fertility market and fertility doctors becoming GOD for infertile couples and experimenting on embryos before implantation should be under surveillance of medical experts and law.

i) The imperfect and abnormal embryos are destroyed and there is no debate whether to destroy such embryos is legal or criminal.

j) Mass discrimination among humans in future can even lead to violence.

k) Genetic and biological risks are yet to be identified.

l) A big danger is awaited in future and immediate action is required to prevent it.

6.5.3 Ethical Assessment

The eight most important aspects to consider in an ethical reflection on the production of designer babies are:

a) the instrumentalization of the child produced in such a way that these children would be treated as commodities;

b) the secondary consequences that could result from the legal authorization of this technique could open the door to other ethically unsuitable techniques, especially sex selection,

c) the benefit that the parents may obtain;

d) the impossibility of obtaining the consent of the child itself;

e) the medical problems that the use of the preimplantation genetic diagnosis technique may cause in the embryo generated;

f) as well as those inherent in the in-vitro fertilization technique;

g) the negative ethical burden involved in the high number of embryos lost with this practice, i.e. the high number of human lives destroyed;

h) and finally, whether or not a medical alternative to the production of designer babies exists, since if so, their generation would be doubly unjustif

6.5.4 Arguments for Designer Babies

a) Children are already "engineered" by their parents. Parents choose where their kids go to school, what they eat, and what family traditions they will have. Parents have always been "designing" their babies in this way.

b) Genetic engineering is only a new tool to design babies. Genetic editing could be used to edit mutations associated with diseases.

c) This could mean that a child with a genetic disease like cystic fibrosis could avoid a lifetime of suffering.

d) Genetic engineering could end some genetic diseases. By eliminating certain genes at the embryo stage, a person will never be able to pass on the disease.

e) This could reduce human suffering caused by genetic diseases.

f) Those who are against genome editing would not have to undergo the procedure for their own children. Other parents could have the freedom to use genetic editing if they want.

6.5.5 Arguments against Designer Babies

a) Many genes have more than one effect. The traits we choose may be accompanied by unwanted changes.

b) A study in mice discovered one genetic change that improved learning. However, the change also increased the mouse's sensitivity to pain.

c) Our genetic "improvements" might also have accidental bad effects.

d) Genetic editing would occur on the embryo, before a child is able to agree to the change. It is unfair to the child to force this change on him or her.

e) Genetic editing of babies would be unfair. Editing genomes would likely be very expensive. This means only rich families could afford genetic engineering for their children. This might mean that the children of rich families may live longer or have other advantages.

f) If a parent chooses a child's traits, that child may feel like they have less control over their life. If a parent pays for genetic engineering to have a child with an amazing voice, that child may feel forced into being a singer. This could unfairly increase pressure on the child to meet high expectations of their paren

6.6 Ethical Considerations in Organ Donation/Transplant

Transplantation is the treatment of choice for end-stage organ disease as it provides a better quality of life and long-term survival to recipients. An organ transplant is a surgical operation where a failing or damaged organ in the human body is removed and replaced with a new one. An organ is a mass of specialized cells and tissues that work together to perform a function in the body. The heart is an example of an organ. It is made up of tissues and cells that all work together to perform the function of pumping blood through the human body. Any part of the body that performs a specialized function is an organ. Therefore, eyes are organs because their specialized function is to see, skin is an organ because its function is to protect and regulate the body, and the liver is an organ that functions to remove waste from the blood.

A graft is similar to a transplant. It is the process of removing tissue from one part of a person's body (or another person's body) and surgically re-implanting it to replace or compensate for damaged tissue. Grafting is different from transplantation because it does not remove and replace an entire organ, but rather only a portion.

Not all organs are transplanted. The term "organ transplant" typically refers to transplants of the solid organs: heart, lungs, kidneys, liver, pancreas and intestines. Advances in immunosuppression, surgical techniques, medical and pharmacological progress have made transplantation possible from cadaveric organ donors starting in 1967, when Christian Barnard had undertaken the world's first cardiac transplant. Transplantation undoubtedly saves lives or improves the quality of life for patients with end-stage organ failure. It is already scientifically proven that there is a substantial long-term survival advantage for renal transplantation compared with dialysis.

6.6.1 Ethical Consideration

The Organ Shortage: the primary ethical dilemmas surrounding organ transplantation arise from the shortage of available organs. Not everyone who needs an organ transplant gets one and in fact, the scales tip quite heavily in the opposite direction. The United Network for Organ Sharing (UNOS) maintains a comprehensive, up-to-date website that gives the status of people awaiting organ transplants. According to their website (updated daily at www.unos.org) over 83,000 people are currently awaiting transplants in the United States. Should someone who has received one organ transplant be given a second transplant? Or should people who have not had a transplant be given priority over those who have already had one?

Should people whose lifestyle choices (smoking, drinking, drug use, obesity, etc.) damaged their organ be given a chance at an organ transplant?

Should suicidal individuals be given an organ transplant? What if they attempted suicide in the past but are not currently contemplating suicide?

Should people who have young children be given an organ transplant over a single person? Over an elderly person? Should age and whether or not a person has children even matter?

Should people who can't afford expensive anti-rejection drugs be passed over for a transplant? Should people who don't have insurance and can't pay for a transplant be allowed to go on the national waiting list?

Should condemned prisoners receive organ transplants? What if they are serving a life sentence without parole?

One way to avoid the ethical problems associated with the shortage of transplantable organs is to increase the number of donor organs. However, fears abound that policies to maximize organ donations could go too far – leading to organ farming or premature declarations of death in order to harvest organs.

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CHAPTER SEVEN:
AQUATIC MACRO INVERTEBRATES

Ibrahim, Baba Usman

Invertebrate organisms are animals without backbones. They existed in two forms micro invertebrates and macro invertebrates. The micro invertebrates are minute in nature and are visible only with the aid of microscope while the macro invertebrates are multicellular and can be visible with the naked eye. The micro invertebrates include the protozoa, coelenterate, Rotifera etc. The macro invertebrates comprise of larger animals, Arthropoda, Mollusca with shells, flat worms platyhelminthes, Annelida etc.

The focus shall be on the aquatic macro invertebrates. The fauna content of fresh waters differs greatly from that of the sea and oceans. Only a comparatively small number of fishes, crustaceans and mollusks can migrate from the sea into fresh waters or vice versa. This mixing of fresh water and marine fauna is observed at the mouths of the large Siberian rivers and inland seas of low salt content in the U.S.S.R. e.g. the sea of Azov and the Baltic Sea. In general, the animal population of rivers and lakes differs considerably from that of the seas. Major group of animals (e.g. the entire phylum Echinodermata, the subphylum Urochodata, the class Cephalopod mollusks etc.) are not represented by even one species in fresh waters. Isolated coelenterate and polychaetae forms are found in fresh waters. The fauna of sponges, bryozoans, gilled snails and many other groups is much poorer in fresh waters than in the sea. Some groups of aquatic animals are more diverse and more abundant in fresh water than in the sea e.g. Cladocerans (water fleas), phyllopods, rotifers, water oligochaetae, archinids and tardigrades. Aquatic insects one of the most important animal groups inhabiting fresh waters are almost entirely absent from the marine fauna. Some fresh water animals are entirely adapted to life in water. All stages of their life cycle occur in an aquatic environment e.g. protozoans, worms, crustaceans, mollusks. Almost all insects live in water during some developmental stages only in the adult stage they leave the water. Spiders are associated in part with aquatic environment.

The aquatic macro invertebrates which is the main focus includes:-

PLATYHELMINTHES

They are triploblastic, acoelomate, unsegmented, flattened animals (flatworms) under leaves or stones, on muds at the bottom of lakes or streams in tropical high lands. They are divided into three classes, two of them Trematodes and Cestoda are parasitic, while the Turbellaria are free living. The larvae of the parasitic forms (flakes) live in the body of certain snails (e.g. Lymnea). The free living kinds are brownish or greenish. They are carnivorous or feed on debris. The planarians are also flatworm inhabiting standing and flowing water. Fig. 1.

NEMATODA

They are round worms. Non segmented. Mostly microscopic or too small to be seen easily. Between lum and l0 um long, with strong flexing movements e.g. *Rhadbolaimus aquaticus.* The fresh water nematodes are found in flowing and standing waters of all

types. Most of them are predators and non-parasitic and protected by their very thick cuticle fig. 2.

ANNELIDA

They are segmented worm, divided into three classes, Polychaetae, Oligochaetae and Hirudinea, those with a sucker at each end, but have no bristles e.g. Hirudinea / sleeches. This group of annelids inhabits fresh waters predominantly and only a few species are marine. Many leeches are parasites of fish, turtles and water fowl. Only a few species e.g. the medicinal leech *(Hirudo medicinealis), Haementeria costata* and the middle Asian limnatis can pierce the skin and feed on the blood of man. Fig.3. The second groups of annelida have no suckers but have bristles present in their body e.g. the earthworms and the fresh water worms. They are large in polluted water in Africa e.g. Yoruba worm *(Limnodrilus sp.),* and the smaller worms found among plant roots in marsh area e.g. Potworms *(Enchytraeus sp.),* and in the mud at the bottom of lake or pond etc. e.g. Tubifex, Nair, and Dero Fig. 3.

Plate: Platyhelminthes (Flat worms) and Nematoda (Round worm)

Source: Phyllis (1965)

AQUATIC MACRO INVERTEBRATES

Plate: Annelida (Hirudinea)

Source: Phyllis (1965)

ARTHROPODA

The arthropoda has jointed limbs in adults and external hard skeleton. They comprise of the following groups:-

The insecta with three pairs of legs in adults and sometimes none in the larvae. A large number of species belonging to class insecta are associated with fresh waters .e.g. water bettles and with very few exceptions insecta not marine. Water bettles and water bugs pass their entire life span in water. Most aquatic insects live in the air during their winged stage, only the eggs, larvae and in some group the pupae are associated with an aquatic environment.

The insecta has three subdivision as follows:-

a. Those that fly over or settle on water, they are further divided into two i.e. those with one pair of wings including mosquitoes e.g. culex, Aedes settle with body parallel to the surface of water, and Anopheles mosquito settle with body at an angle head down. Black fly e.g. Simulium. And those with two pairs of wings including Caddis flies e.g. chimera, Stone flies e.g. Neoperla, Mayflies e.g. Cloeon , Dragon flies e.g. Pantala.

b. Those that run on water surface including pond skaters e.g. Gerrir, water crickets e.g. Velia, Whirling beetles e.g. Gyrinus, water measurers e.g. Hydrometra.

c. Those that live in or under water are subdivided into two (i) those with biting mouth parts e.g. water beetles and (ii) those with piecing mouth parts with forewings leathery comprising of water bugs e.g. Belostoma , lethocerus, water boatman e.g. Notonecta, water scorpion e.g. lacotrephes and water stick insects e.g.. Ranatra. Fig 4.

d. The Arachinida has four pairs of legs e.g. spiders and mites. The spiders (Araneina) are large 1cm or more and are represented in ponds laters and creeks of small rivulets by a single aquatic species, the water spider *(Argyroneta aquatica).* The water mites (Acarina) are small 1mm - 3mm.

The crustacean has more than 4 pairs of legs comprising of two subdivisions fresh water crabs e.g. Deckenia, potamonautes, trichodactylus - they are broad, flattened carapace, and abdomen folded beneath. And the fresh water shrimps e.g. *Palaemon, caridina,* they are longer with cylindrical carapace large abdomen. Fig. 5.

Plate : Insecta (Phyllum Arthropoda)

Source : Phyllis (1965)

Plate : Insecta (Phyllum Arthropoda)

Source : Phyllis (1965)

INSECT LARVAE

Insect larvae form a considerable part of aquatic macro invertebraes, they have obvious mouth parts but no wings. The following were classified based on the position they occupied in the aquatic habitat.

a. Those hanging from surface of water or surface film include Mosquito larvae *(Culex* sp. hang at an angle 1cm), *anopheles* sp Larva hang at an angle 1cm and Rat-tailed Maggot *Eristalis* sp found in stagnant water in some tropical area at 5cm.

b. Those that are motionless in mid-water e.g. lake flies phantom larvae *Chaoborus* sp 1cm.

c. Those on the bottom or amongst vegetation e.g. Blood worm *Chironomus* sp 11/2cm and midge lavae e.g. Tanytarsus *1/2*cm.

d. Those Larvae with three pairs of legs form another classification, with two subdivisions, (i) Those living freely with one (1) tail filament include Alder Flies e.g. *Sialis* rare in the tropic (ii) Those with two (2) tail filaments comprise stone fly larvae e.g. Neoperla, some beetle larvae e.g. Eretes, gyrinus and some caddis larvae usually with two hooks, (iii) those with three (3) tail filaments include dragon fly larvae e.g. Pantala, Aushna, Damsel fly larvae e.g. Ischnura and may fly larvae e.g. Cloeon, Baetis Fig. 6.

AQUATIC MACRO INVERTEBRATES

Plate: Insect larvae

Source: Phyllis (1965)

Plate: Insect larvae

Source: Phyllis (1965)

Plate: Insecta (Phyllum Arthropoda)

Source: Phyllis (1965)

MOLLUSCA

Mollusca are divided into two major groups, Gastropoda and Lamellibranchia. The Gastropoda possess 1 valve to shell, coiled or cap shaped and subdivided into two:

i. Those with operculum and gill comprise of Nerites (neritidae) e.g. Neritina, Apple snail (Ampullaridae) e.g. Pila, Lanistes, Pomaceae, Asolane, and Viviparous snails (Viviparidae) e.g. Bellamaya viyipara.

ii. Those that lack operculum, have lung, but no gill. Comprise of freshwater limpets (Ancylidae) e.g. Bunopia, Ancylus, pond snails Lymnaeidae e.g. Lymnaea, Bulinus and Ramshorn snails (Planorbidae) e.g. Austrlorbis, Indoplanorbis, and Biompholarria Fig. 7.

The diversity of aquatic macro invertebrates is very great, and their classification based on their adaptation to the environment of the habitat, the position they occupied in the habitat and the structure they possess or modification of these structures in relation to functions such as defence i.e. bitting and piercing or modification for nutritional purpose. It is also important to note that some of the aquatic macro-invertebrates are harmful or parasitic e.g. Leech, mosquitoes which are vectors of malaria parasites, while some serve as food for other aquatic inhabitats like fish e.g. earthworms of Oligochaetae group.

Plate: Mollusca (divided into two major groups, Gastropoda and Lamellibranchia)

Source: Phyllis (1965)

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CHAPTER EIGHT:

INTRODUCTORY FISH PARASITIC DISEASES AND PATHOLOGY

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8.1 Introduction

Parasites are organisms that live in or on other animals know as hosts. They extract food from the host by sucking blood fluid or eating the tissues. All fish are potential hosts to many different species of parasites. Parasitic diseases of fish pose a significant threat to fish populations. Parasite is found globally and infects freshwater and marine fishes. They attach to the gills and skin of the fish. Among fish parasites, are obligatory parasites, infesting mostly commercially important fishes. Pathological conditions resulting from parasitic diseases assume high magnitude of epidemics under crowded and other unnatural conditions among fish. Fish pathology deals with the diseases and parasites that effects fish ordinary living. Parasites can be internal (*endoparasites*) or external (*ectoparasites*). Pathogens which can cause fish diseases comprise:

1. *viral* infections, such as *esocid lymphosarcoma* found in *Esox* species.
2. *bacterial infections*, such as *Pseudomonas fluorescens* leading to *fin rot* and *fish drops y*
3. *fungal* infections *water mould* infections, such as *Saprolegnia sp.*
4. *metazoan* parasites, such as *copepods.*
5. unicellular parasites, such as *Ichthyophthirius multifiliis.*
6. Certain parasites like *Helminths* for example *Eustrongylides*

8.2 Bacterial Diseases in Fish

Bacteria are responsible for many fatal diseases in fishes like furunculosis, columnaris, fin and tail rot, vibriosis, dropsy, cotton mouth disease and tuberculosis. Some of the bacterial diseases are:

8.2.1 Furunculosis Disease

Furuculosis disease is caused by *Aeromonas almonicida* in salmon fishes. It is a non-motile, gram-negative bacterium. This disease frequently appears to infect fishes living in the dirty waters containing a large amount of decaying matter. The first symptoms of this disease are appearance of boil like lesions. Others, symptoms are blood-shot fins, blood discharge from the vent, haemorrhages in muscles and other tissues and necrosis of the kidney. Bursting of boils allow the spread of this disease among other fishes and also offer suitable areas for fungus growth. Fishes severely infected with the bacteria die in good number. The disease is controlled on farms by medication or vaccination. Iodine is also used to decontaminate the surface of fertilized eggs to prevent vertical transmission (passage of infection from parent to offspring).

8.2.2 Columnaris Disease

Columnaris disease is caused by *Chondroccus columnaris* and *Cytophaga columnaris* in many freshwater aquarium fish. It is a long, thin, flexible, gram-negative slime bacterium (myxobacteriales). This disease is often associated with low oxygen level. Initially it is

marked by appearance of grayish-white or yellowish-white patches on the body. The skin lesions change to ulcerations and fins may become frayed. Gill filaments are destroyed and

eventually lead to the death of the fish. It is caused by infection of Myxosorida. Cysts appear on the body, internal tissues and organs. Fish becomes weak. Scales become weak, perforated and fall off. Give dip treatment in 10% common salt solution.

8.2.3 Fin and Tail Rot Disease

Fin and Tail Rot disease is caused by *Aeromonas salmonicid* and *A. liquefaciens*. However, protozoans and fungi may also be involved. It is characterized by appearance of white lines along the margins of fins, the opacity usually progresses towards the base eroding them and causing haemorrhage. The fin rays become brittles first and later break leading to the complete destruction of the fins. The infection may also spread on the body surface. Fin and tail rot are associated with poor sanitary conditions in fish ponds and with water pollution in nature.

Disintegrating fins that may be reduced to stumps, exposed fin rays, blood on edges of fins, reddened areas at base of fins, skin ulcers with grey or red margins, cloudy eyes. It is caused by the bacteria Aeromonas. If tank conditions are not good an infection can be caused from a simple injury to the fins/tail. Tuberculosis can lead to tail and fin rot. Basically, the tail and/or fins become frayed or lose colour. Treat the water or fish with antibiotics. A good antibiotic is Chloromycetin (chloramphenicol) or tetracycline. Treatment of 1% $CuSO_4$ is also effective.

8.2.4 Vibriosis Disease

Vibrio bacteria are the causative agents of vibriosis disease in salmon and many other fishes. This disease may occur in waters with low oxygen. These bacteria are small gram-negative bacilli, characteristically curved. Diseased fishes show large, bright coloured, bloody lesions in the skin and muscles, haemorrhages in eyes, gills may bleed with slight pressure, and inflammation of the intestinal tract. If you suspect your fish is infected with Vibriosis, move them to a hospital or quarantine tank. *Test your water chem istry* to make sure your levels are within range, evaluate your fish's diet, and consider any *aggressive tank mates*. Secondary stress goes a long way in making *Vibrio* infections worse. Over-the-counter "antibiotics" are not effective against Vibriosis. There are many known resistant strains of *Vibrio* that require the correct treatment. Once a diagnostic culture and sensitivity has been performed, your veterinarian will determine the best course of action. Antibiotics can be given directly as an injection, formulated into a feed, or may require a water-based treatment.

8.2.5 Dropsy Disease

Pseudomonas punctata is the causative agent of this disease. It is characterized by accumulation of yellow coloured fluid inside the body cavity, protruding scales and pronounced exopthalmic conditions. This is known as Intestinal Dropsy. In case of ulcerative dropsy, ulcers appear on the skin, deformation of back bone takes place and show abnormal jumping. This is a fatal disease in culture systems.

Bloating of the body, protruding scales. Dropsy is caused from a bacterial infection (acromonas) of the kidneys, causing fluid accumulation or renal failure. The fluids in the body build up and cause the fish to bloat up and the scales to protrude. An effective treatment is to add an antibiotic to the food. With flake food, use about 1% of antibiotic and carefully mix it. Antibiotics in 250 mg capsules if added to 25 grams of flake food will be sufficient to treat dozens of fishes. A good antibiotic is Chloromycetin (chloramphenicol), or use tetracycline.

8.2.6 Cotton Mouth Disease

The filamentous bacteria, Flexi bacteria are the causative agent of this disease. The main symptom is appearance of fungus like tuft around the mouth. White cottony patches around the mouth. It looks like a fungus attack of the mouth, so it is called mouth fungus. It is actually caused from the bacterium *Chondrococcus columnaris*. In the beginning a grey or white line appear around the lips and later short tufts arise from the mouth like fungus. This disease may be fatal due to production of toxins and the inability to eat. Hence treatment at an early stage is essential. Penicillin at 10000 units per litre is a very effective treatment. Second

dose should be given in two days, or use Chloromycetin, 10 to 20 mg per litre, with a second dose in two days.

8.2.7 Tuberculosis Disease

Mycobacterium is a disease-causing agent which is difficult to diagnose without pathological examinations. The symptoms are ulcers on body, nodules in internal organs, fin and tail rot, loss of appetite and loss of weight of fish.

Emaciation, hollow belly, possibly sores. Tuberculosis is caused by the Bacterium *Mycobacterium piscium*. Fish infected with tuberculosis may become hollow bellied, pale, show skin ulcers and frayed fins, and loss of appetite. Yellowish or darker nodules may appear on the body or eyes. The main cause for this disease appears to be over-crowding in un-kept conditions. There is no known and effective treatment for this disease. The best thing to do is to destroy the infected fish and, if un-kept conditions or overcrowding is the suspected cause, it is required to take necessary measures.

8.2.8 Bacterial Gill Disease

This disease is caused by *Myxobacteria* in salmon fish. Many bacteria are found in swollen gill lamellae which show proliferation of the epithelium, and symptoms are lack of appetite. This disease is transmitted through water from infected fish. Treatment is by removal of the carbon filter from filtration; however, it must be returned 7 days after the last dose.

8.3 Viral Diseases in Fish

Viruses are transmitted from one host to the other through a structure called virion. Viruses are classified mainly based on external structure, shape, size, capsid structure, RNA and DNA nucleic acids. Viruses cause disease by weakening the host tissue or by forming tumors in the host tissues. There is no treatment for viral diseases, only prophylactic measures have to be taken up. Some of the viral diseases are:

8.3.1 Carp Pox

One of the oldest recognized fish diseases, carp pox is caused by Cyprinid herpesvirus-1 (CyHV-1). Pox lesions may be seen on other species of fish and are sometimes referred to as fish pox. Lesions typically are smooth and raised and may have a milky appearance. They are benign, non-necrotizing areas of epidermal hyperplasia. Severe cases may result in development of papillomatous growths, which may be a site of complicating bacterial infection. Generally, lesions are self-limiting and of minimal clinical significance. Carp pox can be a significant problem in koi because their aesthetic quality, and hence market value, is severely compromised.

