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Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

LECTURE HANDOUTS

L - 1
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BME	

II/IV

Course Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY

Course Teacher : Ms.A.Anjali

Unit

Date of Lecture:

Topic of Lecture: Cell injury &Reversible cell injury

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: I

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

Fundamentals of biochemistry

# **Detailed content of the Lecture:**

Cell damage (also known as cell injury) is a variety of changes of stress that a cell suffers due to external as well as internal environmental changes. Amongst other causes, this can be due to physical, chemical, infectious, biological, nutritional or immunological factors.

**Cell** damage (also known as **cell injury**) is a variety of changes of stress that a **cell** suffers due to external as well as internal environmental changes.

# **REVERSIBLE CELL INJURY (RCI):**

If ischemia or hypoxia is for short period of time, the cell can be reverting back to its normal condition which is known as RCI. In coronary arteries, myocardial contractility is reversed if circulation is quickly restored.

It also depends upon the organ which undergoes hypoxia. Ex, skeletal muscle can with stand for half an hour with cell injury but brain cells can undergo permanent damage within 10 minutes. The pathogenesis of RCI is described below.

- Decrease supply of oxygen, decreases cells aerobic respiration by mitochondria due to decrease ATP generation.
- To maintain the supply of energy to the cell anaerobic glycolysis takes place to generate ATP.
- Decreases glycogen level. This result in increase accumulation of lactic acid. Thus, decreases intracellular pH.
- This causes clumping of nuclear chromatin.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 



**BME** 

## MUTHAYAMMAL ENGINEERING COLLEGE (An Autonomous Institution)



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Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

**LECTURE HANDOUTS** 

II/IV

L - 2

Course Name with Code	: 16BMD11 &PATHOLOGY	&MICROBIOLOGY
Course Teacher	: Ms.A.Anjali	
Unit	: I	Date of Lecture:

Unit

Topic of Lecture: Irreversible cell injury and Necrosis,

## **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:** Irreversible cell injury

- After the initiation of irreversible death, the cell and its organelles start to disintegrate, leading to rupture of the cells.
- Gradually, the cytotoxic edema starts to resolve and interstitial edema develops as the cell membranes disintegrate and the intracellular components become extracellular.
- This results in increased Brownian water motion and marked reduction in diffusion restriction. •
- The neuronal death result in reduction of its markers (NAA). •

Necrosis is the death of body tissue. It occurs when too little blood flows to the tissue. This can be from injury, radiation, or chemicals. **Necrosis** cannot be reversed. When large areas of tissue die due to a lack of blood supply, the condition is called gangrene

#### There are six types of necrosis:

- Coagulative necrosis. •
- Liquefactive necrosis.
- Caseous necrosis. •
- Fat necrosis.
- Fibroid necrosis.
- Gangrenous necrosis.

#### Video Content / Details of website for further learning (if any):

https://nptel.ac.in/courses/102106025/36

**Important Books/Journals for further learning including the page nos.:** Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

**LECTURE HANDOUTS** 

L	-	3	

BME		II/IV
Course Name with Code	: 16BMD11 &PAT	HOLOGY &MICROBIOLOGY
Course Teacher	: Ms.A.Anjali	
Unit	: II	Date of Lecture:

**Topic of Lecture: Apoptosis, Intracellular accumulations** 

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

## Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

Effect of a force that tends to cause rotation about an axis  $M = F \cdot d$  (Nm)

Apoptosis is a form of programmed cell death that occurs in multicellular

organisms. <u>Biochemical</u> events lead to characteristic cell changes (<u>morphology</u>) and death. These changes include <u>blebbing</u>, <u>cell shrinkage</u>, <u>nuclear fragmentation</u>, <u>chromatin</u>

<u>condensation</u>, <u>chromosomal DNA fragmentation</u>, and global <u>mRNA</u> decay. The average adult human loses between 50 and 70 <u>billion</u> cells each day due to apoptosis.<sup>[3][a]</sup> For an average human child between the ages of 8 to 14 years old approximately 20 to 30 billion cells die per day.

In contrast to <u>necrosis</u>, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's life cycle. For example, the separation of fingers and toes in a developing human <u>embryo</u> occurs because cells between the digits undergo apoptosis. Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that <u>phagocytic cells</u> are able to engulf and remove before the contents of the cell can spill out onto surrounding cells and cause damage to them.

INTRACELLULAR ACCUMULATION:

It occur due to accumulation of normal cellular□ substance such as water, lipid, carbohydrates, protein & abnormal substance such as exogenous & endogenous into tissues. Exogenous include minerals or product of infectious□ agents & endogenous include synthetic & metabolic products. It may be transient (reversible) or permanent.□ May be harmless but occasionally harmful.□ It may be in cytoplasm or nucleus.

CAUSES:

Normal endogenous substances is produced at normal or  $\Box$  increased rate but metabolism is inadequate. Abnormal endogenous substances accumulates due to  $\Box$  alteration in protein folding & transport. Normal endogenous substances accumulates because of  $\Box$  inherited defect in enzymes. Abnormal exogenous substance accumulates because of  $\Box$  defect in enzymatic mechanism & transport. Video Content / Details of website for further learning (if any):

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**LECTURE HANDOUTS** 



BME

II/IV

L-4

Course Name with Code	: 16BMD11 &PATHOLOGY &MICROBIOLOGY	
Course Teacher	: Ms.A.Anjali	
Unit	: I	Date of Lecture:

Topic of Lecture: Pathological calcification- Dystrophic and Metastatic.

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

## **Detailed content of the Lecture:**

Pathologic calcification is the abnormal deposition of calcium salts with smaller amounts of iron,

magnesium, and other minerals.

Mechanism of Calcification:

Calcium deposits in the form of hydroxyapatite and involves two phases:

1)Initiation: Initiation of calcium takes place in the matrix vesicles where calcium and phosphate accumulate. Matrix vesicles are extracellular membrane bound vesicles that bud off from other

cells

2)Propagation: The second phase involves further growth of hydrpxyapatite

Types of Pathologic