8.3.2 Koi Herpesvirus:

Koi herpesvirus (KHV), caused by Cyprinid herpesvirus-3 (CyHV-3), was first recognized in 1996. It is widespread in the USA and considered endemic. KHV causes clinical disease in koi and common carp. Goldfish and grass carp are refractory to clinical disease but may serve as carriers. Koi that are exposed, but survive, may also serve as carriers. Clinical disease is seen at water temperatures of 72°–81°F (22°/27°C), with maximal mortality at temperatures of 72°/78°F (22°/25.5°C). Mortality rates can reach 80%/100%.Fish of any age are susceptible, but mortality rates may be higher in younger fish, especially fry. The most obvious lesions are seen on gill tissues, which are severely affected and develop a mottled red and white appearance with obvious hemorrhage in some cases. Affected fish are lethargic, swim at the surface, and may show behavioral signs of respiratory distress. The presence of severe bacterial or parasitic disease may mask the fact that KHV is the primary cause of gill lesions. The disease is transmitted horizontally by exposure to sick or carrier fish and also by exposure to contaminated water, substrate, or equipment. The only effective way to treat Koi that are infected with K.H.V. is with heat therapy. You must have a way to isolate these fish to treat them. You can use aquariums, or smaller isolation ponds to do this. You could set up a temporary isolation pond by using some bricks, back-filling them with dirt, and installing a liner. Make sure you use some bird netting over the top so the Koi will not jump out. Make sure the isolation pond is at least 12 inches deep.

· Remove the Koi from the main pond, into the isolation pond or aquariums making sure that the water you are using in the isolation quarters is the same temperature as the pond water.

· Slowly heat the water up to 86Â°F (no more than 2Â°F per hour). If your water is very cool, due this over a two-day period.

· Leave the fish in the heated water for a 7-day period. Do not feed the fish during this time as they will be stressed out, and the last thing you want is to have an ammonia or nitrite problem in the water.

· After the 7-day heat treatment is finished, slowly cool the water down to 80Â°F. Once you reach this temperature, start an antibiotic treatment with Koi Fix for Food, and Forma-Green in the water...if the fish are still eating. If the fish will not eat, use Koi Fix for Water. The Koi will need to be kept on these antibiotics for a two-week minimum treatment. You may continue these treatments for an extra week if the fish are not quite healed up yet.

8.3.3 Herpes viral Hematopoietic Necrosis

This condition of goldfish is caused by Cyprinid herpesvirus-2 (CyHV-2). It is probably widespread throughout the USA, but it is not a reportable disease. Clinically ill goldfish often are anorectic and exhibit pale gills and ascites. At necropsy, the spleen and kidneys (anterior and posterior) are often enlarged. As is typical for herpesviruses, survivors can be carriers and exhibit clinical signs of disease if subjected to stressors. Water temperatures between 10° and 22°C will result in replication of the virus that can be detected with quantitative PCR.

IHNV can be transmitted through water, by movement of fish, contact with contaminated untreated waste material and by equipment. In areas where IHN is endemic, the number of cases can be controlled by good hygiene, the use of virus-free water supplies and the disinfection of eggs from farms with IHNV. Testing of brood stock is important in identifying carriers; where known carriers are present, the disinfection of eggs with iodine products is recommended.

8.3.4 Herpesvirus of Angelfish

A herpesvirus of angelfish (*Pterophyllum* spp) has been detected by electron microscopy of skin from moribund angelfish. Affected fish produce copious amounts of skin mucus that gives affected fish a grey sheen. Often, these fish have multiple parasitic infestations and bacterial infections, similar to that of KHV in koi. It is suspected that survivors are carriers.

P. scalare is relatively easy to breed in the aquarium, although one of the results of generations of inbreeding is that many breeds have almost completely lost their rearing instincts, resulting in the tendency of the parents to eat their young. In addition, it is very difficult to accurately identify the sex of any individual until it is nearly ready to breed.

Angelfish pairs form long-term relationships where each individual will protect the other from threats and potential suitors. Upon the death or removal of one of the mated pairs, breeders have experienced the total refusal of the remaining mate to pair up with any other angelfish and successfully breed with subsequent mates. Depending upon aquarium conditions, *P. scalare* reaches sexual maturity at the age of six to 12 months or more. In

situations where the eggs are removed from the aquarium immediately after spawning, the pair is capable of spawning every seven to 10 days. Around the age of three years, spawning frequency decreases and eventually ceases. The virus can be spread by water and by movement of live infected fish. Controls are based on prevention of movement of fish and equipment from infected farms to uninfected locations, and by appropriate hygiene measures.

8.3.5 Viral Hemorrhagic Septicemia (VHS)

This disease is caused by an unequal shaped fish virus with RNA. This reportable disease is caused by a *Novirhabdo virus* and is a member of the family Rhabdoviridae. This disease occurs in salmon fishes. Transmission of the disease occurs through the water by a flagellate. Most of the reported hosts are not ornamental fishes, but koi were shown to be susceptible experimentally to genotype IVb. The symptoms are kidney swelling, reduced appetite, obvious distress, erratic spiral swimming, multiple haemorrhages in skeletal muscles, change in body colour, reddish fins. The only control measure is prevention.

8.3.6 Spring Viremia of Carp (SVC)

This acute, virulent, usually hemorrhagic disease of cultured carp is caused by a *Vesiculo virus* that, like VHS, is a member of the Rhabdoviridae family. Historically, it was reported in Europe and the former USSR; however, several outbreaks have been reported in the USA between 2002–2007, in both wild fish and cultured ornamental koi. SVC is considered a foreign animal disease in the USA and must be reported. It causes disease in common carp, including koi, as well as grass, bighead, silver, and crucian carp. Limited experience suggests that common goldfish may be susceptible. Clinical signs are nonspecific and may include darkening of the skin, exophthalmia, ascites, pale gills, hemorrhage, and a protruding vent with thick mucoid fecal casts. Pinpoint hemorrhage in the swim bladder is indicative of SVC, if present. Coinfection with *Aeromonas* or other systemic bacteria may obscure the presence of the virus.

The virus can be spread by water and by movement of live infected fish. Controls are based on prevention of movement of fish and equipment from infected farms to uninfected locations, and by appropriate hygiene measures.

8.3.7 Lymphocystis Disease

This typically chronic, viral infection of wild or captive marine and freshwater fish is caused by an icosahedral DNA virus of the Iridoviridae family. Infection may be manifest by benign, cauliflower-like lesions typically located on fins. The disease affects a wide range of fish and is generally considered to have a global distribution. Within the aquarium trade, painted glass fish and marine tropical fishes such as the anemonefishes

(Pomacentridae), marine angels (Pomacanthidae), and butterflyfishes (Chaetodontidae) are susceptible. Presumptive diagnosis is based on the presence of enlarged fibroblasts (up to 1 mm), which are easily visualized with a light microscope. Microscopic examination typically reveals the appearance of grape-like clusters of virus-laden cells. Diagnosis is confirmed histologically: feulgen-positive cytoplasmic inclusions and a hypertrophied nucleus are pathognomonic and the disease is usually self-limiting but is of aesthetic concern.

Nodular white swellings (cauliflower) on fins or body. Lymphocystis is a virus and being a virus, affects the cells of the fish. It usually manifests itself as abnormally large white lumps (cauliflower) on the fins or other parts of the body. It can be infectious but is usually not fatal. Unfortunately, there is no cure. Fortunately, this is a rare disease. There are two suggested treatments. One treatment is to remove and destroy the infected fish as soon as possible. The other treatment is to simply separate the infected fish foe several months and wait for remission, which usually does occur.

8.4 Fungal Diseases in Fish

Fungal infections (fungal infections are called mycoses) are among the most common diseases seen in temperate fish. Because fungal spores are found in all fish ponds and create problems in stressed fish. Poor water quality can also lead to an increase in fungal infections in an otherwise healthy fish population. Some of the fungal diseases are:

8.4.1 Saprolegniasis

Saprolegniasis is a fungal disease of fish and fish eggs most commonly caused by the *Saprolegnia* species called "water molds." They are common in fresh or brackish water. *Saprolegnia* can grow at temperatures ranging from 32° to 95°F but seem to prefer temperatures of 59° to 86°F. The disease will attack an existing injury on the fish and can spread to healthy tissue. Poor water quality (for example, water with low circulation, low dissolved oxygen, or high ammonia) and high organic loads, including the presence of dead eggs, are often associated with *Saprolegnia* infections. Saprolegniasis is often first noticed by observing fluffy tufts of cotton-like material coloured white to shades of grey and brown--on

skin, fins, gills, or eyes of fish or on fish eggs. These areas are scraped and mounted on a microscope slide for proper diagnosis. Under a microscope. Saprolegnia appears like branching trees called hyphae. With progression of infection fish usually becomes lethargic and less responsive to external stimuli. So, fish under such conditions is a target to predators.

Tufts of dirty, cotton-like growth on the skin, can cover large areas of the fish, fish eggs turn white. Fungal attacks always follow some other health problems like parasitic attack, injury, or bacterial infection. The symptoms are a grey or whitish growth in and

on the skin and/or fins of the fish. Eventually, if left untreated, these growths will become cottony looking. The fungus, if left untreated, will eventually eat away on the fish until it finally dies. Use a solution of phenoxethol at 1% in distilled water. Add 10 ml of this solution per litre. Repeat after a few days if needed, but only once more as three treatments could be dangerous inhabitants. If the symptoms are severe the fish can be removed and treated with small amount of provide one iodine or mercurochrome. For attacks on fish eggs, most breeders will use a solution of methylene blue adding 3 to 5 mg/1 as a preventive measure after the eggs are laid.

8.4.2 Branchiomycosis

Branchiomyces demigrans or "Gill Rot" is caused by the fungi *Branchiomyces sanguinis* (carps)and *Branchiomyces demigrans* (Pike and Tench). Branchiomycosis is a pervasive problem in Europe, but has been only occasionally reported by U.S. fish farms.

Both species of fungi are found in fish suffering from an environmental stress, such as low pH (5.8 to 6.5), low dissolved oxygen, or a high algal bloom. *Branchiomyces* sp. grow at temperatures between 57° and 95°F but grow best between 77° and 90°F. The main sources of infection are the fungal spores carried in the water and detritus on pond bottoms.

Branchiomyces sanguinis and *B. demigrans* infect the gill tissue of fish. Fish may appear lethargic and may be seen gulping air at the water surface (or piping). Gills appear striated or marbled with the pale areas representing infected and dying tissue. Gills should be examined under a microscope by a trained diagnostician for verification of the disease. Damaged gill tissue with fungal hyphae and spores will be present. As the tissue dies and falls off, the spores are released into the water and transmitted to other fish. High mortalities are often associated with this infection.

Avoidance is the best control for Branchiomycosis. Good management practices will create environmental conditions unacceptable for fungi growth. If the disease is present, do not transport the infected fish. Great care must be taken to prevent movement of the disease to noninfected areas. Formalin and copper sulphate have been used to help stop mortalities; however, all tanks, raceways, and aquaria must be disinfected and dried. Ponds should be dried and treated with quicklime (calcium oxide). A long term bath in *A criflavine Neutral* or *Forma-Green* for seven days helps this condition. Ponds should be dried and treated with quicklime (calcium oxide) and copper sulphate (2-3kg / ha). Dead fish should be buried.

8.4.3 Icthyophonus Disease

Icthyophonus disease is caused by the fungus, *Icthyophonus hoferi.* It grows in fresh and saltwater, in wild and cultured fish, but is restricted to cool temperatures (36° to 68°F). The disease is spread by fungal cysts which are released in the faeces and by cannibalism

of infected fish. Because the primary route of transmission is through the ingestion of infective spores, fish with a mild to moderate infection will show no external signs of the disease. In severe cases, the skin may have a "sandpaper texture" caused by infection under the skin and in muscle tissue. Some fish may show curvature of the spine. Internally, the organs may be swollen with white to grey-white sores. Diseased fish shows curious swinging movements hence the disease is called as swinging disease. Along with liver, particularly severely affected organs are: - spleen (salmonids), heart (herring), kidney (salmonids), gonads, brain (salmonids), gills (salmonids), and musculature and nerve tissue behind the eyes (sea fish).

Salt-like specks on the body fins. Excessive slime. Problems in breathing (Ich invades the gills), clamped fins, loss of appetite. Ich, white spot disease, whatever the name, this is the most common malady experienced in the home aquarium. Luckily, this disease is also easily cured if noticed in time. Ich is actually a protozoa called *Ichthyophthirious multifiliis*.

There are three phases to the life cycle of these protozoa. Normally, to the amateur aquarist, the life cycle is of no importance. However, since Ich is susceptible to treatment at only one stage of the life cycle, an awareness of the life cycle is important.

Adult Phase is embedded in the skin or gills of the fish, causing irritation (with the fish showing signs of irritation) and the appearance of small white nodules. As the parasite grows it feeds on red blood cells and skin cells. After a few days it bores itself out of the fish and falls to the bottom of the aquarium. In the cyst phase after falling to the bottom, the adult parasite forms into a cyst with rapid cell divisions occurring. Finally, the free-swimming phase occurs after the cyst phase, about 1000 free swimming young swim upwards looking for a host. If a host is not found within 2 to 3 days, the parasite dies. Once a host is found the whole cycle begins a-new.

The drug of choice is quinine hydrochloride at 30 mg per litre (1 in 30000). Quinine sulphate can be used if the hydrochloride is not available. The water may cloud but this will disappear. By reducing the time (with raised temperature) of the phases, you should be able to attack the free-swimming phase effectively. Most commercial remedies contain malachite green and /or copper, which are both effective.

8.5 Spoilage of Fish-Process and Its Prevention

The foods are usually classified as less perishable, moderately perishable and highly perishable in order to understand their perishable nature. Cereals, nuts and grains are included in less perishable and more stable category, vegetables as moderately perishable and seafood's as highly perishable food items. Seafood's are less stable because of their high moisture content and availability of nutrients for the growth of microorganisms. Ambient temperature plays a crucial role to alter the stability of a product. Highly perishable foods like seafood's have low tolerance to ambient temperature, while

moderately perishable items like fruits and vegetables have increased tolerance and non-perishable items are least affected.

8.5.1 Causative Factors of Spoilage

Spoilage is the indicative of post-harvest change. This change may be graded as the change from absolute freshness to limits of acceptability to unacceptability. Spoilage is usually accompanied by change in physical characteristics. Change in colour, odour, texture,

colour of eyes, color of gills and softness of the muscle are some of the characteristics observed in spoiled fish. Spoilage is caused by the action of enzymes, bacteria and chemicals present in the fish. In addition, the following factors contribute to spoilage of fish. High moisture content, high fat content, high protein content, weak muscle tissue, ambient temperature and unhygienic handling.

8.5.1.1 Enzyme Action

The Rigor mortis is a physical effect on the muscle tissue of fish caused by chemical changes following the death. In live fish, its movements are controlled by chemical signals which cause the euthymic contraction (stiffing) and relaxation of the muscles. This produces swimming action. After the death, the normal circulatory system breaks down and chemical signals leak into the muscle causing them to stiffen. This process is known as Rigor Mortis. In other words, in live fish the glycogen present in the muscle is converted to carbon dioxide and water after supply of oxygen to the cells. After the death of fish, the blood circulation stops and the supply of oxygen is prevented. The enzymes present in the muscle convert glycogen into lactic acid. The pH of the fish muscle falls. The formation of the lactic acid continues till the supply of glycogen is completely used up.

After the completion of rigor mortis, muscle stiffness gradually decreases accompanied by increase in pH, ending up in softening of muscle. This is followed by breakdown of proteins by enzymes. This process is called as autolysis. Thus, autolysis can be described as an internal breakdown of the structure of the protein and fats due to a complex series of reactions by enzymes. Autolysis of protein starts immediately after rigor and creates favorable conditions for the growth of bacteria. Another important action of the enzymes is that it affects the flavor of fish. The components responsible for the taste and flavor of the fish are changed by the enzymatic action. An example is the progressive, degradation of ATP to AMP and Hypoxanthine. Hypoxanthine is produced by the breakdown of ATP which is a main component of fish muscle nucleotide. The accumulation of Hypoxanthine imparts a bitter taste in the fish muscle accompanied by loss of fresh fish flavor. Thus, the estimation of Hypoxanthine content in fish indicates the degree of freshness. Enzymatic action also causes decomposition in the fish known

as belly bursting. The belly bursting is caused by the action of digestive enzymes present in the gut of the fish. The black spot formation in shrimps is also caused by the action of the enzymes on the amino acid. The black colour is due to the

formation of Melanin (Black Pigment) by the action of enzyme tyrosinase on tyrosin present in the shrimps. Black spots present a poor appearance and therefore, are not acceptable.

8.5.1.2 Bacteria Action

The freshly caught fish will be almost free from bacteria but the surface slime, gills and intestine may contain considerable load of bacteria. When the fish is dead, these bacteria start attacking the flesh causing spoilage and produce undesirable compounds. The nature and type of bacteria present in a fish depends upon the water from where it is caught and methods used for handling of the fish after its catch. The important changes brought out by the action of the bacteria in fish are as follows:

1. Reduction of TMAO to TMA: Marine fish contains a small percentage of odourless TMAO which is reduced to an offensive smelling TMA by the action of bacteria.
2. Breakdown of Amino Acids and formation of Primary Amines: the bacterial action of amino acids present in the fish muscle leads to formation of primary amines. Examples are formation of histamine from histidine, arginine from glutamic acid etc. This bacterial action may cause food poisoning in extreme cases.
3. Breakdown in Urea: the high concentration of urea in the flesh of some fishes is degraded to ammonia by the microorganisms. The formation of ammonia is accompanied by an offensive odour.

8.5.1.3 Chemical Action

The most common chemical action which causes spoilage is the oxidative rancidity in fatty fishes. The levels of peroxide value and free fatty acid content both a measure of oxidative rancidity is considered an index of quality of fat fishes.

8.6 Prevention/Reduction of Spoilage.

The activity of organism can be controlled, reduced or even retarded by proper handling and immediate lowering of the temperature. The chilling of the fish immediately after catch and holding the fish at $0^{o}C$ by proper icing will reduce the spoilage. In case of shrimps, removing head immediately after catch will reduce the rate of spoilage. In the case of big fishes, beheading and eviscerating will reduce the enzymatic actions which cause spoilage.

The spoilage is reduced or prevented in a number of ways like drying, salting, chilling, canning and freezing. Chilling is a means of short-term preservation of seafoods achieved by

the reduction in temperature using ice. Freezing is the most satisfactory method currently available for a long-term preservation of seafood.

It is, in fact by far the best way of preventing fish from spoilage, since fish continues to remain in almost the same natural conditions even after freezing. It is effective for retaining flavour, colour and nutritive value of seafoods. Freezing is a process by which the water in the fish muscle is crystallized into ice. The crystallization will be complete at -40°C. After freezing, the fish must be stored at a temperature maintained constantly at -18°C or below. Fluctuation in this temperature will cause spoilage of products. If there is a wide variation in the temperature recrystallisation takes place.

Dehydration is another important reaction of a physical nature caused by the evaporation of ice due to differences in vapour pressure over the product surface and in the air of the store room. Loss of the moisture by evaporation of ice causes the product surface to dry resulting in dull appearance and even discolouration in some cases. The evaporated water eventually condenses and freezes on the cooling surfaces of the store room and the transfer of moisture from the product will be continuous. Proper glazing and packaging eliminate this evaporation.

8.7 Zoonotic Parasites in Fish

8.7.1 *Mycobacterium*

Organisms in the genus *Mycobacterium* are non-motile, acid-fast rods. Two species, *M. fortuitum* and *M. marinum*, are recognized as pathogens of tropical fish. Humans are typically infected by contamination of lacerated or abraded skin with aquarium water or fish contact. A localized granulomatous nodule (hard bump) may form at the site of infection, most commonly on hands or fingers. The granulomas usually appear approximately 6-8 weeks after exposure to the organism. They initially appear as reddish bumps (papules) that slowly enlarge into purplish nodules. The infection can spread to nearby lymph nodes. More disseminated forms of the disease are likely in immunocompromised individuals. It is possible for these species of mycobacterium to cause some degree of positive reaction to the tuberculin skin test.

8.7.2 *Aeromonas*

Aeromonad organisms are facultative anaerobic, gram-negative rods. These organisms can produce septicemia in infected fish. The species most commonly isolated is *A. hydrophilia*. It is found worldwide in tropical fresh water, and is considered part of the normal intestinal microflora of healthy fish. Humans infected with *Aeromonas* may show

a variety of clinical signs, but the two most common syndromes are gastroenteritis (nausea, vomiting and diarrhea) and localized wound infections. Again, infections are more common and serious in the immunocompromised individual.

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CHAPTER NINE:

EPIDEMIOLOGY

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9.1 Introduction

Epidemiology can be referred to a branch of medical science that deals with the study of the distribution and patterns of health-events, health-characteristics and their causes or influences in a well-defined population mainly by the use of statistical tools; and as well as the application of such study results to control health problems. Epidemiology, however, is the cornerstone method of public health research and practice, and helps inform policy decisions and evidence-based medicine by identifying risk factors for disease(s) and targets for preventive medicine and public policies. Epidemiologists are involved in the design of studies, collection and statistical analysis of data, and Interpretation and dissemination of the results. They use a collection of data and a broad range of biomedical and psychosocial theories in a repeated manner to generate or expand theory, test hypotheses, and try to make educated, informed assertions about which relationships are causal, and how exactly they are able to cause the disease or illness.

In recent years, epidemiology has significantly contributed to improve methods used in clinical research, however, it is nearly impossible to assume with precision how even the most simple physical systems behave beyond the immediate future, much less the complex field of epidemiology; for epidemiologists, the key is in the term inference. Major areas of epidemiological study include investigation of outbreak, disease surveillance and screening, biomonitoring, and clinical trials to compare treatment effects. Epidemiologists rely on a number of other scientific disciplines such as biology (to better understand disease processes), biostatistics (to make efficient use of the data and draw appropriate conclusions), and exposure assessment and social science disciplines (to better understand proximate and distal risk factors, and their measurement).

9.1.1 Etymology

Epidemiology is derived from Greek epi, meaning "upon, among", demos, meaning "people, district", and logos, meaning "study", which literally means "the study of what is upon the people"(Nutter,1999).

The distinction between 'epidemic' and 'endemic' was first drawn by Hippocrates, to distinguish between diseases that are 'visited upon' a population (epidemic) from those that 'reside within' a population (endemic). The term 'epidemiology' appears to have first been used to describe the study of epidemics in 1802 by the Spanish physician

Villalba in *Epidemiologia*, *Espanola* (Carol, *et al.,* 1998). Epidemiologists also study the interaction of diseases in a population, a condition known as a syndemic. However, the term epidemiology is now widely applied to cover the description and causation of not only epidemic disease, but of disease in general, and even many non-disease health-related conditions, such as high blood pressure and obesity.

9.1.2 History

Ancient Era

The first epidemiologist was Hippocrates, a Greek physician who isreferred to as the father of medicine. He first examined the relationships between the occurrence of disease and environmental influences and he framed the terms endemic (for diseases usually found in some places but not in others) and epidemic (for diseases that are seen at sometimes but not others) (Alfredo, 2004). One of the earliest theories on the origin of diseases was that it was primarily the fault of human luxury. This was expressed by philosophers such as Plato and Rousseau, and social critics like Jonathan Swift.

In the middle of the 16th century, Girolamo Fracastoro, a doctor from Verona was known as the first to propose a theory that diseases are caused by tiny, microscopic particles and such particles were alive. According to him, those particles were considered to be able to spread by air, multiply by themselves and to be destroyable by fire. In this way he refuted Galen's miasma theory of poison gas in sick people. He then wrote a book in 1543, titled *De contagioneet contagiosis morbis*, in which he promoted personal and environmental hygiene to prevent diseases. The development of a sufficiently powerful microscope by Anton van Leeuwenhoek in 1675 provided visual evidence of living particles consistent with a germ theory of disease.

Modern Era

In the 19th century, Dr. John Snow referred to as the father of modem epidemiology was famous for his investigations into the causes of cholera epidemics.His work demonstrates the classic sequence from descriptive epidemiology to hypothesis generation to hypothesis testing (analytic epidemiology) to application (Snow, 1963). He began with noticing the significantly higher death rates in two areas supplied by Southwark Company. His identification of the Broad Street pump as the cause of the Soho epidemic is considered the classic example of epidemiology. He used chlorine in an attempt to clean the water and had the handle of the pump removed, thus ending the outbreak (Snow, 1963). This has been perceived as a major event in the history of public health and regarded as the founding event of the science of epidemiology, having helped shape public health policies around the world. Other pioneers include Danish physician Peter Anton Schleisner, who in 1849 related his work on the prevention of the epidemic of neonatal tetanus on the Vestmanna Islands in Iceland. Another important pioneer was

Hungarian physician IgnazSemmelweis, who in 1847 brought down infant mortality at a Vienna hospital by instituting a disinfection procedure. His findings were published in 1850. Disinfection did not become widely practices until British surgeon Joseph Lister 'discovered' antiseptics in 1865 in light of the work of Louis Pasteur.

In the early 20th century, mathematical methods were introduced into epidemiology by Ronald Ross, Anderson GrayMckendrick and others. Another breakthrough was the 1954 publication of the results of a British Doctor's study, led by Richard Doll and Austin Bradford Hill, which lent very strong statistical support to the suspicion that tobacco smoking was linked to lung cancer.

The Profession

Some epidemiologists work 'in the field'; i.e., in the community, commonly in a public health/health protection service and are often at the forefront of investigating and controlling disease outbreaks. Others work for non-profit organizations, Universities, Hospitals and larger government entities such as the Centers for Disease Control and Prevention (CDC), the Health Protection Agency, The World Health Organization (WHO), or National Agency for Food and Drug Administration and Control NAFDAC, Standard Organization of Nigeria. Epidemiologists can also work in for-profit organizations such as pharmaceutical and medical device companies in groups such as market research or clinical development.

The Practice

Epidemiologists employ a range of study designs from observational to experimental and epidemiological studies are aimed, where possible, at revealing unbiased relationships between exposures, biological agents, stress, or chemicals to morbidity or mortality. The identification of causal relationships between these exposures and outcomes is an important aspect of epidemiology. Modern epidemiologists use informatics as a tool.

The term 'epidemiologic triad' is used to describe the intersection of Host, Agent, and Environment in analyzing an outbreak. Diseases occur when host, agent and environment are not balanceddue to new agent, change in existing agent, change in number of susceptibilities in the population,and environmental changes that effect the agent or growth of agent. Therefore, there must be a unique combination of events, i.e. a harmful agent that comes into with a susceptible host in a proper environment.

Figure 1: The "epidemiologic triad" of infectious disease summarizes the factors that influence an infection, and the measures you might take to combat the infection. Source: Ian McDowell In: Johnson Y. J. (2018)

Epidemiologists tend to use synonyms for the **5 W's**: diagnosis or health event (what), person (who), place (where), time (when), and causes, risk factors, and modes of transmission (why/how) (Kobayashi, 2020).

Advocacy

Epidemiologic evidence is often used to advocate both personal measures like diet change and corporate measures like removal of junk food advertisement, with study findings disseminated to the general public to help people to make informed decisions about their health, often the uncertainties about these findings are not well communicated; news articles often prominently report the latest result of one study with little mention of its limitations, bias, warnings, or context. The Epidemiology Forum (IEF) guidelines suggests that advocacy is appropriate, it recommend separating the roles of scientist and advocate, and the Council of International Organizations of Medical Sciences (CIOMS) guidelines recommend advocacy dependent on the quality of epidemiologic research and on causal interpretations of the data (Weed, 1994).

Epidemiology Terminologies

Agents - are biological, physical, or chemical factors that contribute to the occurrence of a disease. Biological agents such as viruses and bacteria are often necessary causes for (infectious) diseases. Chemical agents such as poisons or allergens, or physical agents such a

radiation, noise, or heat, are all non-biological agents that are frequently not necessary causes for a disease but contributing factors.

Attributable risk – is a measure of association in cohort studies and experimental studies. The attributable risk is a difference measure and calculated as the difference between the incidence of the outcome in the exposed group (or intervention) and the incidence of the outcome in the unexposed group (or control).

Bar Chart (univariate) -is a graphical display of a categorical variable. A barchart consists of separate disjointed rectangles representing the frequencies of observations of the different categories. In a bar chart, the heights of the bars directly reflect the frequencies.

Basic reproductive rate - is the average number of people directly infected by an infectious case during its infectious period, when the case enters a completely susceptible population. The basic reproductive rate is the theoretical potential of an infection to spread in an entirely susceptible population.

Categorical variable - is a characteristic with defined categories, such as gender (categories: male and female) or blood group (categories: A, B, AB, 0). Categorical data have to be recorded in exhaustive and exclusive categories that is, there must be enough categories so that each observation fits into a category (exhaustive), and one category only (exclusive).

Chronic disease - a long-lasting, persistent or recurrent disease. Chronic diseases often lead to a loss of function, impairment, and long-term disabilities. Typical chronic diseases include cardiovascular diseases, cancer, diabetes mellitus, asthma, and musculoskeletal diseases. These are diseases with complex aetiologies.

Acute disease - Acute diseases come on rapidly, and are accompanied by distinct symptoms that require urgent or short-term care, and get better once they are treated.

Classical public health epidemiology – generally aims to investigate distributions and causes of diseases in populations.

Clinical epidemiology - studies the diagnosis, prognosis, and therapies of patients. Clinical epidemiology is conducted in a clinical setting, usually by clinicians, with patients as the participants.

Clinical equipoise - Clinical trials comparing two different treatments can only be ethically justified if there is no convincing evidence that one treatment is better than the other. This prerequisite has been called clinical equipoise.

Closed cohort - is a cohort in which membership begins at a defined time or with a defining event and ends only with observed the study outcome, the end of eligibility for membership, or the end of the study period.

Cluster sampling - is a form of probability sampling that involves sampling in naturally occurring clusters such as schools, households, or suburbs. In single-stage cluster sampling, a random sample of clusters is drawn and within each selected cluster either all units of analysis or a random sample of units are observed. An example of a two-stage cluster sampling is randomly sampling schools in Australia, randomly sampling classes within selected school, and within each selected class all students are invited to participate.

Conceptual research hypothesis – is another word for an initial research idea.
Confidence interval – is part of inferential statistics. A confidence interval allows the following statement about the unknown population parameter by taking exclusively information from a sample into account: The true but unknown population parameter lies within a (1-α)-confidence interval with a probability of 1-α. In most cases α is set to 5% and as a consequence 95% confidence intervals are calculated.

Determinant-centred epidemiology - is epidemiological research that investigates the effect of a specific determinant or exposure on health outcomes. For example, nutritional epidemiology investigates the effect of diet on health.

Diagnostic test – is a test applied to a person in order to determine the health status of the person. In contrast to a screening test, a diagnostic test is usually applied to symptomatic persons. Diagnostic tests are often used to confirm diseases suggested by symptoms and other circumstantial evidence.

Directionality – is the inner logic of an analytical study design. A study follows a forward directionality when first the exposure groups are defined based on the study factor and then the participants are followed-up to detect the outcome. Studies of "backward" directionality first define groups based on the outcome and then look backwards to exposure status of

participants. "Non-directional" means that both exposure and outcome are observed simultaneously in one group of participants.

Disease-centred epidemiology – is epidemiological research that focuses on only one disease or defined group of diseases and investigates distribution and determinants of this disease or group of diseases. For example, cancer epidemiology investigates distribution and determinants of cancers.

Dynamic cohort - is a cohort that gains and loses members throughout its existence. Most cohorts in epidemiology are dynamic.

Endemic – means the occurrence of a disease in a population or region at 'normally' expected levels. Endemic implies that the disease is able to maintain itself in a population or region without cases entering the population or region from outside.

Environment – in an epidemiological context refers to the habitat in which the biological agent and the host exist, survive or originate.

Epidemic – means the occurrence of a disease in a population or geographical region at clearly higher levels than 'normally' expected.

Epidemic curve – is a graphical display of the distribution of cases of an outbreak by time of onset.

Epidemiological (demographic) transition – is the transition from high mortality rates, usually caused by infectious diseases, to lower mortality rates mainly caused by chronic diseases in a country or region. It is usually accompanied by a transition from high to low fertility rates. The theory of demographic transition evolved to explain the rapid changes in population structure as observed during industrialization of western countries in the 19th and 20th century.

Epidemiological process – is an idealised concept on how to conduct epidemiological research. It is a cyclic process governed by the scientific method. Current theory and knowledge inform of a research idea. A study design is chosen to investigate the research idea. An operational, falsifiable, research hypothesis is formulated. Tools are developed to collect data in a standardised way. Data is collected, collated and statistically analysed. The results of this analysis confirm or reject the operational research hypothesis. The results of the study are published and thereby integrated into the current theory and knowledge.

Ethics - is the part of philosophy that deals with moral issues such as good and evil, right and wrong, what is just, etc.

Evidence-based practice - is an approach to health care where health professionals use the best currently available evidence possible. Evidence-based practice uses the most appropriate and most current information available to make optimal clinical decisions for individual patients.

Health – is a state of complete physical, mental, and social well-being; not merely the absence of disease or infirmity

Host – is a person or other animal that harbours an infectious agent.

Incidence - is a measure of disease frequency. Incidence quantifies the number of new cases (incident cases; i.e. people newly acquiring a disease or an attribute) in a population at risk of developing the disease over a given period of time.

Incidence rate - is the number of new cases (i.e. people newly acquiring a disease or an attribute) developing during a specific period of time divided by the total disease-free person-time of observation seen in the population at risk.

Incubation period - is the time interval between exposure to a sufficient cause of the disease and the onset of symptoms. The incubation period = Induction period + Latency period.

Infectious disease (Communicable disease) - is an illness caused by transmission of a specific infectious biological agent or its toxic products (= necessary cause) from an infected person, animal, or reservoir to a susceptible host. The transmission can occur directly or indirectly from a plant or animal host, vector, or the inanimate environment.

Infectious disease epidemiology – is the part of epidemiology that focuses on infectious diseases. Infectious disease epidemiology raises very specific questions about agents, transmission routes, and immunization. Infectious disease epidemiology provides models explaining occurrence and development of infectious disease outbreaks.

Point prevalence – is the total number of people with a disease or an attribute divided by the total number of people in the population at a given point in time.

Population at risk - are all people under observation who initially do not have the disease or the attribute but are "at risk" of acquiring the disease or the attribute.

Positive predictive value - of a diagnostic or screening test is the probability that a person with a positive test result will actually have the disease.

Prevalence - is a measure of disease frequency. Prevalence quantifies the number of existing cases (prevalent cases; i.e. people with a disease or an attribute) in a population at a point in time or during a period of time. Prevalence is also the number of existing cases of a disease in a population at a given time.

Prevalence odds-ratio – is a measure of association used in cross-sectional studies. The prevalence odds-ratio compares the odds of the prevalence of the outcome in the exposed group with the odds of the prevalence of the outcome in the unexposed group.

Primary prevention – are public health efforts that are directed towards the stage of susceptibility of a disease. Primary prevention aims to prevent or reduce "exposure" and thus the possibility of the disease occurring. An example of primary prevention is the "Slip, Slop, Slap" campaign to reduce sun exposure and hence prevent skin cancer.

Relative risk – is a ratio measure of association in cohort studies and experimental studies. The relative risk is the incidence of the outcome in the exposed (or intervention) group divided by the incidence of the outcome in the not-exposed (or control) group.

Reliability (Consistency, repeatability, precision, or reproducibility) – of 'measurements' means that if "measurements" were repeated with the same participants by the same or a different health professional, the results of the repeated "measurements" would be very similar or even identical to the first findings. The "measurements" might be responses to questions, results of diagnostic tests, or physical measurements such as height or weight. Also important is the reliability of the overall results of an

epidemiological study (i.e. the amount of random error involved) which is assessed with statistical techniques such as confidence intervals and statistical hypothesis testing.

Representative uniformity – is a pre-requisite for the internal validity of an epidemiological study. Representative uniformity means that the sample(s) represent the target population. Representative uniformity implies that there is no selection bias.

Target population - is the population about which one wants to draw conclusions. The actual population, and with that, the sample may or may not be representative of the target population. The target population is partly defined by the exclusion and inclusion criteria of a study. When conducting a study, it is most important to define the target population first to ensure appropriate sampling.

Tertiary prevention - are public health efforts that are directed towards the clinical stage of a disease. Tertiary prevention aims preventing or minimising the progression of a disease or its consequences. A randomised controlled trial that aims to identify best treatment for a disease is an example of tertiary prevention.

9.2 Types of Epidemiologic Studies

The three major epidemiologic studies are case series/cross-sectional studies, case-control, and cohort

a. Case series or cross-sectional studies

Case-series may refer to the qualitative study of the experience of a single patient, or group of patients with a similar diagnosis, or to a statistical technique comparing periods during which patients are exposed to some factor with the potential to produce illness with periods when they are unexposed. Case series identify unusual features of a disease or of individuals and may lead for example to the formulation of new aetiological hypotheses(Hennekens and Buring 1987), the identification of a new disease, or the identification of adverse effects to a certain exposure.

b. Case control studies

A case-control study starts by categorizing groups according to the outcome (e.g. disease present or absent) and then looks back to establish the study factor (e.g. exposure present or absent). In case-control studies, individuals suffering from the studied disease are compared with controls who do not have the disease and exposure is recorded retrospectively (Ressing, 2010). Results of a case-control study are often expressed as exposure odds-ratios OR and is unable to estimate relative risk RR. Case control studies, however, select subjects based on their disease status. A group of individuals that are disease positive (the "case" group) is compared with a group of disease negative individuals (the "control" group). The control group should ideally come from the same population that gave rise to the cases. The case control study looks back through time at potential exposures that both groups (cases and controls) may have encountered. A 2x2 contingency table is constructed; displaying exposed cases (A), exposed controls (B), unexposed cases (C) and unexposed controls (D). The statistics generated to measure

association is the odds ratio (OR), which is the ratio of the odds of exposure in the cases (A/C) to the odds of exposure in the controls (B/D), i.e. OR = (AD/BC).

Cases Controls

Expose d A B

Unexposed C C D

If the OR is clearly greater than 1, then the conclusion is "those with the disease are more likely to have been exposed," whereas if it is close to 1, then the exposure and disease are not likely associated. If the OR is far less than one, then this suggests that the exposure is a protective factor in the causation of the disease. Case control studies are usually faster and more cost effective than cohort studies, but are sensitive to bias (such as recall bias and selection bias).

c. Cohort studies

A cohort study starts by defining groups by the study factor (e.g. exposure present or absent) and then follows-up these exposure groups to detect the outcome (e.g. disease present or absent).Individuals exposed to specific risk factors are compared with individuals not exposed to these factors in a cohort study, and the incidence of diseases or mortality in these two groups is observed (Ressing, 2010). A cohort study is able to estimate relative risk since incidences are observed. The data from a cohort study allow the estimation of incidence rate and mortality rate as simple descriptive measures of frequency, as well as relative risk (RR) or hazard ratio (HR) as comparative effect measures. Standardized incidence ratios (SIR) or standardized mortality ratios (SMR) are used for comparison with the general population (Ressing, 2010).The RR compares the risk of health event among one group with the risk among other group. It is calculated by dividing the risk of disease for an exposed individual by the risk of disease for a non-exposed individual(Sauerbrei, 2009).

Prospective studies have many benefits over case control studies. The RR is a more powerful effect measure than the OR, as the OR is just an estimation of the RR, since true incidence cannot be calculated in a case control study where subjects are selected based on disease status. Temporality can be established in a prospective study, and confounders are more easily controlled for. However, they are more costly, and there is a greater chance of losing subjects to follow-up based on the long time period over which the cohort is followed.

9.3 Validity and Error

9.3.1 Validity

A diagnostic test is valid if the results of the diagnostic test are correct, that is, if the test is able to differentiate correctly between diseased people and people free of the disease.

Validity, often refers to the overall result of an epidemiological study. The results of an epidemiological study are called valid if no bias (i.e. no systematic error that distorts the results) is present. Different fields in epidemiology have different levels of validity. One way to assess the validity of findings is the ratio of false-positives (claimed effects that are not correct) to false-negatives (studies which fail to support a true effect). Validity is usually separated into two components:

Internal validity is dependent on the amount of error in measurements, including exposure, disease, and the associations between these variables. In other words,internal validity refers to the inner workings of a study i.e. the design used, variables measured, correct analysis (Bovbjerg, 2020). Good internal validity implies a lack of error in measurement and suggests that inferences may be drawn at least as they pertain to the subjects under study.

External validity pertains to the process of generalizing the findings of the study to the population from which the sample was drawn (or even beyond that population to a more universal statement). External validity is however, truth beyond a study. A study is external valid if the study conclusions represent the fact for the population to which the results will be applied because both the study population and the reader's population are similar enough in important characteristics (Gay, 2022).External validity can therefore, only occur if the study is first internally valid.

9.3.2 Error

Error, on the other hand, is the difference recorded between the calculated or measured value and the true outcome. This type of error is thus explained:

Random Error

Random error exists in all studies, because to some extent, it exists in all measurements. It is a type of error that is governed by chance, and is the result of fluctuations around a true value because of sampling variability. The smaller the random error in a study the more reliable are the results of the study. Standard statistical methods are used to quantify random error and the role it may or may not have played in the interpretation of a study's results.Random error occurs in every epidemiological study due to natural or biological variation. It can occur during data collection, coding, transfer, or analysis. Random errors cannot be eliminated entirely, and by correctly interpreting *p*-values and confidence intervals (CIs), we can place our results in the appropriate context (Bovbjerg, 2020).

Examples of random error include: question not properly structured, poor interpretation of an individual response from a particular respondent, or a typographical error during coding. Random error affects measurement in a transient, inconsistent

manner and it is impossible to correct for random error. Random error is assessed during the statistical analysis of the data collected in a study.

Precision is inversely related to random errorand is a measure of random error. Therefore, to reduce random error is to increase precision. Confidence intervals are computed to demonstrate the precision of relative risk estimates. The narrower the confidence interval, the more precise the relative risk estimate.

There are two basic ways to reduce random error in an epidemiological study. The first is to increase the sample size of the study. In other words, add more subjects to your study. The

second is to reduce the variability in measurement in the study. This might be accomplished by using a more precise measuring device or by increasing the number of measurements.

Systematic Error

Systemic error is a type of error which acts on the results of a study in a systematic way. It is on the other hand, *consistent*, repeatable error associated with faulty equipment or a flawed experiment design (Glen, 2022).The smaller the systematic error in a study the more valid are the results of the study. Like random error, systematic error occurs in every epidemiological study. Study design features such as randomization, blinding and matching are used to minimize systematic error. An example is a plastic tape measure that becomes slightly stretched over the years, resulting in measurements that are slightly too high. The validity of a study is dependent on the degree of systematic error.

It is quite difficult to detect and prevent systematic error. So, in order to avoid this error, the researcher should know the limitations of the equipment in use and understand how the experiment works. This can help identify areas that may be prone to systematic errors.

9.3.3 Bias

Bias, refers to systematic errors, meaning that they disproportionately affect the data in one direction only. Bias can be minimized with correct study design and measurement techniques, but it can never be omitted entirely. All studies have bias because humans are involved, and humans are inherently biased.

Selection Bias

Selection bias refers to a distortion in the effect measure resulting from the manner in which the people are selected for the sample. In other word, selection bias occurs when study subjects are selected or become part of the study as a result of a third, unmeasured variable which is associated with both the exposure and outcome of interest. If it occurs,

the sample(s) do not represent the target population and it can threaten the internal validity of a study.Selection bias adversely affecting internal validity occurs when the exposed and unexposed groups (for a cohort study) or the diseased and non-diseased groups (for a case-control study) are not drawn from the same population (Beckmann and Beckmann, 1990). Examples of selection bias are volunteer bias (the opposite of which is non-response bias)in which participants and non-participants differ in terms of exposure and outcome.

Confounding Bias

Confounding bias may occur if the effect of the study factor on the outcome is mixed in the data with the effect of another variable (confounder). Whether confounding truly exists in a study can only be assessed during data analysis. Confounding has traditionally been defined as bias arising from the co-occurrence or mixing of effects of extraneous factors, referred to as confounders, with the main effect(s) of Interest. A confounder is thus a third variable—not the exposure, and not the outcome (Goldstein, 2016)—that biases the measure of association we calculate for the particular exposure/outcome pair.

Information Bias

Information bias refers to a distortion in the effect measure, due to measurement error or misclassification of participants for one or more variables. It occurs when the measurement of either the study factor or the outcome is systematically inaccurate. Information bias most times arises from systematic error in the assessment of a variable (Rothman, 2002).

9.4 Guidelines for Assessing Causality

Causality (Disease aetiology) – is about relating causes to their effects. In the context of epidemiology, causality is about identifying the causes of disease. Sir Austin Bradford Hill in 1965 detailed criteria for assessing evidence of causation (Hill, 1965). These guidelines are sometimes referred to as the Bradford-Hill criteria.

1. **Strength:** A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.
2. **Consistency:** Consistent findings observed by different persons in different places with different samples strengthen the likelihood of an effect.
2. **Specificity:** Causation is likely if a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.
3. **Temporality:** The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that

delay).Biological gradient: Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an Inverse proportion is observed: greater exposure leads to lower incidence.

4. **Plausibility:** A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).

5. **Coherence:** Coherence between epidemiological and laboratory findings increases the likelihood of an effect. However, Hill noted that ".... lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations Experiment: Occasionally it is possible to appeal to experimental evidence ".

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17

CHAPTER TEN: PLANT DISEASES

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Disease in plants can be defined as any disturbance brought about by a pathogen or due to environmental factors which interferes with the physiological function of a plant. Agrios (2005) has defined disease in plants as a series of invisible and visible response of plant and tissues to a pathogenic microorganism or environmental factors that result in adverse change in the form, function, or integrity of the plant parts. According to Nutter *et al.*, (2006), disease is a malfunctioning process that is caused by continuous irritation.

10.1 Concept of Disease in Plants

A plant is healthy when it can carry out its physiological functions to the best of its genetic potential. These functions include:

1. Normal cell division
2. Differentiation and development
3. Absorption of water and minerals from the soil.
4. Photosynthesis and translocation of photosynthetic products.
5. Reproduction and the storage of food supplies.

Whenever plants are disturbed by pathogens or by environmental factors one or more of these functions are altered with beyond certain deviation from the normal, then the plants become diseased. The specific mechanisms by which diseases are produced vary considerably with the causal agent and sometimes with the plant. At first the reaction of the plant to the disease-causing agent is at the site of affliction, which is of chemical nature and invisible. Soon, however, the reaction becomes more widespread and histological changes take place that manifest themselves macroscopically and constitute the symptoms of the disease.

10.2 Classification of Plant Diseases

Plant diseases are classified according to the following criteria:

1. Symptoms they cause e.g. Root rots, cankers, wilts, leafspots, blights, mosaic etc.
2. Plant organ they affect e.g. root diseases, stem diseases, fruit diseases etc.
3. Types of plants affected e.g. field crops diseases, vegetable diseases, fruit tree diseases, diseases of ornamental plants etc. (Agrios, 2005).

However, the most useful criteria for classifying plant diseases are the type of pathogen that causes the diseases. On this basis plant diseases are classified as:

1. Infectious plant diseases which include:
    i. Diseases caused by fungi
    ii. Diseases caused by bacteria
    iii. Diseases caused by mycoplasmas
    iv. Diseases caused by parasitic higher plants
    v. Diseases caused by viruses

vi. Diseases caused by nematodes
vii. Diseases caused by protozoa.

2. Non infectious diseases
   i. Inadequate temperature
   ii. Lack or excess light
   iii. Inadequate oxygen
   iv. Nutrient deficiencies
   v. Mineral toxicities
   vi. improper agricultural practices etc.

Indirectly, environmental factors that cause a plant to be stressed may result in the plant's gradual decline. Decline results in the plant being more susceptible to disease organisms. Because of this, diagnosing plant diseases can be tricky. The real cause of a problem may be the stress factors, with the disease simply being a secondary factor. Three critical factors or conditions must exist for disease to occur: a susceptible host plant, a pathogen, and the right mix of environmental conditions. The relationship of these factors is called the disease triangle. If only a part of the triangle exists, disease will not occur. Understanding the disease triangle helps us understand why most plants are not affected by the many thousands of diseases that exist. Pathogens are microorganisms that cause disease. Because they are living, they are called biotic (bye-AH-tick) agents or causes. Pathogens can be fungi (FUN-geye), bacteria, viruses, mycoplasmas (MY-crow-plas-mahs) or nematodes (KNEE-ma-toads). Each has a different life cycle, which includes an infectious stage (Agrios, 2005).

Most pathogens are host-specific to a particular plant species, genus or family. For instance, blackspot of rose will not attack marigolds or lettuce. Some diseases, such as the powdery mildews, produce similar symptoms on different plants. However, the fungi involved are usually host-specific. The rose powdery mildew fungus will not infect zinnias or turfgrass or vice-versa.

A susceptible host has a genetic makeup that permits the development of a particular disease. The genetic defense against a disease is called disease resistance. This resistance can be physical characteristics of the plant (fuzzy or waxy leaf surfaces), chemical characteristics (enzymes that kill pathogens and lack of enzymes) and growth patterns (ability to block off diseased tissue or outgrow damage). Plants also may be disease-tolerant. Even though infected with a disease, they can grow and produce a good crop or maintain an acceptable appearance. The plant outgrows the disease and symptoms are not apparent or at a damaging level (Zadoks, 2001).

Certain environmental conditions must exist for disease pathogens to cause infection. The specific conditions vary for different pathogens. High moisture and specific temperature ranges, for example, are necessary for many fungal diseases. These conditions must continue for a critical period of time while the pathogen is in contact with the host for infection to occur. Moisture, temperature, wind, sunlight, nutrition and soil quality affect plant growth. If one of these factors is out of balance for the

culture of a specific plant, that plant may have a greater tendency to become diseased. For example, lilacs growing in shade are more likely to be infected with powdery mildew than those growing in full sunlight. Often gardeners believe that their plants have become diseased overnight. This may be true in the case of damping-off. More often, however, much has occurred before symptoms are seen. There are five stages in disease development: inoculation, incubation, penetration, infection and symptoms (Zadoks,2001).

INOCULATION

The pathogen must be introduced (inoculated) to the host plant. Most pathogens cannot move on their own, but must be carried to the host plant. This is done by rain, wind, insects, birds and people (Nutter *et al.*, 2006).

INCUBATION

The second stage of disease development is incubation. The pathogen changes or grows into a form that can enter the new host plant. In many fungal diseases, the pathogen arrives on the plant as a spore which must germinate before it can grow into the plant.

PENETRATION

The third stage is penetration or the point at which the pathogen actually enters the host plant. Once the fungal spore germinates, it sends out thread-like tubes call hyphae. These penetrate the plant through wounds or natural pores. Wounding roots of bedding plants during transplanting provides entry for root-rotting fungi. The mouthparts of an insect also result in openings for penetration.

Figure 1.Penetration of disease in plant.

Source: Agrios, 2005

Indirect Penetration:

Penetration of plant tissues by a pathogen through natural openings

INFECTION

The fourth stage is infection. The pathogen grows within the plant and begins damaging the plant tissue.As the pathogen consumes nutrients, the plant reacts by showing symptoms. Symptoms are evidence of the pathogens causing damage to the plant. Symptoms include mottling, dwarfing, distortion, discoloration, wilting, and shriveling of any plant part (Nutter *et al.*, 2006).

SYMPTOMS

As the pathogen consumes nutrients, the plant reacts by showing symptoms. Symptoms are evidence of the pathogens causing damage to the plant. Symptoms include mottling, dwarfing, distortion, discoloration, wilting, and shriveling of any plant part. As shown in figure 2.

Figure 2. Symptoms of a diseased plant.

Source: Agrios, 2005

PLANT PATHOGENS

FUNGI

The majority of phytopathogenic fungi belong to the *Ascomycetes* and the *Basidiomyce tes*. The fungi reproduce both *sexually* and *asexually* via the production of *spores* and other structures. Spores may be spread long distances by air or water, or they may be soil borne. Many soil inhabiting fungi are capable of living *saprotrophically*, carrying out the part of their lifecycle in the *soil*. These are known as facultative saprotrophs (Wright, 2012).

Fungal diseases may be controlled through the use of *fungicides* and other agriculture practices, however new *races* of fungi often *evolve* that are resistant to various fungicides.

Biotrophic fungal pathogens colonize living plant tissue and obtain nutrients from living host cells. Necrotrophic fungal pathogens infect and kill host tissue and extract nutrients from the dead host cells (Wright, 2012).

Figure 3: *Rice blast*, a necrotrophic fungus
Source: Agrios, 2005

Significant fungal plant pathogens include:

Ascomycetes

- *Fusarium* spp. (causal agents of *Fusarium* wilt disease) e.g .*Fusarium oxysporum* in guava and tomato fruits.

- *Thielaviopsis* spp. (causal agents of: canker rot, black root rot, *Thielaviopsis* root rot)
- *Verticillium* spp.
- *Magnaporthegrisea* (causal agent of blast of rice and gray leaf spot in turf grasses) (Camire *et al.*, 2009).

Basidiomycetes

- *Rhizoctonia* spp.(causal agent of rice sheath blast, and jute plant).

- *Phakosporapachyrhizi* (causal agent of soybean rust)
- *Puccinia* spp. (causal agents of severe rusts of virtually all cereal grains and cultivated grasses)

Oomycetes

The*oomycetes* are not true fungi but are fungus-like organisms They include some of the most destructive plant pathogens including the *genus Phytophthora*, which includes the causal agents of potato late blight and *sudden oak death*. Particular species of oomycetes are responsible for*root rot*. (Camire *et al.*, 2009).

Despite not being closely related to the fungi, the oomycetes have developed very similar infection strategies. Oomycetes are capable of using effect or proteins to turn off a plant's defenses in its infection process. Plant pathologists commonly group them with fungal pathogens (Camire *et al.*, 2009)..

Significant oomycete plant pathogens

- *Pythium* spp.(causal agent of ginger rhizome rot and soft rot in tomato).

- *Phytophthora* spp.; including the causal agent of the *Great Irish Famine (1845–1849)*

Bacteria

Figure 4. Crown gall disease caused by an *Agrobacterium*

Source: Agrios, 2005

Most *bacteria* that are associated with plants are actually *saprotrophic*, and do no harm to the plant itself. However, a small number, around 100 known species, are able to cause disease. Bacterial diseases are much more prevalent in *sub-tropical* and *tropical* regions of the world. Most plant pathogenic bacteria are rod-shaped (*bacilli*). In order to be able to colonize the plant they have specific pathogencity factors. Five main types of bacterial pathogenicity factors are known: uses of Cell wall-degrading enzymes, *Toxins*, Effect or proteins, *Phytohormones* and *Exopolysaccharides* (Jackson, 2009).

Pathogens such as *Erwinia*, use Cell wall-degrading enzymes to cause *soft rot*. *Agrobact erium* changes the level of *auxins* to cause tumours with phytohormones. *Exopolysaccharid es* are produced by bacteria and block *xylem* vessels, often leading to the death of the plant.

Significant bacterial plant pathogens:

- *Burkholderia*

- *Proteobacteria*

  - *Xanthomonas* spp (causal agent of bacterial blight in rice).

- *Pseudomonas* spp. (causal agent of bacterial wilt in potato).
- *Pseudomonas syringae in tomato*, causes tomato plants to produce less fruit, and it "continues to adapt to the tomato by minimizing its recognition by the tomato immune system (Jackson, 2009).

Phytoplasmas (Mycoplasma-like organisms) and spiroplasmas

Phytoplasma and *Spiroplasma* are a genre of bacteria that lack cell walls, and are related to the *mycoplasmas*, which are human pathogens. Together they are referred to as the *mo l-*

licutes. They also tend to have smaller *genomes* than true bacteria. They are normally transmitted by sap-sucking *insects*, being transferred into the plants *phloem* where it reproduces.

Figure 5.*Tobacco mosaic virus*

Source: Agrios, 2005

Viruses, viroids and virus-like organisms

There are many types of *plant virus*, and some are even *asymptomatic*. Under normal circumstances, plant viruses cause only a loss of *crop yield*. Therefore, it is not economically viable to try to control them, the exception being when they infect *perennia l* species, such as fruit trees (Popp and Hantos, 2011).

Most plant viruses have small, single-stranded *RNA genomes*. These genomes may encode only three or four *proteins*: a *replicas'*, a coat protein, a *movement protein*, in order to allow cell to cell movement though *plasmodesmata*, and sometimes a protein that allows transmission by a vector. Plant viruses must be transmitted from plant to plant by a *vector*. This is often by an *insect* (for example, *aphids*), but some *fungi*, *nematodes*, and *pro tozoa* have been shown to be viral vectors.eg. Rice turgo, maized warf mosaic, downy mildew of lettuce,citrus tristeza and bean mosaic (Popp and Hantos, 2011).

Nematodes

Figure 6.*Root-knot nematode* galls

Source: Agrios, 2005

Nematodes are small, multicellular wormlike creatures. Many live freely in the soil, but there are some species that parasitize plant *roots*. They are a problem in *tropical* and *s ubtropical* regions of the world, where they may infect *crops*. Potato cyst nematodes (*Globoderapallida* and *G. rostochiensis*). Root knot nematodes have quite a large host range, whereas cyst nematodes tend to be able to infect only a few species. Nematodes are able to cause radical changes in root cells in order to facilitate their lifestyle.e.g. Root knot of vegetables, cereal cyst nematodes, soybean cyst nematode and white tip disease of rice (Sharma and Thakur, 2007).

Protozoa

There are a few examples of plant diseases caused by *protozoa*. They are transmitted as *zoospores* that are very durable, and may be able to survive in a resting state in the soil for many years. They have also been shown to transmit plant *viruses*. When the motile zoospores come into contact with a *root hair* they produce a plasmodium and invade the *roots*.

Parasitic higher plants

Parasitic plants such as mistletoe and dodder are included in the study of phytopathology. Dodder, for example, is used as a conduit either for the transmission of viruses or virus-like agents from a host plant to a plant that is not typically a host or for an agent that is not graft-transmissible.eg. causal agents witch weed disease of sugar

cane, rice, and small grains , broomrapes disease of tomato and tobacco, dodder disease in onions, sugar beets and potato (Sharma and Thakur, 2007),

10.3 Diagnosis of Plant Diseases

For diagnosis of a plant disease, it is important to first determine whether the disease is caused by a pathogen or due to environmental factors. For disease caused by pathogens e.g. Fungi, bacteria, parasitic higher plants, nematodes, viruses are characterized by the presence of these pathogens on the surface of these plants or inside the plants. The presence of such pathogens at an active state on the surface of the plant would indicate that they are probably the cause of the disease. However, if no pathogen could be found transmitted from a diseased plant, then it would have to be assumed that the diseases is caused by environmental factor (Zadoks, 2001).

10.3.1 Identification of a Previously Unknown Disease

When a pathogen is found on a diseased plant, the pathogen is identified by reference to special manuals, but, however, if the pathogen is known to cause such a disease, then the diagnosis of the disease may be considered completed. If, however, the pathogen found to be the cause of the disease but no previous reports have been made, then the following steps are taken.

1. The pathogen must be found to be associated with the disease in all the diseased plants.
2. The pathogen must be isolated and grown in pure culture on nutrient media.
3. The pathogen from the pure culture must be inoculated on healthy plants of the same species, and it must produce the same disease on the inoculated plants.
4. The pathogen must be isolated in pure culture again and its characteristics must be exactly like those observed.

The above steps are known as Koch's postulates, if all the above steps have been followed then the isolated pathogen is identified as the pathogen responsible for the disease (Agrios, 2005).

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CHAPTER ELEVEN:

INTRODUCTORY ANIMAL BIOTECHNOLOGY

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11.1 Introduction

Biotechnology is defined as 'the use of organisms or their components in industrial or commercial processes, which can be aided by the techniques of genetic manipulation in developing e.g. novel plants for agriculture or industry'. Biotechnology can also be defined as using scientific process to get new organisms or new products from organisms. This may involves changing organism in some way to get a desired trait. It is used to promote the production of desired products by organisms.

Biotechnology is said to be multidisciplinary. This means that it involves many disciplines, or branches of learning. All areas of the life sciences, such as microbiology, biology, biochemistry, botany, zoology, agricultural sciences, medicines, food science, and genetics are branches of biotechnology. Computer science and related computer applications are used in biotechnology. Similarly, engineering and the physical sciences such as physics, chemistry are often involved. Biotechnology is carried out in two major levels;

a. Organismic biotechnology: this is working with complete, intact organism or their cells. The organisms are not genetically changed with artificial means. A major goal of organismic biotechnology is to improve organisms and the condition in which they grow. One typical example of organismic biotechnology is cloning. Cloning is the process of producing new organisms from cell or tissue of existing organisms. It does not alter genetic makeup. It uses existing genetic characteristics to achieve desired traits in new organism.

b. Molecular biotechnology/ Recombinant Deoxyribonucleic Acid (DNA) technology: this is changing the genetic make-up of organisms. It is achieved by altering the structure and parts of cells, particularly the genetic material. Genetic engineering, molecular mapping and similar procedures are used in molecular biotechnology. Genetic engineering is changing the genetic information in a cell. Organisms produced by genetic engineering are called transgenic organisms. Transgenic means that the genetic material in an organism has been altered.

Biotechnology helps meet human needs. Three of these needs are food, clothing and shelter. The products of plants and animals are used in manufacturing food, clothing and materials for homes. Biotechnology is also used to make products that are more desirable, such as the conversion of milk into cheese.

11.2 History of Biotechnology

The nature and uses of biotechnology have changed over the years. Older uses are still important in some areas, these include making cheese, baking bread etc. these older methods have served as the foundation on which modern biotechnology has been built. The amount of science needed to use the new methods in biotechnology has greatly increased in recent years with the advent of computers, communication and other technologies where biotechnology now includes DNA structure, gene expression and recombinant DNA methods. The stages of biotechnology development are;

a. **Ancient Biotechnology:** this includes important development in agriculture and food production that is dated to human civilization.

b. **Classical Biotechnology:** this makes wide spread use of the methods from ancient biotechnology, especially fermentation. The methods were adapted to industrial production. Many of the methods that emerged through classical biotechnology are widely used today.

c. **Modern Biotechnology:** this is the manipulation of the genetic material within organisms. It is based on genetics and the use of microscopy, biochemical methods and related sciences and technologies. This is often known as genetic engineering.

11.3 The Roles of Animals in Biotechnology

Animals have important roles in biotechnology. As most of the biotechnology work begins in a laboratory. Researchers attempt to answer questions about animals through laboratory experiment. Without the use of animals, humans might be in danger.

11.3.1 Animal Models

Scientist have developed models for animal research. One uses live animals, in the other model do not. These helps alleviate problems that might arise about the well-being of animals. Overall, four models apply for the use of animals.

a. **Model 1:** living Animals: these animals are living and usually have no threat to their well-being. They are known as the laboratory or scientific animals. As seen in agriculture /biological research, where experimental group of animals are used.

b. **Model 2:** living animal tissues/ systems: animal tissue can be cultured in laboratory. This saves the use of the whole animal, feeding, housing, cleaning up expenses.

c. **Model 3:** non-living systems: this involves using non-living mechanical models that reflect animal activity. These often relates to skeletal movement and locomotion. Artificial replacement parts, such as hip joints, can be studied using non-living systems.

d. **Model 4:** computer and mathematical approach: computer simulations with virtual reality and other uses help in biotechnology. Computer modelling may be done with a proposed biotechnology practice before it is tested with animals.

11.3.2 Laboratory Animals

A laboratory animal is an animal used for laboratory or research purposes. Note that laboratory could be cages, pastures, or other facilities where the animals can be controlled and observed. The significance of research with animals includes:

a. Scientist having base of information about animals.
b. People know about the nutritional needs of animals.
c. People know about proper care for animals.
d. Having animals to be more productive.

11.3.3 Animals Species

Most animals are subjected of study at one time or another. The commonest species used in research laboratories are: mice, rats, hamsters, guinea pigs, rabbit, cats, dogs and non-primates. A primate is an animal with thumb and forefingers opposition whilst a non-human primate is an animal similar to humans, but is not human e.g. Monkeys and chimpanzees. In agriculture, when information is needed from research, cattle, hogs, sheep, horse, fish and others are used. Where their results were carefully studied to determine future recommendations.

11.3.4 Transgenic Animals and Products.

Several uses for animals of animals to produce drugs have been investigated. A few have been developed to the level of satisfactory use. Pigs have been used to manufacture human haemoglobin Pigs are used as sources of organ in human in xenograft. Xenograft is the practice of grafting an organ or a tissue from one species into another. Transgenic mice have been used to produce human antibodies; the antibodies are needed to fight some kinds of diseases. Transgenic mice have been used to model disease that occurs in human e.g. modelling of sickle cell anemia, atherosclerosis and prostate cancer. White cattle have been used to produce lactose in tolerance milk. They are also used to control disease such as mastitis in dairy cows. Mastitis is a disease of mammary glands that interferes with milk production.

11.3.4.1 Methods of Creating Transgenic Animal

The only proven method is by the microinjection of DNA into the embryos which are in one-cell or two-cell stages. There are three general steps well decorated for the transgenic development sheep, goats, cattle's and pigs.

a. **Step 1:** collect embryos: timing is important, super ovulation is promoted in donor females. With proper stimulation, far more embryos can be obtained than would be natural result of the reproduction process.

b. **Step 2:** injection embryos: microscopy is used to locate embryonic cells. The pronuclei within the cells are identified. A pronucleus is the haploid (single) nucleus of the sperm or ovum that have been united in fertilization to form zygote. A zygote is the one-cell embryo formed by the union of sperm and ovum. The appropriate DNA is determined and through the injection process, picked and used using microscope.

c. **Step 3:** zygote culture: following microinjection, the zygotes are placed in the oviduct of a recipient female. This implies that the female must be a proper stage in the oestrous cycle. This transfer may either involve surgical or non-surgical method. The embryo develops much as a normal embryo. Some may be carried to full –term, others may be removed in various stages of development.

All of the steps in this were proven to involve hazards in the survival of the zygotes. Moving about, inspecting, injecting and other procedures injure and kill zygotes. The success rate for the birth of live transgenic animals is often low. It ranges from less than 1% in cattle to more than 1% in goats. Mice have a nearly 3% success rate.

11.4 Techniques of Biotechnology

To understand different biochemical events of prokaryotic and eukaryotic cells at molecular level and be able to characterize, isolate and manipulate molecular components of cells and organisms, wide arrays of bio-physico-chemical techniques are used thus;

a. **Polymerase Chain Reaction (PCR):** This is a versatile technique for copying DNA, and allows a single DNA sequence to be copied repeatedly, or altered in predetermined ways; essentially when it is used for repeated replication of a defined segment of DNA.

b. **Gel Electrophoresis:** This is a common method in which molecules (DNA, Ribonucleic Acid (RNA) and proteins) are separated based on the rates of their migration in an electric field. A gel, usually formed from agarose or polyacrylamide, is placed between two buffer compartments containing electrodes. The sample is then pipetted into preformed slots in the gel, and the electric field is turned on; the gel acts like a sieve, selectively retarding the movement of larger molecules. Smaller molecules

therefore move through the gel more rapidly, allowing a mixture of nucleic acids to be separated on the basis of size.

c. **Expression of Cloned Genes:** this is the situation where molecular cloning enables the determination of the nucleotide sequences of genes that provides new approaches to obtaining large amounts of proteins for structural and functional characterization.

d. **Nucleic Acid Hybridization:** Nucleic acid hybridization is a method used for the detection and analyzes of sequences of homologous DNA. This enables the mapping of genes, to chromosomes, the analysis of gene expression, and the localization of proteins to sub-cellular organelles. In this way, it is possible to study genetic differences between organisms or individuals. Hybridization can be achieved by two methods:

a) Southern blotting. Southern blotting is a method for probing for the presence of a specific DNA sequence within a DNA sample and it enables a researcher to determine not only whether a particular sequence is present within a sample of DNA, knowing the sequences present and the size of the restriction fragments that contain these sequences.

b) Northern blotting is when Messenger RNA subjected to hybridization analysis; in an analogous process. It is used for the study of the expression patterns of a specific type of RNA molecules which is essentially a combination of denaturing RNA gel electrophoresis and a blot. In this process RNA is separated based on size and is transferred to a membrane that is then probed with a labeled complement of a sequence of interest. It is used to determine whether a particular gene is made into mRNA, how much of that mRNA is present, and whether the abundance of that specific mRNA changes at different stages of development or in response to certain regulatory signals that control gene expression.

e. Restriction Fragment Length Polymorphism Analysis: this is when the DNA fragments that result from cutting a particular piece of DNA with a specific restriction enzyme give a characteristic pattern of bands upon gel electrophoresis. Fact band corresponds to a DNA restriction fragment of a certain length. Such differences are called restriction fragment length polymorphisms (RFLPs) serving as genetic marker for a particular location in the genome. RFLPs analysis is important in the diagnosis of genetic disorders and in forensic applications.

11.5 Tools of Deoxyribonucleic Acid Science

The DNA code of any organism can be analyzed. This includes plants, animals, bacteria and other organisms. Most of these procedures begin with;

a. **DNA isolation:** is the process of extracting and separating DNA from all other cell materials. Different procedures can be used to isolate 'clean DNA'. The procedure varies with the cells/tissues being used.

· **Step 1:** Break open the cell wall/membrane. This is done with liquid nitrogen and/or grinding. The step varies with the source of DNA. A mortar and pestle may be used to crack and grind seed cells, such as wheat germ. A food blender containing sucrose may be used with animal tissues, such as a thymus gland. The solution thereof is strained through cheesecloth to remove clumps and gristle (low speed centrifuging may help settle the nuclei to the bottom of a test tube and leave the cellular material in suspension).

· **Step 2:** Digest the cellular components: this is often done by heating with a detergent. With wheat germ, a small amount of detergent solution is mixed with the ground powder. The wheat germ solution is heated to 60^{o}C for 10 minutes. With the thymus solution, a small amount is put into a small test tube. EDTA solution is added to weaken membranes and inactivate DNA digesting enzymes.

· **Step 3:** Separate the polar compounds. This involves using a detergent solution such as sodium dodecyl sulfate (SDS). The detergent dissolves the lipids (fat) in the nuclear membranes. Salts such as NaCl, may be added to the solution. Gently stirring the solution with a slow circular motion promotes the process. Procedures may vary slightly depending on the source of the DNA. Some involves using chloroform/ethanol.

b. **Polymerase chain reaction (PCR):** is a procedure that uses controlled temperature and the enzyme, *taq polymerase* to replicate pieces of DNA. This technique allows scientists to make many copies from a few target DNA molecules. Essentially, by multiplying the molecules exponentially. DNA can be made visible and thus analyzed. Taq polymerase is the DNA replication enzyme found in bacteria that live in hydrophilic vents in the ocean (*Thermus aquaticus*). In its natural environment, the bacteria work at very high temperatures. Thus, temperature is used to control PCR reaction. PCR is a three-step process performed in a piece of equipment called thermocycler. The machine alters the temperature at each step of the process and the process is repeated many times. The steps are:

i. *Separation:* heat to 95^{o}C to separate the DNA strands

ii. *Annealing:* cool to 35 – 58^{o}C for the primers to bond to complimentary DNA regions.

iii. *Extension:* warm to 72^{o}C for Taq polymerase to build a new DNA strand primed region.

NB: each cycle represents an exponential step in DNA production: 1st cycle = 2^{n} = 2 copies; where n is the number of cycles.

OR

The purpose of sequencing is to determine the order of the nucleotides of a gene. For sequencing, we don't start from gDNA (like in PCR) but mostly from PCR fragments or cloned genes. There are three major steps in a sequencing reaction (like in PCR), which are repeated for 30 or 40 cycles.

a. Denaturation at 94°C: During the denaturation, the double strand melts open to single stranded DNA, all enzymatic reactions stop (for example: the extension from a

previous cycle).

b. Annealing at 50°C: In sequencing reactions, only one primer is used, so there is only one strand copied (in PCR: two primers are used, so two strands are copied). The primer is jiggling around, caused by the Brownian motion. Ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break anymore.

c. Extension at 60°C: This is the ideal working temperature for the polymerase (normally it is 72 °C, but because it has to incorporate dideoxynucleotides triphosphates (ddNTP's) which are chemically modified with a fluorescent label, the temperature is lowered so it has time to incorporate the 'strange' molecules. The primers, where there are a few bases built in, already have a stronger ionic attraction to the template than the forces breaking these attractions. Primers that are on positions with no exact match, come loose again and don't give an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3'side (adding dNTP's or ddNTP's from 5' to 3', reading from the template from 3' to 5' side, bases are added complementary to the template). When a ddNTP is incorporated, the extension reaction stops because a ddNTP contains a H-atom on the 3rd carbon atom (dNTP's contain a OH-atom on that position). Since the ddNTP's are fluorescently labeled, it is possible to detect the color of the last base of this fragment on an automated sequencer.

c. Gel electrophoresis: is a process of using an electrical field in agar to separate DNA and RNA molecules based on molecular size. It provides a sequence of the DNA fragments. DNA sequencing is the process of determining the order of the nucleotides in DNA fragments. The chain termination sequencing is most commonly used. The chain has three steps DNA synthesis, gel electrophoresis and DNA detection.

OR

This is done on an acrylamide gel, which is capable of separating a molecule of 30 bases from one of 31 bases, but also a molecule of 750 bases from one of 751 bases. All this is done with gel electrophoresis. DNA has a negative charge and migrates to the positive side. Smaller fragments migrate faster, so the DNA molecules are separated on their size.

d. Sequence alignment: To compare two or more sequences, it is necessary to align the conserved and un-conserved residues across all the sequences (identification of locations of insertions and deletions that have occurred since the divergence of a common ancestor). These residues form a pattern from which the relationship between sequences can be determined with phylogenetic programs. When the sequences are aligned, it is possible to identify locations of insertions or deletions since their divergence from their common ancestor. There are three possibilities:

a) *The bases match:* this means that there is no change since their divergence.

b) *The bases mismatch:* this means that there is a substitution since their divergence.

c) *There is a base in one sequence, no base in the other:* there is an insertion or a deletion since their divergence.

Figure: The comparison of sequences.

A good alignment is important for the next step: the construction of phylogenetic trees. The alignment will affect the distances between 2 different species and this will influence the inferred phylogeny. There are several programs available on the net for aligning sequences. These are all based on different mathematical models to compare two or more sequences with the most optimal score for matching bases with a minimum number of gaps inserted (because you can insert a huge number of gaps, so every base will match another).

e. DNA profiling techniques: is identifying an organism based on regions of DNA that vary greatly from one organism to another. DNA profiling is also known as DNA typing/fingerprints.

11.6 DNA fingerprinting

DNA fingerprinting, also called DNA typing, DNA profiling, genetic fingerprinting, genotyping, or identity testing, in genetics. It is method of isolating and identifying variable elements within the base-pair sequence of DNA (deoxyribonucleic acid). The technique was developed in 1984 by British geneticist Alec Jeffreys, after he noticed that certain sequences of highly variable DNA (known as minisatellites), which do not contribute to the functions of gene, are repeated within genes. Jeffreys recognized that each individual has a unique pattern of minisatellites (the only exceptions being multiple individuals from a single zygote, such as identical twins). DNA fingerprinting is a law-enforcement technology in which a small sample of hair, semen, blood, other tissue can be subjected to DNA analysis and the owner identified with high certainty. DNA fingerprints depend on the genetic differences between individuals, we have called such genetic differences DNA markers; they are also called DNA polymorphisms.

The term polymorphism literally means "multiple forms", the term DNA polymorphism refers to a wide range of variations in nucleotide base composition, length of nucleotide repeats, or single nucleotide variants. DNA polymorphisms are important as genetic markers to identify and distinguish alleles at a gene locus and to determine their parental origin, there are different types of genetic polymorphism:

a. **Single Nucleotide Polymorphism (SNP):** these allelic variants differ in a single nucleotide at a specific position. At least one in a thousand DNA bases differs among individuals. The detection of SNPs does not require gel electrophoresis, this facilitates large-scale detection. A SNP can be visualized in a Southern blot as a Restriction

Fragment Length Polymorphism (RFLP) if the difference in the two alleles corresponds to a difference in the recognition site of a restriction enzyme. Single Nucleotide Polymorphism (SNP) in genes and non-coding parts of the genome is considered as a worthwhile tool for the biodiversity assessment.

b. **Simple Sequence Length Polymorphism (SSLP):** these allelic variants differ in the number of tandem repeated short nucleotide sequences in noncoding DNA. Genetic variation in the cervix mitochondrial genome has been utilized largely in population genetic analysis or phylogenetic studies. Mitochondrial DNA (mtDNA) sequence variation is highly appropriate for phylogenetic analysis amongst closely related species, as compared to nuclear DNA markers. This is because mtDNA shows more rapid evolution (especially the hypervariable D-loop region), maternal inheritance and the absence of recombination, although there is some evidence that recombination may occur in the mtDNA of animals.

11.6.1 Procedure of Creating a DNA Fingerprint

First obtaining a sample of *cells*, such as skin, hair, or *blood* cells, which contain DNA. The DNA is extracted from the cells and purified. There are several methods for DNA extraction such as: conventional method with phenol chloroform and commercial DNA extraction kit or tissue kit. The application depends on the type of samples and techniques for each laboratory. It should adjust DNA to the optimal concentration before used.

In Jeffreys's original approach, which was based on *restriction fragment length polymor phism* (RFLP) technology, the DNA was then cut at specific points along the strand with *proteins* known as *restriction enzymes*. The enzymes produced fragments of varying lengths that were sorted by placing them on a gel and then subjecting the gel to an *electri c current* (*electrophoresis*): the shorter the fragment, the more quickly it moved toward the positive pole (anode). The sorted double-stranded DNA fragments were then subjected to a blotting technique in which they were split into single strands and transferred to a nylon sheet. The fragments underwent autoradiography in which they were exposed to DNA probes - pieces of *synthetic* DNA that were made radioactive and that bound to the minisatellites. A piece of *X-ray* film was then exposed to the fragments, and a dark mark was produced at any point where a radioactive probe had become attached. The resultant pattern of marks could then be analyzed.

Note;

ü Genomic DNA is amplified by using single or multiplex polymerase chain reaction (PCR) technique.

ü DNA fragments of PCR products produced for each marker are separated on acrylamide gel electrophoresis or automate DNA sequencing.

ü This technique is species-specific, requiring the development of suitable genetic makers for each species.

ü More number of markers will enhance power of the analysis.

ü The set of 6-17 microsatellite markers were used in various species of animals such as horse, cow, dog, cat, bird and elephant while 12-13 markers for the DNA fingerprint were used in human for DNA fingerprint.

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11.6.2 Techniques of DNA Profiling

Over past 3 decades, the fundamental DNA technology developments-restriction enzymes coupled with Southern-blot hybridization, sequencing and PCR have contributed to a burst of applications in multiple research areas, including genetic variation and diversity in chickens.

Restricted fragment length polymorphism (RFLP): originally, RFLP referred to analysis of band patterns derived from DNA cleavage using restriction endonuclease enzymes based on SNP. RFLP and related techniques are usually modifications of the Southern blot method when the whole genomic DNA or its fraction is cut with restriction enzymes, transferred to a membrane and hybridized with radiolabeled or fluorescent probes. The latter can be cloned fragments of endogenous avian viruses, particular nuclear genes, MHC genes, EST, or mitochondrial DNA (mtDNA) genes. Individual or pooled RFLP patterns can easily be compared with identify variation within and among populations studied. The technique is time consuming but might still be useful in species for which no or little sequence information is available.

PCR-Based techniques: amplification of noncoding or coding regions of a genome using PCR has revolutionized molecular genetics research and provided an impressive variety of new markers to tackle diversity problems:

a. **Random amplified polymorphic DNA (RAPD):** the random amplified polymorphic DNA technique employs single short primers of random sequence, usually 10-mers, which produce multiband patterns similar to DNA fingerprints. No sequencing information is needed before genotyping. Use of RAPD markers to study vertebrate genetic diversity was thought to be promising and they were heavily exploited in the 1990s. However, because of poor PCR reproducibility and dominance mode of inheritance, they are no longer markers of choice.

b. **Amplified fragment length polymorphism (AFLP):** AFLP molecular markers have been an important tool to enrich existing genetic maps in plants, bacteria and less widely in animal genomes. As developed by Keygene (Keygene N.V., Wageningen, The Netherlands), the amplified fragment length polymorphism technique involves the restriction of genomic DNA, followed by ligation of adaptors complimentary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. Although this type of markers is popular, especially among plant researchers, there are just a few examples of its application to examine genetic variation in

vertebrates. Like RAPD markers, AFLP markers are characterized by a dominant nature, which is a main disadvantage of this technique. Microsatellites, these types of single-locus markers are also known as short (or simple) tandem repeats, simple sequence repeats, or simple sequence-length polymorphisms and belong to a variable number of tandem repeat loci, the most extensively used class of highly polymorphic molecular markers. Unlike all the above techniques, prior sequence information of flanking regions is necessary to develop these markers. Major advantages of microsatellites are that they are detectable by PCR representing unique sequences in the genome that can be mapped and easily be exploited for many genetic applications. Also, they show extensive allelic differences in length, mainly based on variation in the number of repeats and partly on polymorphism of flanking regions.

11.6.3 DNA Fingerprinting Applications

a. Crime scene (forensic analysis in murder, rape, and other violent crimes).
b. Human relatedness (Paternity)
c. Animal relatedness
d. Anthropology studies
e. Disease-causing organisms
f. Food identification
g. Detecting infectious species of bacteria
h. Human remains
i. Monitoring transplants
j. Analysis of old or ancient DNA.
k. Mapping the human genome
l. Tumor biology
m. Transplantation medicine
n. Medical microbiology
o. Application of DNA fingerprinting in animal breeding.
p. Estimate genetic variation
q. Population identification by mixed DNA from more one sample.
r. Identification of genes affecting traits of INTEREST
s. Evaluation of genetic diversity
t. Measure progress of selection.
u. Prediction of Hybrid Vigour (Heterosis)
v. Inbreeding calculation
w. Marker-Assisted Selection.
x. Transgenesis.

11.7 Biotechnology Laboratory Hygiene/Safety

11.7.1 Operational Guidelines

a. Food and drink are not to be stored or prepared in laboratories or chemical storerooms. All food and drink should be consumed in specially designated areas such as canteen or pantry.

b. Use appropriate personal protective equipment (laboratory coats, disposable gloves, and safety glasses).

c. Protective clothing must be removed when leaving the working areas.

d. Gloves must not be worn outside the laboratory.

e. Wash your hands regularly when working with chemical reagents, especially before meals or snack.

f. Smoking in laboratories is prohibited.

g. Do not store personal items such as street clothing, backpacks, etc. on work benches.

h. The working areas should be cleared up and kept clean. The benches must be disinfected before and after use according to the hygiene concept.

i. Only the equipment and materials actually needed must remain on the workbenches. Stocks are stored only in the designated areas or cupboards.

j. If you have long hair, ensure that it is properly tied back.

k. DO NOT mouth pipette. Always use a pipette filler or other pipetting device.

l. Wearing of contact lenses in the lab is strongly discouraged. If it is unavoidable, notify your supervisor and co-workers so that this information is known in the event of a chemical splash in the eyes.

11.7.2 Chemicals Guidelines

a. A number of chemicals used in any molecular biology/biotechnology laboratory are hazardous.

b. You are strongly urged to make use of the information on the MSDS (Material safety data sheet) prior to using a new chemical and certainly in the case of any accidental exposure or spill.

c. The instructor/lab manager must be notified immediately in the case of an accident involving any potentially hazardous reagents.

d. The following chemicals are particularly noteworthy; however, they are not harmful if used properly:

a) PHENOL - can cause severe burns

b) ACRYLAMIDE - potential neurotoxin

c) ETHIDIUM BROMIDE - carcinogen

e. Always wear gloves when using potentially hazardous chemicals and never mouth-pipette them.

f. If you accidentally splash any of these chemicals on your skin, immediately rinse the area thoroughly with water and inform the instructor.

g. Discard the waste in appropriate containers.

h. Keep all noxious and volatile compounds in the fume hood.

i. Work with foul-smelling or toxic substances and highly flammable gases must only be carried out in the fume hood. The additional safety measures required in the particular case must be observed.

11.7.3 Disposal of Buffers and Chemicals

a. Any uncontaminated, solidified agar or agarose should be discarded in the trash, not in the sink, and the bottles rinsed well.

b. Any media that becomes contaminated should be promptly autoclaved before discarding it.

c. Petri dishes and other biological waste should be discarded in Biohazard containers which will be autoclaved prior to disposal.

d. Organic reagents, e.g. phenol, should be used in a fume hood and all organic waste should be disposed of in a labelled container, not in the trash or the sink.

e. Ethidium bromide is a mutagenic substance that should be treated before disposal and should be handled only with gloves.

f. Ethidium bromide should be disposed of in a labelled container.

11.7.4 Ultraviolet light

a. Exposure to ultraviolet light can cause acute eye irritation. Since the retina cannot detect UV light, you can have serious eye damage and not realize it until 30 min to 24 hours after exposure.

b. Therefore, always wear appropriate eye protection when using UV lamps.

c. Use UV goggles and common sense when working with the UV lightbox.

11.7.5 Electricity

a. The voltages used for electrophoresis are sufficient to cause electrocution.

b. Cover the buffer reservoirs during electrophoresis.

c. Always turn off the power supply and unplug the leads before removing a gel.

11.7.6 Glassware and Plastic Ware

a. Glass and plastic ware used for molecular biology must be scrupulously clean. Dirty test tubes, bacterial contamination and traces of detergent can inhibit reactions or degrade nucleic acid.

b. Glassware should be rinsed with distilled water and autoclaved or baked at 150 degrees C for 1 hour.

c. For experiments with RNA, glassware and solutions are treated with diethyl-pyrocarbonate to inhibit RNases which can be resistant to autoclaving.

d. Plastic ware such as pipettes and culture tubes are often supplied sterile. Tubes made of polypropylene are turbid and are resistant to many chemicals, like phenol and chloroform.

e. Polycarbonate or polystyrene tubes are clear and not resistant to many chemicals.

f. Make sure that the tubes you are using are resistant to the chemicals used in your experiment.

g. Micro pipette tips and microcentrifuge tubes should be autoclaved before use.

11.7.7 Equipment Use

a. It is to everyone's advantage to keep the equipment in good working condition.

b. As a rule of thumb, don't use anything unless you have been instructed in the proper use.

c. This is true not only for equipment in the lab but also departmental equipment.

d. Report any malfunction immediately.

e. Rinse out all centrifuge rotors after use and in particular if anything spills.

f. Please do not waste supplies - use only what you need. If the supply is running low, please notify either the instructor/lab manager before the supply is completely exhausted.

g. Occasionally, it is necessary to borrow a reagent or a piece of equipment from another lab. Except in an emergency, notify the instructor.

11.7.8 Micropipettes

a. Most of the experiments you will conduct in this laboratory will depend on your ability to accurately measure volumes of solutions using micropipettes.

b. The accuracy of your pipetting can only be as accurate as your pipette and several steps should be taken to ensure that your pipettes are accurate and are maintained in good working order.

c. They should then be checked for accuracy following the instructions given by the instructor. If they need to be recalibrated, do so.

d. Ensure the use of appropriate pipette tip during pipetting.

e. Do not drop the pipette on the floor. If you suspect that something is wrong with the pipette, first check the calibration to see if your suspicions were correct, then notify the instructor.

11.7.9 Curative Measures

a. Fire extinguisher

b. First-Aid Box

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CHAPTER TWELVE: MANAGEMENT OF ZOOLOGICAL GARDEN

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Zoological Garden: is a place where wild animals are kept in captivity for public viewing. And because they are in captivity a new dimension is introduced into their management. The important factors are: -

a. The health of the animal,
b. Food and feeding habits of the animals,
c. Habitats of the animals, and
d. The safety and comfort of the visitors

12.1 Factors Considered in Establishing Zoological Garden:

i. Location: choose a site which is easily accessible with plenty of room for expansion.

ii. Topography of the area: i.e. whether the land is a level ground or a slope. The area should be level and not a slope of hill and the soil should be well drained and not a water-logged area.

iii. The place should have a natural source of water either river or stream, because artificial supply of water may fail, and there may be some animals that may require natural water for habitat e.g. Fish, Hippopotamus and Crocodile.

Zoo animal can be kept in zoological garden by taken care to: -

i. Prevent break out of diseases
ii. By keeping the animal on balance diet

1. Prevention of break out of disease:

i. Animals should not be placed in a condition which could create a stress e.g overcrowding animal together may cause this. A stress individual is highly predisposed to disease.

ii. Bad topography may also bring about stress among animals e.g. those living on a hill or slope area.

iii. Quarantine: A new animal for zoo has to be isolated in a place for some days before being introduced into the zoo. Quarantine makes sure that new animals coming into the zoo/game reserve do not introduce disease.

iv. Vaccination: Animal in the zoo can be save from disease by vaccination and immunizing then against diseases e.g. immunization against rabies.

v. Vector Control: Habitats of vectors of diseases should be destroyed or Biological control method used to eliminate the vectors e.g. mosquito can be control biologically by using Tilapia fish.

vi. Disinfection of Pen: This can be done either by physical or chemical method. Chemical disinfectant is by the use of DDT, Dettol e.t.c. or by physical disinfection of heating.

(ii) By keeping the animal on balance diet

12.2 Principles of Zoo Animal Feeding

§ The nutrient need of a giving animal is in the same proportion recognized for the same species of the domestic kind e.g. cat, dog can be taken to represent carnivores, while cow and sheep represent herbivore.

§ The smaller the animal, the larger the size of food supply. Small size animal requires more food because they lose more energy.

§ 10-12% of the diet for herbivore should be protein, while for carnivore its diet should contain 21% protein.

§ In the case of lactating animal, the diet should contain more protein than the prescribe ones.

§ One of the aspects that are common to both manager of zoo and wild life is the management of people. Thus, pen in a zoo is constructed in such a way that contact between people and the animals are eliminated. This ensures that the animals are not over fed, by the visitors and also the visitors are not exposed to any risk.

§ In visitor managements, adequate provision should be done for the visitors such as rest spot, shades provided by planting trees around the zoo. All this increases the visitors comfort.

§ Visitor centre should also be build, here there should be food available to the visitors and also comfortable resting place at the visitor centre.

12.3 What Role can Zoological Garden Play in Conservation?

Zoological garden has a role to play in preserving species that ate endangered or threatened. E.g. wild dog are endangered species in Nigeria.

Endangered species are threatened toward extinction.

Rare Threatened Endangered Extinction

Giraffe and cheater are endangered species in Nigeria.

While Elephant and some antelope are threatened in Nigeria but not endangered.

12.4 International Organizations and Conservation Clubs

The international organizations and conservation clubs, that protect natural environment and wildlife includes the followings:-

i. World wild life fund (WWF)
ii. International union of conservation of natural resources (IUCN)
iii. International council for Bird preservation (ICBP)
iv. Nigerian conservation foundation (NCF)

NCF is found in Nigeria protecting the natural environment.

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CHAPTER THIRTEEN: WILDLIFE DISEASES

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13.1 Introduction

Wildlife refers to animals and other living things including vegetations living in a natural undomesticated habitat. While on the other hand, diseases are conditions in living that is manifested in pathological symptoms not resulting from physical injuries. Thus, wildlife diseases can be described as conditions in wildlife that results in pathological symptoms from the internal working conditions of the body system and not as a result of direct of physical injury

Diseases of wildlife can cause significant illness and death to individual animals and can significantly affects wildlife populations. Wildlife species can also serve as natural hosts of certain diseases that affects human (zoonosis). The diseases agents or parasites that causes these zoonotic diseases can be through the bite of arthropod vectors such as mosquitoes ticks, and mites that have previously fed on an infected animal (World Health Organization, 2021). These zoonotic diseases are primarily diseases acquired within a specific locality, and secondarily, disease of occupation and a vocation. Plague, tularaemia and leiptospirosis have been acquired in the handling and skinning of rodents, rabbits and carnivores. Human have usually acquired disease like Colorado tick fever, Rocky Mountain spotted fever, and Lyme disease because they have spent time in optimal habitats of diseases vectors and hosts.

13.2 DISEASES: THEIR SYMPTOMS, DIAGNOSIS, PREVENTION, AND TREATMENT

Below are some of the diseases that are associated with wildlife, their symptoms, diagnosis, prevention and treatment.

13.2.1 TULAREMIA (RABBIT FEVER)

Tularaemia (also known as rabbit fever or deerfly fever) is an infectious disease of ticks and rabbits that is caused by a bacterium, *Francisella tularensis*). The disease was first described in Japan in 1837. Its name relates to the description in 1911 of a plague-like illness in the ground squirrels in Tulare County, California (hence the name tularemia) and the subsequent work done by Dr. Edward Francis. Tularaemia occurs throughout North America and in many parts of Europe and Asia. *Francisella tularensis* is found worldwide in over a hundred species of wild animals, birds and insects. Some examples of animals, other than rabbits, that carry tularemia are meadow mice, ground hogs (woodchucks), ground squirrels, tree squirrels, beavers, coyotes, muskrats, opossums, sheep, and various game birds. There are two common ways through which humans can contract tularaemia from the bite of an infected tick, deerfly (*Chrysops discalis*), or

mosquito: 1) when transmitted to humans by insects; the exposed body surfaces are bitten by the vectors and this results in pain and fever that is sudden and very severe: 2) when broken skin such as cuts and abrasions comes into direct contact with an infected rabbit carcass (mostly from the rabbit species of the genus *Sylvilagus;* which are also known as the cottontails)(Center for the Disease Control and Prevention, 2021;Croddy and Krcalova, 2001). Other ways of spreading the disease are drinking contaminated water, inhaling dust from contaminated soil, or handling contaminated pets or paws of animals. However, Human-to-human transmission of tularemia is highly uncommon.

Symptoms

In humans, tularaemia may appear in two forms depending on how a patient contracted the disease. The most common form is usually acquired through the bite of an infected tick (especially deer ticks and dog ticks) or from contact with infected rabbits. (Note: dog ticks are also called wood ticks and that deer ticks are the primary cause for a very serious disease called Lyme disease) (CDC, 2015; Kwit *et al.,* 2016). Patients will develop an ulcer at the site of infection and lymph glands become inflamed and swollen. Severe fever and flu-like symptoms may accompany the ulcer or lesion. Symptoms start to show within 1-14 days after contracting the disease, with 3-5 days being most common. The fever generally lasts for 3 to 6 weeks if no type of antibiotic therapy is used to combat the bacteria. Patients with the less common form of tularemia, which occurs mainly after inhalation of bacteria, typically experience sudden chills, fever, weight loss, abdominal pains, tiredness, and headaches. Patients with the above forms and symptoms of tularaemia may develop an unusual pneumonia that can induced fatality.

Symptoms of the disease in a rabbit are a white spotted liver, swollen spleen, and an ulcerated or raw area about ¼ inches in diameter which is where the animal was bitten by a tick or deer fly and thus infected.

Transmission

A report of tularaemia outbreaks indicates two primary modes of disease transmission. An increase in the number of reported cases in the eastern and midwestern United States during fall and winter coincides with hunting season when hunters are skinning rabbits (CDC, 2015; Eisen *et al.,* 2008). In the southwestern and western United States, the incidence of tularemia is highest during summer months due to tick bites. The risk of contracting tularemia from rabbits is greatest when handling rabbits after the hunt during the cleaning process. Hunters skinning rabbits are advised to wear protective rubber gloves to reduce the risk of contracting the bacteria that cause tularemia when broken skin comes into contact with an infected carcass or a live, infected rabbit. It is also advised that rabbit meat should be thoroughly cooked before eating. Bacteria that

cause tularemia can live for weeks in water, soil, carcasses, and hides, and for years in frozen rabbit meat (Enderlin *et al.,* 2004).

Prevention

Rubber, plastic, or latex gloves should be worn while skinning or handling rabbits, especially if there is an open cuts or abrasions. Wild rabbit and rodent meat should be cooked thoroughly before eating (WHO, 2021). Although this doesn't imply that the meat has to be overcooked that it is not bloody in the middle which is a sign that the meat is still raw or uncooked (Tarnvik, 2007). To avoid the transmission through the bites from the vectors when outdoors, insect repellent apparels that contain DEET (for skin) and/or repellents containing Permethrin (for clothing only) should be used. Tick checks should also be conducted every two to three hours if spending a lot of time outdoors where ticks are in large numbers. If stricken by the ticks, careful removal from the spot is actually recommended immediately with the aid of a tweezers. It is wisely suggested that dead rabbits found in the outdoors are not picked up unnecessarily.

Treatment

Doctors recommend a preventive vaccine for people at high risk of contracting tularemia. Untreated, tularemia (Type B) carries a mortality rate of 5-15%, even higher to about 35% with the typhoidal form (Type A). Appropriate antibiotics lower both mortality rates to about 1%. If there is a suspicion of an infection of tularaemia, adequate professional medical care should be sought immediately. Antibiotics such as streptomycin, gentamicin, and tobramycin have been reported to be effective in the treatment the infectious bacteria (Weber *et al.,* 2012; Dennis *et al.,* 2004; Morner, 1994). Long-term immunity will follow recovery from tularemia. However, reinfection has been occasionally reported in laboratory workers that had not received the Tularemia vaccine (CDC, 2021).

13.2.2 RABIES

Rabies is a zoonotic disease (a disease that is transmitted to humans from animals) that is caused by a virus of the genus *Lyssavirus*. The disease infects both the domestic and wild animals, and is transmitted to people through close contact with infected saliva via bites or scratches from the animals (WHO, 2023). The distribution of the Rabies is widespread on all continents with the exception of Antarctica; however, more than 95% of human deaths occurred in Asia and Africa. Once symptoms of the disease develop, rabies is nearly always fatal.

Symptoms

The incubation period for rabies is typically 1–3 months, but may vary from <1 week to >1 year. The initial symptoms of rabies are fever and often pain or an unusual or unexplained tingling, pricking or burning sensation (paraesthesia) at the wound site. As

the virus spreads through the central nervous system, progressive, fatal inflammation of the brain and spinal cord develops. Two forms of the disease can follow CDC, 2021). People with furious rabies exhibit signs of hyperactivity, excited behaviour, hydrophobia and sometimes aerophobia. After a few days, death occurs by cardio-respiratory arrest. Paralytic rabies accounts for about 30% of the total number of human cases. This form of rabies runs a less dramatic and usually longer course than the furious form. The muscles gradually become paralyzed, starting at the site of the bite or scratch. A coma slowly develops, and eventually death occurs. The paralytic form of rabies is often misdiagnosed, contributing to the underreporting of the disease.

Diagnosis

No tests are available to diagnose rabies infection in humans before the onset of clinical disease, and unless the rabies-specific signs of hydrophobia or aerophobia are present, the clinical diagnosis may be difficult. Post mortem, the standard diagnostic technique is to detect rabies virus antigen in brain tissue by fluorescent antibody test (Pearson, 1998). Currently, the diagnosis is usually done through testing the saliva, spinal fluid and skin samples.

Transmission

People are infected through the skin following a bite or scratch by an infected animal. Dogs are the main host and transmitter of rabies. They are the source of infection in all of the estimated 55 000 human rabies deaths annually in Asia and Africa. Bats are the source of most human rabies deaths in the United States of America and Canada. Bat rabies has also recently emerged as a public health threat in Australia, Latin America and Western Europe. Human deaths following exposure to foxes, raccoons, skunks, jackals, mongooses and other wild carnivore host species are very rare. Transmission can also occur when infectious material – usually saliva – comes into direct contact with human mucosa or fresh skin wounds. Human-to-human transmission by bite is theoretically possible but has never been confirmed (Rappini *et al.,* 2007). Rarely, rabies may be contracted by inhalation of virus-containing aerosol or via transplantation of an infected organ. Ingestion of raw meat or other tissues from animals infected with rabies is not a source of human infection.

Treatment after Exposure

Effective treatment soon (within a few days, but as soon as possible) after exposure to rabies can prevent the onset of symptoms and death. Post-exposure prevention consists of local treatment of the wound, administration of rabies immunoglobulin and immediate vaccination.

Local Treatment of the Wound

Removing the rabies virus at the site of the infection by chemical or physical means is an effective means of protection. Therefore, prompt local treatment of all bite wounds and scratches that may be contaminated with rabies virus is important. Recommended first-aid procedures include immediate and thorough flushing and washing of the wound for a minimum of 15 minutes with soap and water, detergent, povidone iodine or other substances that kill the rabies virus. The recommended post-exposure prophylaxis depends on the type of contact with the suspected rabid animal (Ryan and Ray, 2004).

13.2.3 PLAGUE (*Yersinia pestis* Infection)

Plague, caused by a bacterium called *Yersinia pestis* mostly found in small mammals and their fleas. It is characterised by the periodic disease outbreaks in rodent populations, some of which have a high death rate. Once there is an outbreak, hungry infected fleas that have lost their normal hosts seek other sources of blood, thus increasing the increased risk to humans and other animals frequenting the area (Trevisanato, 2007). Epidemics of plague in humans usually involve house rats and their fleas with an epidemic that continue to occur in some developing countries with more rural areas. Many other rodent species, for instance, prairie dogs, wood rats, chipmunks, and other ground squirrels and their fleas, suffer plague outbreaks and some of these occasionally serve as sources of human infection. Deer mice and voles are thought to maintain the disease in animal populations but are less important as sources of human infection. Other less frequent sources of infection include wild rabbits, and wild carnivores that pick up their infections from wild rodent outbreaks. Domestic cats (and sometimes dogs) are readily infected by fleas or from eating infected wild rodents. Cats may serve as a source of infection to persons exposed to them. Pets may also bring plague-infected fleas into the home. Between outbreaks, the plague bacterium is believed to circulate within populations of certain species of rodents without causing excessive mortality (Office International des Épizooties, 2000). Such groups of infected animals serve as silent, long-term reservoirs of infection.

Distribution

In the United States during the 1980s, plague cases averaged about 18 per year. Most of the cases occurred in persons under 20 years of age. About 1 in 7 persons with plague died. Worldwide, there are 1,000 to 2,000 cases each year. During the 1980s epidemic plague occurred each year in Africa, Asia, or South America. Epidemic plague is generally associated with domestic rats (CDC, 2021). Almost all of the cases reported during the decade were rural and occurred among people living in small towns and villages or agricultural areas rather than in larger, more developed, towns and cities.

Transmission

Plague is transmitted from animal to animal and from animal to human by the bites of infective fleas. Less frequently, the organism enters through a break in the skin by direct contact with tissue or body fluids of a plague-infected animal, for instance, in the process of skinning a rabbit or other animal (kwit *et al.,* 2019). Plague is also transmitted by inhaling infected droplets expelled by coughing, by a person or animal, especially domestic cats, with pneumonic plague. Transmission of plague from person to person is uncommon but does occur as an important factor in plague epidemics in some developing countries.

Diagnosis

The pathogenomic sign of plague is a very painful, usually swollen, and often hot-to-the touch lymph node, called a bubo. This is usually accompanied with fever, extreme exhaustion. A history of possible exposure to rodents, rodent fleas, wild rabbits, or sick or dead carnivores should serve as a signal of potential plague infection. Onset of bubonic plague is usually 2 to 6 days after a person is exposed (CDC, 2021; Nelson *et al.,* 2021). Initial manifestations include fever, headache, and general illness, followed by the development of painful, swollen regional lymph nodes. Occasionally, buboes cannot be detected for a day or so after the onset of other symptoms. The disease progresses rapidly and the bacteria can invade the bloodstream, producing severe illness, called plague septicemia.

Once a human is infected, a progressive and potentially fatal illness generally begins unless specific antibiotic therapy is given. Progression leads to blood infection and, finally, to lung infection. The infection of the lung is termed *plague pneumonia*, and it can be transmitted to others through the expulsion of infective respiratory droplets by coughing (CDC, 2021; Nelson *et al.,* 2021). The incubation period of primary pneumonic plague is 1 to 3 days and is characterized by development of an overwhelming pneumonia with high fever, cough, bloody sputum, and chills. For plague pneumonia patients, the death rate is over 50%.

Prevention

Plague will probably continue to exist in its many localized geographic areas around the world, and plague outbreaks in wild rodent hosts will continue to occur. Attempts to eliminate wild rodent plague are costly and futile. Therefore, primary preventive measures are directed toward reducing the threat of infection in humans in high-risk areas through three techniques - environmental management, public health education, and preventive drug therapy.

Antibiotics may be taken in the event of exposure to the bites of wild rodent fleas during an outbreak or to the tissues or fluids of a plague-infected animal. Preventive therapy is also recommended in the event of close exposure to another person or to a pet animal with suspected plague pneumonia. For preventive drug therapy, the preferred antibiotics are the tetracyclines, chloramphenicol, or one of the effective sulphonamides (CDC, 2021; Nelson *et al.,* 2021).

Treatment

As soon as a diagnosis of suspected plague is made, the patient is isolated, and local and state health departments notified. Confirmatory laboratory work should be initiated, including blood cultures and examination of lymph node specimens if possible. Drug therapy should begin as soon as possible after the laboratory specimens are taken. The drugs of choice are streptomycin or gentamycin, but a number of other antibiotics are also effective (OIE, 2000). Those individuals closely associated with the patient, particularly in cases with pneumonia, should be traced, identified, and evaluated. Contacts of pneumonic plague patients should be placed under observation or given preventive antibiotic therapy, depending on the degree and timing of contact

Epidemic plague is best prevented by controlling rat populations in both urban and rural areas. This goal has been reached in the cities, towns, and villages of most developed countries (James and Berger, 2006). It has not been achieved in either the rural or urban areas of many developing countries where the threat of epidemic plague continues to exist. Control of plague in such situations requires two things: 1) close surveillance for human plague cases, and for plague in rodents, and 2) the use of an effective insecticide to control rodent fleas when human plague cases and rodent outbreaks occur.

13.2.4 MYCOBACTERIUM TUBERCULOSIS INFECTION

Tuberculosis is one of the oldest of the recognized diseases in humans and animals. It is caused by a species of pathogenic bacteria of family Mycobacteriaceae. It was not until 1882, that the cause of the disease became known. Dr. Robert Koch, the noted German scientist, proved that a microorganism could be isolated from the characteristic tubercules that form during the typical TB disease process. The rod-shaped organism as seen under the microscope varies in size, from about 1 to 4 microns (about 6,000-16,000 laid end to end equals 1 inch). Koch showed that these same "rods" can cause the typical tuberculosis disease when inoculated into another animal. Although it was originally called Koch's bacillus, the organism was later given the scientific name of *Mycobacterium tuberculosis* as it is still known today.

The disease may be contracted in a variety of ways and affects many organs of the body (Todar, 2016). Most of the time, the bacilli are inhaled via infected droplets from an infected person's or animal's lungs, or by ingesting contaminated food, milk or water. Once the bacilli get into a susceptible organism, a number of events occur which cause the characteristic disease. It generally starts in the areas where the exposure occurs. The lungs are often attacked, but other parts of the body can be affected. When it gets into the lymph system, it can travel to all parts of the body. Wherever the bacilli lodge, invader-fighting white blood cells are attracted to the bacilli invaders and attempt to ingest them. The bacillus has a waxy coating that is very resistant to the white cells attempts to digest it. The invader fighting cells then attempt to isolate bacilli inside a hard nodule and a "tubercle" is thus formed (CDC, 2015). If the wall of the tubercle is dense and effective in encapsulating all the mycobacterium bacteria, there will be little advancement of the disease and the tubercule may even calcify. But, if the bacilli are not completely contained, a spreading lesion will ensue. Bacilli may escape from the lesion and move to other parts of the body. Wherever invader cells lodge, the disease process starts over again. During the process of the disease development and expansion, the animals become emaciated, lethargic, organs become damaged and the animal weakens and dies.

Diagnosis

Tuberculosis lesions cannot always be seen or palpated, so clinical signs are not reliable for a diagnosis. The intradermal tuberculin test is still the most important diagnostic test for TB. Radiography is useful for imaging lesions in non-human primates and small animals. Discharges and sputum can be examined microscopically, but diagnosis other than the tuberculin test requires culturing tissue samples on selective media which can take 4-8 weeks (Ryan and Ray, 2004). The intradermal test made from mycobacterial antigen is the one most used for large animals. A positive reaction includes skin swelling. The test is not perfect as there are cross reactions with other strains of mycobacteria and false negatives may occur under certain conditions. Current research efforts are trying to find improved diagnostic methods. An interferon-based assay has recently been developed and has proven beneficial. Other tests are also being evaluated.

Control Measures.

The trend toward intensive agriculture has made control more difficult. Also, the presence of wild animals as reservoirs of the *Mycobacterium* (e.g. badgers in the UK, brush-tail possums in New Zealand, and white-tailed deer in the US) make it very difficult to eradicate the disease. Generally, there are three approaches currently used to the control the disease in domestic animals: 1) test for TB with the intradermal tuberculin test and either slaughter, 2) segregate or 3) treat with drugs

(CDC, 2018). The removal of reacting presumed infected animals is the only assured approach to eradicating the disease. This is however limited where pastured animals are infected by exposure to diseased wild animals. The use of drugs is discouraged due to exposure of humans to animals being treated, possible development of drug resistant strains, and the expense to producers. The efficacy of a live vaccine made from the attenuated strain of *Mycobacterium tuberculosis* BCG (Bacilli Calmette-Guerin) has proven variable and use of this vaccine may confound interpretation of current diagnostic tests. Improved vaccines are currently being evaluated in research trials.

13.2.5 Lymphocytic Choriomeningitis (LCM)

Lymphocytic choriomeningitis (LCM), is a *rodent*-borne viral infectious disease that presents as *aseptic meningitis*, *encephalitis* or *meningoencephalitis*. Its causative agent is the Lymphocytic Choriomeningitis Virus (LCMV), a member of the family *Arenaviridae*. The name was coined by *Charles Armstrong* in 1934. In the Gale Encyclopedia of Medicine, second edition, lymphocytic choriomeningitis (LCM) is defined as "a viral infection of the membranes surrounding the brain and spinal cord and of the cerebrospinal fluid. The name is based on the tendency of an individual to have abnormally high levels of lymphocytes during infection. Choriomeningitis is cerebral meningitis in which there is marked cellular infiltration of the *meninges*, often with a lymphocytic infiltration of the *choroid plexuses* (Albarino *et al.,* 2007). LCMV is an enveloped virus with a helical *nucleocapsid* containing an RNA genome consisting of two single-stranded RNA segments. The L strand is ambisense RNA and encodes the polymerase and z protein while the S strand is ambisense and encodes the nucleo-protein and glycoproteins. The first *arenavirus*, lymphocytic choriomeningitis virus (LCMV), was isolated in 1933 by Charles Armstrong during a study of *an epidemic in St. L ouis*. Although not the cause of the outbreak, LCMV was found to be a cause of nonbacterial or *aseptic meningitis*. LCMV is naturally spread by the common *house mous e*, *Mus musculus*. Once infected, these mice can become chronically infected by maintaining virus in their blood and/or persistently *shedding virus* in their urine.

Infection

Infected female mice are reported to usually transmit infection to their offspring (*vertical transmission*), which in turn become chronically infected. Other modes of mouse-to-mouse transmission include nasal secretions, milk from infected dams, bites, and during social grooming within mouse communities. Airborne transmission also occurs. The virus seems to be relatively resistant to drying and therefore humans can become infected by inhaling infectious *aerosolized* particles of rodent urine, faeces, or saliva, by ingesting food contaminated with virus, by contamination of *mucus membranes* with infected body fluids. Direct exposure to cuts, abrasions or other open wounds to virus-

infected blood is another way of acquiring this infection. The only documented cases of transmission from animals have occurred between humans and mice or hamsters (Jahrling and Peters, 1992). Cases of lymphocytic choriomeningitis have been reported in North and South America, Europe, Australia, and Japan, particularly during the 1900s. However, infection may occur wherever an infected rodent host population exists.

Symptoms

LCMV infection manifests itself in a wide range of clinical symptoms, and may even be asymptomatic for immunocompetent individuals. Onset typically occurs between one or two weeks after exposure to the virus and is followed by a biphasic febrile illness. During the initial or prodromal phase, which may last up to a week, common symptomatic features include fever, lack of appetite, headache, muscle aches, *malaise*, nausea, and/or vomiting. Less frequent symptoms include a sore throat and cough, as well as joint, chest, and *parotid* pain (CDC, 2005). The onset of the second phase occurs several days after recovery, and consists of symptoms of meningitis or encephalitis. Pathological findings during the first stage consist of *leukopenia* and *thrombocytopenia*. During the second phase, typical findings include elevated protein levels, increased leukocyte count, or a decrease in glucose levels of the cerebrospinal fluid).

Diagnosis

Current or previous infection can be detected through a blood test. However, some authors note that such complement-fixation tests are insensitive and should not be used for diagnosis. Timeliness of diagnosis is important not only in expediting treatment of infected persons, but also in preventing further LCM transmission to other workers and animals (CDC, 2005). Clinical diagnosis of LCM can be made by the history of *prodrom e* symptoms and by considering the period of time before the onset of meningitis symptoms, typically 15–21 days for LCM.

Pathological diagnosis of congenital infection is performed using either an *immunofl uorescent* antibody (IFA) test or an enzyme *immunoassay* to detect specific antibody in blood or cerebrospinal fluid. Diagnoses are subject to methodological shortcomings in regard to specificity and sensitivity of *assays* used. For this reason, LCMV may be more common than is realized.

Treatment

Currently, there is no LCMV - specific treatment for humans; although *nucleosid e* analog *ribavirin* is used in some cases due to the inhibitory effect the agent has *in vitro* on arena viruses. However, there is not sufficient evidence for efficacy in humans to support routine use. Early and intravenous ribavirin treatment is required for

maximal efficacy, and it can produce considerable side effects (CDC, 2005). Use of ribavirin during pregnancy is generally not recommended, as some studies indicate the possibility of *teratogenic* effects. If aseptic meningitis, encephalitis, or meningoencephalitis develops in consequence to LCMV, hospitalization and supportive treatment may be required. In some circumstances, anti-inflammatory drugs may also be considered. In general, mortality is less than one percent.

13.2.6 HISTOPLASMOSIS INFECTION

Histoplasmosis is a fungal infection that can infect cats, dogs, and people. It is caused by *Histoplasma capsulatum*, which is found in the soil that contains most of the droppings from the afore-mentioned animals. It enters the body through the lungs. The disease causes a variety of respiratory and intestinal symptoms. Other symptoms include; fever, cough fatigue and body aches. Histoplasma is predominantly isolated in areas that are moist and humid and grows best in soils that contain nitrogen-rich organic matter such as bird or bat droppings. Cats and dogs can both be infected (Assi *et al.,* 2007). Infections are more common in outside cats and dogs, particularly in hunting dogs. Animals of any age can get histoplasmosis. However, most infections occur in animals under four years of age.

Transmission

Cats get infected by inhaling the spore-like particles of the fungus that inhabit the soil. These tiny particles are small enough to reach the lower respiratory tract (lungs). A few cases of infection in strictly indoor animals have been reported and it is suspected that potting soil or dirt brought in from outside may be the source of infection in these animals (CDC, 2020).

Symptoms

The symptoms are varied and depend somewhat on the severity of the infection. Histoplasmosis infections start in the lung. As the fungal organisms replicate, the animal can develop a respiratory form of the disease. Many healthy animals will recover from the milder respiratory infections on their own. In other animals, particularly ones with a poor or deficient immune system, the respiratory infection may become more severe or the infection may spread to the gastrointestinal (GI) system, lymph nodes, spleen, liver, or eyes. The most common symptoms in the cat or dog are weight loss, fever, loss of appetite, and depression. Laboured breathing with increased lung sounds is present, and many animals may also have a cough (Assi *et al.,* 2007). In addition to these symptoms, both cats and dogs may be anaemic and have pale gums.

Diagnosis

Diagnosis of histoplasmosis is often made from information obtained from the history, symptoms, x-rays of the chest and abdomen, and by finding the organisms in the infected tissue (Assi *et al.,* 2007). A needle aspirate or biopsy of the infected tissue can often yield some of the small budding fungal organisms.

Prevention

There is no vaccine to protect against histoplasmosis. The best prevention is to avoid areas where histoplasmosis is known to be a problem. Areas where large numbers of birds or bats roost should also be avoided. Infections are not transmitted between infected animals or between animals or humans. While humans can get the infection, they get it from the fungal spores in the soil just like animals do.

Treatment

In many simple cases of the respiratory form of histoplasmosis, treatment may not be necessary because the animal will clear the infection on its own. But because of the risk of the infection spreading or becoming more severe, treatment is often initiated as soon as a positive diagnosis is made. The treatment of choice is an oral antifungal drug. The most commonly used ones are itraconazole or fluconazole. Ketoconazole is sometimes used when cost is a consideration, though it may not be as effective and can be more toxic than itraconazole. The treatment usually lasts several months or longer. The success in treating histoplasmosis is very good if the correct treatment is used and instituted before the animal becomes too debilitated (CDC, 2020).

13.2.7 GIARDIASIS

Giardiasis is an intestinal infection caused by a parasitic protozoan (single celled organism) called *Giardia lamblia*. These germs are found mostly on surfaces, in soil, food or water that has been contaminated with faeces from infected animals. These protozoans are found in the intestines of many animals, including dogs and humans. This microscopic parasite clings to the surface of the intestine, or floats free in the mucous lining the intestine (Beer *et al.,* 2017; Nakao *et al.,* 2017). Giardia occurs in two forms: a motile feeding stage that lives in the intestine, and a non-motile cyst stage that passes in the faeces. The giardia trophozoite - which is the active stage of the organism - inhabits the small intestine of the dog. The trophozoite stage is tear-drop shaped, binucleated, and has four pairs of flagella. It attaches to the cells of the intestine with its adhesive disc and rapidly divides to produce a whole population of trophozoites. As they detach, they may be swept down the intestine. If intestinal flow is fast then they may

appear in the faeces. However, if they have time, encystment occurs as the parasite travels to the large intestine (CDC, 2020). The cyst is fairly resistant, and can survive for several months outside of a host's body as long as sufficient moisture is provided. The cyst is oblong in shape with four nuclei that are sometimes distinctly visible. Mature cysts are usually found in the faeces of infected animals. Other animals become infected by ingesting the cysts that passed from the body in faeces. These ingested cysts then break open inside the small intestine to release the motile feeding stage (trophozoite). Giardia increases their numbers by each organism dividing in half which is called binary fission.

Transmission

Giardia lives and reproduces in the small intestine of host animals. Giardia trophozoites, the free-living stage of the organism, form infective cysts that are passed out in the faeces (Beer *et al.,* 2017; Nakao *et al.,* 2017). If the cysts are present in a wet or damp environment they can survive in a viable state for a few weeks to several months. Giardia infections are transmitted via ingestion of trophozoites or cysts in contaminated water or food. If a giardia cyst is ingested, the cyst wall is broken down during the digestive process and the trophozoite stage begins to colonize the upper small intestine. Transmission also occurs by direct contact, especially with asymptomatic carriers. More recently, giardiasis has also been recognized as being able to be sexually transmitted. Giardia is so prevalent throughout North America because it is highly contagious. The ingestion of as few as one or more giardia cysts may cause the disease, as contrasted to most bacterial illnesses where hundreds to thousands of organisms must be consumed to produce illness (Beer *et al.,* 2017; Nakao *et al.,* 2017). Giardia causes its unpleasant effects on the body not by invading the tissues, but simply by being in the way. It multiplies to the point where it sorts of paves the lining of the intestine and blocks normal digestion (malabsorption). This causes only partially digested food to get lower in the digestive tract than it should, causing diarrhea.

Symptoms

The trophozoites divide to produce a large population, and then they begin to interfere with the absorption of food. Clinical signs range from none in asymptomatic carriers, to mild recurring diarrhea consisting of soft, light-coloured stools, to acute explosive diarrhea in severe cases. Other signs associated with giardiasis are weight loss, listlessness, fatigue, mucus in the stool, and anorexia (Beer *et al.,* 2017). These signs are also associated with other diseases of the intestinal tract, and are not specific to giardiasis. These signs, together with the beginning of cyst shedding, begin about one-week post-infection. There may be additional signs of large intestinal irritation, such as straining and even small amounts of blood in the faeces (CDC, 2021; Beer *et al.,* 2017). Usually, the blood picture of affected animals is normal, though occasionally there is a

slight increase in the number of white blood cells and mild anaemia. Without treatment, the condition may continue, either chronically or intermittently, for weeks or months.

Diagnosis

Diagnosing giardia is not easy. Diagnosis can be done in one of two ways: via faecal sample by a Vet or via educated evaluation of clinical findings by the breeder/owner or the Vet. Faecal sample analysis is not straightforward. Even when a flare is at its worst, the cysts will not be shedding in every single stool. Therefore, a negative report does not rule out giardia. The most thorough way to assess is to collect a sample from every single stool produced for 48 to 72 hours and have a Vet examine it using the giardia test kit. The giardia test is a monoclonal antibody-based ELISA for the rapid detection of *Giardia lamblia* cysts antigen in stool specimens and serves as an in vitro aid in the diagnosis of giardiasis (Beer *et al.,* 2017).

Treatment

Infection may be treated using one of a number of different drugs that are available through Vet Supply Stores. The treatment of choice is often with Metronidazole (brand name Flagyl). Metronidazole kills off the giardia and reduces the numbers to the level the dog's immune system can handle. Adaptations that may be made to try to improve the success rate of a treatment regime include extending the duration and dose of the treatment. Care must obviously be taken with this approach to make sure that an adequate safety margin is always maintained (Beer *et al.,* 2017). Another approach is to retreat after an interval of one week. Alternatively, repeat faecal samples may be collected one week after the treatment and dogs which are still passing cysts can be identified and treated. It should be recognized that, when treating a large number of dogs, whichever of these treatment strategies is adopted, there may be one or two dogs that remain as carriers of infection that will act as a potential source for reintroducing the infection into your entire kennel.

Prevention

It is very difficult to prevent the entry of an infection that is known to be carried by a percentage of normal dogs into a kennel. However, an initial period of isolation for all new entrants into kennels, for perhaps ten days, would reduce the risk of an infected dog spreading a large number of cysts around the main kennel area (CDC, 2021). All dogs could be observed and any infection present, which in the case of giardia might be exacerbated by the stress of entry in kennels, could be identified and treated before entry into the main kennels, Dogs should be prevented from access to foul water that may contain large numbers of cysts (e.g: river-flooded paddocks). Small numbers of cysts may

occasionally be present in the potable water supply but the risk of this being a major source of infection is small (CDC, 2021). It is best to use chlorinated water for the dog's drinking water as much as possible. When using non-chlorinated water from a well, lake, or stream, there is need to chlorinate the water. To chlorinate drinking water: Use only liquid bleach that contains 5.25% sodium hypochlorite as its only active ingredient and avoid soap. Use a scant 4 drops of Clorox bleach per quart of water or 2 teaspoons per 10 gallons. As an alternate method of purification, you can also boil all of your dog's drinking water. To make sure the water is completely free from living bacteria and protozoans, there is need to bring the water to a rapid boil for a minimum of five (5) minutes. However, cool moist conditions favour the survival of the organism; therefore, simply by keeping everything clean, disinfected, and dry there will be a lot of extermination of these parasites (Daily *et al.,* 2010).

13.2.8 HANTAVIRUS PULMONARY SYNDROME (HPS)

Hantavirus (Sin Nombre virus) belongs to the bunyaviridae family of viruses that is mostly found in the Wild rodents (cotton and rice rats, the white-footed mouse), but particularly deer mice (*Peromyscus spp*). The disease is recognised as one of the severe respiratory and occasionally fatal infection of human. The main source of the disease is the infestation of the wild rodents that shed the virus through urine, droppings, and saliva (Vaheri *et al.,* 2008; Evander *et al.,* 2007) HPS is majorly transmitted to humans through a process called aerosolization. Humans become infected by breathing in these infectious aerosols.

Symptoms

In the carrier organisms, they are usually asymptomatic with no outward visible signs of illness, however in human, there are flu-like symptoms such as fever, chill, muscle ache and headache. There is possible likelihood of a respiratory failure which may lead to death. A case of renal syndrome has also been reported in some humans (CDC, 2021).

Treatment

Since diagnosing this infection is difficult, however, when there are symptoms of a typical influenza, the test can also be carried out for it. Although there is no accepted drug for the treatment of the virus, however, practicing a good personal hygiene such as washing of hands after handling animals or nest materials. Since Hantavirus can persist for a few days in the environments. It is recommended that wearing of gloves and facemasks when working with rodents or in potentially contaminated areas should be practised. In case of a development of fever or respiratory illness in any of the handlers of the wild rodents, medical attention should be sought immediately (Heyman and Vaheri, 2008).

13.2.9 BRUCELLOSIS

Brucellosis is a zoonotic disease that is highly contagious. The illness is also known as Bang's disease, Crimean fever, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, Rock fever, or undulant fever. It is caused by the ingestion of unsterilized milk or meat from infected animals or close contact with their secretions (OIE, 2000). The transmission of the disease from human to human is through sexual contact or from mother to child which although is rare but possible. The causative bacteria, Brucella spp. are small, gram-negative, non-motile, Non-spore-forming and rod shaped (coccobacilli). They function as facultative intracellular parasites causing chronic disease, which usually persists for life. Symptoms include profuse sweating and joint and muscle pain. Brucellosis has been recognized in animals including humans since the 20th century. Species infecting domestic livestock are B. melitensis (goats and sheep), B. suis (pigs), B. abortus (cattle and bison), B. ovis (sheep), B. canis (dogs). B. abortus also infects bison and elk in North America. Brucella species have also been isolated from several marine mammal species (pinnipeds and cetaceans). The bacterium, Brucella abortus is the principal cause of brucellosis in cattle. The bacteria are shed from an infected animal at or around the time of calving or abortion. Once exposed, the likelihood of an animal becoming infected is variable, depending on age, pregnancy status, and other intrinsic factors of the animal, as well as the number of bacteria to which the animal was exposed (Pearson, 1998). The disease has been classified as an occupational hazard for people who worked in the livestock sector. The most common clinical signs of cattle infected with Brucella abortus are high incidences of abortions, arthritic joints and retained after-birth. There are two main causes for spontaneous abortion in animals.

The causative agent of brucellosis in *dogs* is *Brucella canis*. It is transmitted to other dogs through breeding and contact with aborted foetuses. Brucellosis can occur in humans that come in contact with infected aborted tissue or semen. The bacteria in dogs normally infect the genitals and *lymphatic system*, but can also spread to the *eye*, *kidney*, and *intervertebral disc* (causing *discospondylitis*). Symptoms of brucellosis in dogs include abortion in female dogs and *scrotal* inflammation and *orchitis* (inflammation of the testicles) in males. Fever is uncommon. Infection of the eye can cause *uveitis*, and infection of the intervertebral disc can cause pain or weakness. Blood testing of the dogs prior to breeding can prevent the spread of this disease. It is treated with antibiotics, as with humans, but it is difficult to cure (WHO, 2021)

Symptoms

Brucellosis in humans although very rare is associated with the consumption of unpasteurized milk and soft cheeses made from the milk of infected animals, primarily

goats, infected with *Brucella melitensis* and with occupational exposure of laboratory workers, veterinarians and slaughterhouse workers. Some vaccines used in livestock, most notably *B. abortus* strain 19, also cause disease in humans if accidentally injected (CDC, 2021). Brucellosis induces inconstant *fevers*, sweating, weakness, *anaemia*, *headach es*, *depression* and muscular and bodily pain.

The symptoms are like those associated with many other *febrile* diseases, but with emphasis on muscular pain and sweating. Flu-like symptoms are also very common for the infection. The duration of the disease can vary from a few weeks to many months or even years. In the first stage of the disease, *septicaemia* occurs and leads to the classic triad of undulant fevers, sweating (often with characteristic smell, likened to wet hay) and migratory *arthralgia* and *myalgia*. If untreated, the disease can give origin to focalizations or become chronic. The focalizations of brucellosis occur usually in bones and joints and *spondylodiscitis* of lumbar spine accompanied by *sacroiliitis* is very characteristic of this disease. *Orchitis* is also frequent in men.

Treatment

Antibiotics like *tetracyclines*, *rifampicin*,andthe *aminoglycosides streptomycin* and *gentamici n* areeffective against *Brucella* bacteria. However, the use of more than one antibiotic is needed for several weeks, because the bacteria incubate within *cells*. However, it should be noted that the main way of preventing brucellosis is by using fastidious hygiene in producing raw milk products, or by *pasteurizing* all milk that is to be ingested by human beings, either in its unaltered form or as a derivate, such as *cheese* (Ryan and Ray, 2004).

13.2.10 EBOLA VIRUS DISEASE

Ebola Virus Disease previously called Ebola haemorrhagic fever is severe and fatal in human. Although it is a very rare disease, the virus is transmitted to human from wild animals and quickly spread among the population through its human-to-human transmission (WHO, 2021; CDC, 2021). It causes acute and serious illness that easily kill an infected person if left untreated. It first appeared in Congo near River Ebola from where it got its name. However, the outbreak is presently restricted to Africa with the West Africa been the most hit. An index case of Ebola was reported in Nigeria on 20th July, 2014 from a Liberia-American named Patrick Sawyer. All in all, 20 cases were reported with 8 deaths. The Ebola virus belongs to Filoviridae which includes other two genera.

Transmission

Fruit bats of the family Pteropodidae were first believed to the natural host of the Ebola virus. The transmission of the virus is through human contact with the animal

secretions such as blood, organs or other body fluid of the carrier organisms. This further spread to human through the direct contact with an infected person secretion (WHO, 2021; CDC, 2021).

Symptoms

The virus has an incubation period of between 2 to 21 days with manifesting symptoms that include sudden debilitating fever, muscle pain, headache and sore throat. These are subsequently followed by vomiting, diarrhea, skin rash, impaired kidney and liver function. In some severe cases, internal and external bleeding occurred.

Diagnosis

It is difficult to distinguish Ebola virus disease from some infectious diseases but through certain medical approaches such as antigen detection test, virus culture isolation, reverse transcription polymerase, the virus can be detected (WHO, 2021; CDC, 2021).

Treatment

Isolation and early incubation of infected patient is a way of managing the disease. Frequent rehydration and treatment of disease-specific symptoms is another method of improving the infect person survival. Presently, there are no vaccines to cure the virus, however, trial vaccines are been tested for safety purpose (CDC, 2021; WHO, 2021).

13.2.11 TOXOCARIASIS

This is a parasitic disease that is caused by the infection of a type of roundworm found in dogs or cats. The disease can result in a serious illness eye, and organ damages. This disease affect children, dog or cat owners and those that have affection for dirt (CDC, 2021). The infection occurs when people accidentally swallow dirt that has been contaminated with dog faeces containing *Toxocara* eggs. Though very rare, infection can also be gotten through the consumption of undercooked meat containing *Toxocara* larvae. However, it is not spread like cold or flu that is transmitted by person-person contact.

Symptoms

Majority of those infected with Toxocariasis especially Adults doesn't exhibit symptoms and get sick, however, children may fall sick and show symptoms such as organ damage, breathing issue, stomach pain, vision problems.

Prevention

The prevention of toxocariasis simply entails adopting hygienic practices especially around the dogs and cats. The practice may include teaching children the importance of washing hands after playing with dirt. Sandboxes should be covered and animals should be restricted to their confines. Dog and cat wastes should be disposed off appropriately. And finally, regular check-up of pets to avoid infection with *Toxocara* should be adopted (CDC, 2021).

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CHAPTER FIFTEEN: ORNAMENTAL PLANTS AND CASH CROPS

Yunusa Audu

15.1 SCOPE AND CONCEPT

Gardening which was only an art and science in the earlier days has now emerged as a huge industry. With the importance and need of gardening in improving and conserving the environment being strongly felt now, the concept of landscaping and gardening is growing rapidly. Ornamental gardening and landscaping has expanded as a multi-faceted industry encompassing activities such as propagating and rearing ornamental plants, landscaping, production of growing media, pots and other accessories, etc., generating huge employment opportunities and simultaneously promoting activities that would improve the environment.

15.2 Introduction

Botany is the field of basics science dealing with the study and inquiry in to the forms, function, development, diversities, reproduction, evolution and uses of plants and their interaction within the biosphere. It also includes the investigation of their uses and the other parameters of importance wherever found (Liddell & Scott 1940)

Economic botany: Is the study of relationship between people (individuals and cultures) and plants. Economic botany intersects many field including established discipline such as Agronomy, anthropology, archeology, chemistry economic, ethnobotany, forestry, horticulture, pharmacognosy and pharmacology etc. (Arber 1928)

15.3 Ornamental Plants

Are all those plants which are cultivated with the main aim of being marketed and used for decorative purposes. With a few exceptions (such are the flowers of daylilies *Haemerocallis* or those of nasturtiums *Tropaeolum*) they are not edible, or at least, not used primarily as food sources. It is justified by the fact that ornamental plants are, and have always been, an indispensable part of human life: planted outdoors, they improve our environment, while in indoor use they contribute to our health, well-being and creativity (Saraswathi *et al.,* 2018)

Method of Cultivation: protected cultivation and open-ground cultivation.

According to the application, the two main groups are plants used for indoor decoration and plants used for outdoor decoration.

Protected cultivation (plants grown and used mainly for indoor decoration), subdivided to:

§ Cut flowers e.g. roses, carnations, chrysanthemums (dendranthemums), gerberas, lilies etc.

§ Cut foliage

§ Pot plants (flowering pot plants and foliage pot plants) e.g. bonsaic, cacti and ficus

§ Bedding plants and balcony plants (annual, biennial and partially perennial ornamentals, grown under cover but used mainly outdoors) e.g. marigold and petunias

Open ground cultivation (plants grown mainly for outdoor decoration or as a starting material for protected cultivation), with a further subdivision as follows:

§ Woody nursery stocks (deciduous trees and shrubs, broadleaved evergreens, climbers, and conifers, grown either in the field or in containers),

§ Herbaceous perennials (usually container- or pot-grown in perennial nurseries),

§ Rose bushes (grown in specialized rose-nurseries),

§ Flower bulbs, corms, and tubers (grown for forcing in greenhouses or for planting out in the open),

§ Open-ground cut flowers and cut foliage,

§ Dried flowers, and

§ Some bedding plants, grown (or finished) in the open.

15.3.1 Importance of Ornamental Plants

Ornamental trees, shrubs and flowers have various uses, hence their importance for our well-being.

1. **Landscaping:** flowers are used to beautify residential and office buildings as well as playground, the plants whose flowers are attractive, colorfuland sweet smelling are used for this purpose.

2. **Oxygen Production:** The fundamental benefit provided by plants is their production of oxygen; thus providing the atmosphere with the element that allows humans to breathe and live on this planet.

3. **Carbon Sinks:** Plants take in carbon dioxide and convert it to carbohydrates (sugars). These sugars provide the plant with energy to grow. As the plant or parts of the plant die, the decomposition of the plant material returns the carbon to both the soil and the atmosphere.

4. **Urban Shade, Green Space and Location of Plants:** Ornamental shrubs and trees such as Odan-ficus spp and almond tree can be strategically located to engineer a more pleasant environment in which to live. They can be placed around pools to provide shade but also to reduce the glare that is reflected up to surrounding buildings; they can be placed in front of houses to prevent lights from vehicles or the street shining into the house; and they can also play a role in reducing noise from highways.

5. **5. Wind break:** Closely spaced tall ornamental shrub and trees are usually planted close to buildings to act as wind break and add beauty of the areas.eg teak,Ashoka (police tree)

6. **Screening and delineation of areas:** Ornamental plant can be used to screen a place such as house from the prying eyes of strangers and also used to delineate area from one another by using it for demarcation.

7. **Decoration:** Live fresh flowers put in vases are used to decorate living rooms Some putted ornamental plants are raised and placed at the porches of the houses or offices at wedding, funeral, workshop seminars beautiful flowers are used for decoration, thus adding color to the occasion.

8. **Indoor Air Quality Improvement:** Ornamental Plants continue to function as atmospheric filters indoors as they do outdoors and enhance the air quality of confined environments.

9. **Water Management and Erosion Control (retention, filtration, purification, flood control):** Ornamental plants can also remove pollution through filtration and purification processes and can also assist in flood control by retaining water.

10. **Wildlife Attraction, Preservation and Biodiversity:** Ornamental plants can also provide environments that encourage the presence of wildlife both during production of the plant (e.g. Christmas trees and other nurseries) and at the end use in the garden or parkland (Saraswathi *et al.,* 2018).

15.4 Cash Crop

A cash crop or profit crop it is an agricultural crop which is grown to sell for profit. The cash crop is equated with plantation of crop such as coffee, tea, cotton, cocoa, tobacco, oilseed, and fruits etc. The term is used to differential marketed crops from subsistence crops, which are those fed to the producers own family or livestock feed. Thus, cash crops may be placed on a continuum, from pure home consumption to pure cash crop. Cash crops are sold on domestic markets and foreign markets (Reeves & Weihrauch, 1979). Cash crops are usually crops which attract demand in more developed nation, and hence have export value.

15.4.1 Types of Cash Crop

Cash crop is distinguished in to two types. First, crops that are exclusively grown for sale, which include crops that are non-food, such as cotton, coffee, tobacco, cocoa or tea. Second, crops that are produced with a 'marketable surplus', which include food crops that may be consumed by the household or sold on markets, such as Guinea corn, rice, millet and maize, but also certain fruits and vegetables (Reeves & Weihrauch, 1979).

15.4.2 Importance of Cash Crop

- Income generation
- Generation of employment
- It promotes economic diversification

Summary

Botany is the field of basics science dealing with the study and inquiry in to the forms, function, development, reproduction and uses of plants and their interaction on earth. Economic botany, is a science that deal with the study of plants and it relationship between people in the environment. Economic botany intersects many fields including established discipline such as Agronomy, anthropology, archeology, ethnobotany, forestry, horticulture etc. Economic botany is catogorised in to Ornamental plants and cash crops. Ornamental plants these are plants that are mainly cultivated for market and decoration purpose e.g. roses, cacti and ficus etc. while cash crop are crops that cultivated to generating profit not for household consumption only. These crops are usually attract demand and also have export value. e.g. Coffee, rice, groundnut, maize, cotton, tobacco and tea. These plants are of great importance for our living, as it act as sources of income generation, sources food we eat, provision of employments and also promotes economic diversification, provision of oxygen, landscaping, carbon dioxide sink and decoration (Armitage, 1994)

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CHAPTER SIXTEEN: CONSERVATION OF NATURAL RESOURCES

Ibrahim, Baba Usman

Conservation of natural resources is the wise use and safe keeping of natural resources for the good of mankind, to ensure their continuous availability and to preserve the original nature of the environment.

16.1 Natural Resources

Natural resources are useful things provided by nature. Soil, water, wildlife, forest, minerals, sunlight and air are examples of natural resources. Natural resources can be divided into:

(a) Replaceable (Renewable) resources, and

(b) Irreplaceable (Non-renewable) resources

Replaceable (Renewable) Resources: are those which can only be replaced by offspring that is by reproduction, e.g. trees and animals.

Irreplaceable (Non-renewable) Resources: are those resources that cannot be replaced when the initial stock is used up. e.g petroleum, coal, gold, diamond, copper and Iron. Therefore, these sources must be carefully managed to avoid their shortage until acceptable substitutes are found.

16.1.1 Conservation and Development of Natural Resources

Conservation starts with superstitions belief. In certain area some species of animals are not use for food due to superstitions belief e.g. vulture throughout the whole world. However, in western part of Nigeria, they cannot be found, because they are utilized for traditional medicine by herbalist. This superstition is not enough to protect the animal in view of population increase.

Thus, the first major step in conservation was taken by the kings, they have their own forest, but now not only the kings and princes are protecting the environment by having area reserved for wild animals. There are international organizations and conservation clubs, who protect natural environment and wildlife:

(a) World wild life fund (WWF)

(b) International union of conservation of natural resources (IUCN)

(c) International council for Bird preservation (ICBP)

(d) Nigerian conservation foundation (NCF)

The first person that observe that human resources is increasing and the resources they consume is decreasing is Malthus.

Malthus in the 19^{th} century warned about decreasing resources of the world and the increasing in human population, and had writing about the conflicting between the two.

There are certain resources that can be renewed e.g. wild life and the non – renewable resources e.g. minerals.

Thus, he warned against possible conflicting between increasing in population and the means of sustaining them i.e. food

16.2 Why Do We Need to Conserve?

(a) **For Educational Purpose:** certain environmental phenomenons are better understood when pursue visually e.g. explain inter specific relationship or competition in game reserve. The student learns and understand more by viewing the animal in their natural environment.

(b) **Aesthetic and Protection of Natural Heritage:** By conserving the wildlife of an area its natural beauty is maintained, and the natural heritage conserved, e.g. people derived pleasure by looking at wildlife in their natural environment.

(c) **Recreation:** Game reserves and natural parks are common, because people derived great pleasure in watching animals, in their natural habitat. There are also other activities carried out in the reserves e.g. sport hunting, however this has not been practice in Nigeria due to low population game reserves of wild animals.

(d) **Tourism:** The economic value of wild life resulting from tourism made wildlife conservation to be encouraged. Thus, tourism boost wildlife industry and this in turn boost hotel industry, food industry, and transportation industry. All of these industries benefit from tourism and they come under tourist industry. The activities of people on tour of a game reserve require these facilities.

(e) **Employment:** Wildlife conservation often employment opportunity to people e.g game warden.

(f) **Scientific Research:** Animals from game reserve serve as a reserviour specimen for research. e.g. America imports 100's of monkey from India for medical researchers. Drugs are first tested on wild animals (e.g rats, monkey (primates), before being introduced to human beings. Without wild life human being has to be used for testing drugs.

(g) **Meat Production:** In most tropical countries. The soil is very poor and they found that game cropping is the best means of utilizing such a marginal land. This marginal land can be utilized for production of meat by rearing wild animal in those places. E.g. Hill or mountainous area. Game cropping recur in game reserves e.g. over population of wild animal- they are killed and sold to the public to maintain wild life population, such situation has not occurred in Nigeria because population of wild animals are still low.

16.2.1 How to Get a Place Secured as A Wildlife Reserve

This is initiated by conservation individual or organization:

a. Survey of the area to be reserved: i.e the area where game reserve is to be made is demarcated after survey.

b. Inventory of the resources of the area to be preserved i.e notice the type of vegetation type of animals and other things in the area including number of organisms i.e. population census by walking through the whole reserve area to get number of the animals and the kinds of animals.

16.2.2 Principles and Prospects of Forestry Conservation Practices

16.2.2.1 Conservation of Forests

Forest is fast dwindling due to indiscriminate felling of trees for various uses for various uses such as firewood and furniture.

Bush burning is a major disaster in forests. Air and soil pollution are also contributors to the dwindling forests. Therefore, to prevent loss of our forests and to have continuous maximum benefits from them, conservative methods are necessary.

16.2.2.2 Methods of Conserving Forest

1. **Laws:** There are established laws that regulate the felling of trees i.e cutting down of timber trees. Permission must be obtained before any timber is felled, from ministry of Agriculture are Natural resources.
2. **Forest Guards:** Forest guards are usually employed to guard and protect the forest against misuse.
3. **Selective Cutting of Trees:** Only mature trees are allowed to be felled for timber. The young trees are left to grow to replace the felled trees.
4. **Planting of new Trees (Afforestation):** In order to preserve the forest, new trees are planted to replace the felled ones. It is now a common practice to plant trees. The slogan is that where you remove one tree, plant two. If trees are not planted, there will be shortage of trees in future.
5. **Forest Reserves:** Government acquires forests as forest reserves. People are not allowed to farm, hunt or cut down trees, in these reserves except by permission.
6. **No Bush Burning:** Bush burning is prohibited and forbidden by law. Anybody caught is severely punished.
7. **Government:** Government should bring the cost of coal, cooking gas and electricity to a level which the poor can afford. This will prevent the excessive use of firewood which is one of the causes of deforestation.

16.2.3 Benefits of Forest Conservation

i. It ensures preservation and continuous availability of timber and other economic trees.

ii. It helps to preserve the fertility of the top soil by preventing soil erosion.

iii. It prevents desert encroachment that is desertification

iv. It preserves the natural habitats of animals hence increase in animal population. And with the increase in animal populations, animal proteins i.e meat will be more available for men.

v. It encourages rainfall which plants need to grow well. Healthy growth plants lead to abundant food supply.

vi. Forest act as wind brakes to strong winds, hence prevent strong winds from destroying man's properties.

vii. Some plants are used for medicines. Their conservation will ensure continuous availability, and therefore promote good health.

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CHAPTER SEVENTEEN: CULTIVATION AND PURIFICATION OF VIRUSES

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17.1 Cultivation

Many viruses can be grown in cell cultures or in fertile eggs (embryonated eggs) under strictly controlled conditions or inoculation into suitable host animal.

17.1.1 Embryonated Eggs

These are more practical for cultivation of viruses, ethical and economic and for ease of handing and relative freedom from contaminants. A developing chick – embryos are immune – deficient thus favours the growth of viruses. Most viruses will grow or can be adapted to grow hi fertile eggs, and some may kill the embryo or may produce visible evidence of specific infection on the Choricallantoic Membrane (C.A.M). haemagglutinnating viruses in allantioc and amnionic fluids will cause haemagglutinnation when incubated with appropriate species of erythrocytes (red blood cells). According to Willey , J. M. et al., (2008), there are four routes of inoculating – eggs for viral cultivation:

- C.A.M. used for many pox and some herpes viruses.
- All nontoxic cavity used for ortho– paramyxo and rhabdo–viruses.
- All nontoxic cavity used for ortho–and paramyxo–viruses.
- Yolk sac used for many touaviruses.

17.1.2 Animal Host

Ideally, the natural host of a viruses or closely related species should be used for animal inoculation. This mode of viral cultivation is not always practical on ethical or economic ground, while there is also the possibility of latent infection with the virus under consideration. The route of inoculation of the animals is an important factor due to the specific affinity some viruses have for certain tissues, e.g. intracerebral–inoculation of mice with rabiesvirus: subcutaneous inoculation of swine vesicular disease virus into pigs. Animal may show clinical signs of infection and these must be observed, or biopsy material taken for examination. Necrosis must be conducted thoroughly and any microscopic abnormalities and histological changes noted. Serology may be necessary fro the presence of specially acquired antibodies.

Isolation or demonstration of the virus may be attempted by egg inoculation or tissue culture, and by electron microscopy. Neutralization of virus with a specific antiserum herb re inoculation of animals will, of course prevent the occurrences of infection.

The growth of virus in animal hosts is still used for primary isolation of certain viruses and for studies of the pathogenesis of viral disease and viral on cogenesis.

17.1.3 Cell Cultures

The availability of cell grown in – vitro has facilitated the identification and cultivation of newly isolated viruses and the characterization of the previously known ones. Cell cultures have been on the success since the advent of antibiotics and fungicides which have made it possible to prevent contamination of cultures. The introduction of trysin facilitated monolayer growth of cells. Chemically defined growth media have been produced to satisfy the nutritional requirements of many different types of cells . According to Medigan, M.T. *et al.,* (2009). There are 3 basic types of cell culture:

(a) Primary cultures are made dispersing cells (usually with trysin) from freshly removed host tissues. In general, they are unable to grow for more than a few passages in culture e.g. Monkey Kidney cells and Human – amnion cells.

(b) Secondary cultures (semi – continuous cells) are also known as Diploid cells. They all undergone a change that allow their limited cultures (up to 50 passages) but which retain their normal chromosome pattern e.g Human embryo lung.

(c) Continuous cell lines are cultures capable of more prolonged, perhaps indefinite growth that have been derived from diploid cell lines or form malignant tissues. They invariably have altered and irregular numbers of chromosomes. The types of cell culture used for virus cultivation depend on the sensitivity of the e cells to a particular virus Continuous cell Lines is also referred to as Heteroploids cells lines e.g Hela cells derived from human cervical cancer.

Conclusion

Viruses can be cultivated via several means including embryonated eggs, animal host and cell cultures.

Summary

The best method for culturing viruses is cell cultures as even the most difficult virus can grow in cell lines suitable as host .

17.2 Purification

For purification, the starting material is usually large volumes of tissue culture medium, body fluids or infected cells, pure virus is important so as to have meaningful studies o the properties and molecular biology of the virion. The first frequently involved concentration of the virus particles by precipitation with ammonium and elution can be used to concentrate orthomyxoviruses. Once concentrated, virus can then be separated I mm materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.

According to Pelczar, M.J. *et al.,* (2001), more than one step is usually necessary to achieve adequate concentration. A preliminary purification will remove no n–virus material: the first step may include centrifugation while the final purification step almost always involves density gradient centrifugation. The band of purified virus may be detected by optical methods, by following radioactivity if the virus is radiolabelled, or by assaying infectivity.

Viruses can also be purified by high-speed centrifugation in density gradients of Cesium Chloride (CsC1), potassium tartarate, potassium citrate of Sucrose. The gradient material of choice is the one that is least toxic to the virus. The virus particles migrate to me equilibrium position where the density of the solution is equal to their buoyant density and form a visible band. Virus bands are harvested by puncture through the bottom of the plastic centrifuge lube and assayed for infectivity.

In column chromatography, virus is bound to substance such as DEAE or phosphocellulose and then eluted by changes in pH or Salt concentration. Zone electrophoresis permits the separation of virus particles from contaminant on the basis of charge. Specific antisera also can he used to remove virus particles from host materials

Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contain variable amounts of envelope per particle, the virus population is heterogeneous in both size amid density. It is very difficult to achieve complete purity of viruses. Small amounts oc cellular materials tend to adsorb to particles and this co purity with the virion. The minimal criteria for purity are a homogenous appearance in electron micrographs and the failure of additional purification procedure to remove contaminants without reducing infectivity.

17.2.1 Centrifugation

Centrifugation as a purification and characterization procedure:

Ultracentrifuge: A centrifuge is capable of generating large centrifugal fields by rotating samples at 20,000 – 100,000 rpm. Centrifugal forces of greater than 100,000 X gravity can be generated.

17.2.2 Sedimentation Coefficient

§ Rate at which a macro molecule sediment under a defined gravitational force.

§ This parameter is influenced by both the molecular weight and shape of a macromolecule (larger and more spherical sed. Faster).

§ The basic unit is the Svedberg (S) which is 10^{-13} sec.

§ This value can be used to estimate molecular weights in conjunction with other values.

Buoyant density – Density at which a virus or other macromolecule neither sinks nor floats when suspended in a density gradient (e.g. C_sC1_2 or source).

17.2.1.1 Types of sedimentation Medium

1. Aqueous Buffer (Water based) – Can be used to separate molecules with widely different S values (ex. Nuclei from ribosomes)

2. Sucrose or glycerol gradients or cushions (isokenetic or rate – zonal) – A fixed concentration or a linear gradient of these agents in buffer is used. The compounds increase the density and viscosity of the medium therefore, decreasing the rate at which macromolecule sediment through them and prevening the sedimentation molecules with densities less than the medium. General approach is to pour "cushion" of material at the bottom of the centrifuge tube and centrifuge the virion onto the cushion (cushion need not always be used). By controlling the time and speed of centrifugation a significant purification can be obtained. Since most macromolecules have greater densities than these mediums separation is based on S values. T us can be used to separate molecules with relatively close S values.

3. C_sC1 gradient centrifugation (isopycnic or buoyant density) – A linear gradient of these compounds in buffer is prepared in the centrifuge tube. As the concentration of the compound is increased the density of the medium increases in the tube Density is low at the lop am high at the bottom. Macromolecule centrifuged through is ill form a band at a position equal to their buoyant density. Useful for separating molecules of different densities even when the densities are very close. Drawback is that C_sC1 can permanently inactivate some viruses.

Conclusion

Virus can then be separated from materials by differential centrifugation density gradient centrifugation, column chromatography and electrophoresis.

Ultracentrifuge is capable of generating large centrifugal fields by rotating samples at 20,000–100,000 rpm

Summary

§ Viruses can be purified from tissue culture mediums.

§ Virus can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.

§ Viruses can also be purified by high-speed centrifugation in density gradients of Cesium Chloride (C_sC1), potassium tartarate, potassium citrate of sucrose.

Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contain variable amounts of envelope per particle, the virus population is heterogeneous in both size amid density.

stain background hut not the – virus particles) or shadowing tech n 'nines (place specimen a support and direct a vaporized heavy metal across the sample at an angle. This creates a region where relatively link: metal deposits just behind the viral particle (resulting in a shadow).

X – ray crystallography involves the analysis of crystallized virus. Virus crystals are symmetrical structures composed of many isometric viruses. The atoms of the crystal will diffract X – rays in a structure dependent manner. This approach has been used to analyze the structure of the viruses at the molecular level. Resolution at the Armstrong level (10^{-10} meters, in the bond length range) is possible.

A purified physical particle should fulfill the following criteria before is identified as a virus panicle:

1. The particle can be obtained only from infected cells or tissues.
2. Particles obtained from various sources are identical regardless of the cellular species in which virus is grown.
3. The degree of infective activity of the preparation vanes directly with the number of particles present.
4. The degree of destruction of the physical particle by chemical or physical means is associated with a corresponding loss of virus activity.
5. Certain properties of the particles and infectivity must be shown to be identical such as their sedimentation behavour in the ultracentrifuge and their PH stability curves
6. The absorption spectrum of the purified physical particle in the ultraviolet range should coincide with ultraviolet inactivation spectrum of the virus.
7. Antisera prepared against the infective virus should react with the characteristic particles and vice versa. Direct observation of an unknown virus can be accomplished by electron microscopic examination fo aggregate fromate in a mixture of antisera and crude virus suspension.
8. The particles should be able to induce the characteristic disease in – vivo (if such experiment are feasible).
9. Passage of the particles in tissue culture should result in the production of progeny with biologic and serologic properties of the virus.

17.3 Preservation of Virus

The preservation of virus is an important sensitive area in virology, it is necessary to preserve viruses after being purified to research purposes and in the development of vaccines. Viruses cannot be preserved on ordinary laboratory media as in most bacteria or fungi. They are preserved as follows:

17.3.1 Freezing

A large wide- mouthed thermo jar or insulated carton, half filled with pieces of solid Co_2 (dry ice), serves for transport and storage of material containing viruses. If dry ice is unavailable,

17.3.2 Cultivation and Purification of Viruses

The specimens should be kept co id and transported on ordinary. The temperature in a dry ice storage cabinet is close to -76^0C. Electric deep freezer can maintain temperatures of -50^0C to -105^0C.

17.3.3 Lyophilization

This procedure consists of rapid freezing at lo\v temperature (in a bath containing Alcohol and dry ice) and dehydration from the frozen state at high vacuum: 1.0-50% of normal plasma or serum in the fluid menstruum protects the virus to be frozen and dried. The plasma or serum must not contain neutralizing antibodies. Skimmed milk also another "protective" menstruum in which virus-containing material may he suspended.

17.4 Ethics in a Virology Laboratory

The virology lab is a place where the scientist needs to take special caution in addition to normal laboratory practices:

- § You must wear a sterile laboratory coat every time.
- § You must wear a shoe cover.
- § You mustn't eat in the laboratory.
- § You must not wear make ups, jewelry or wear your hair down in the laboratory.
- § The work benches must be free of unnecessary items such as bags.
- § Nose mask and sterile gloves must be available at all times.
- § You mustn't talk when working with RNA viruses as RNAases are everywhere and may degrade your RNA genome.
- § Safety signs must be in appropriate places and on chemicals.
- § Proper storage of chemicals before and alters use.
- § Proper labeling of samples and chemicals.
- § The laboratory must be clean at all times.

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CONCLUSION

Freezing is best for presetting samples during active research and lyophilisation is using preserving viruses for a long time. Good laboratory practices are essential for obtaining excellent results from research

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SUMMARY

1. Lyophilisation and freezing are methods of preserving viruses
2. Good laboratory practice is important in getting good results from virus studies.

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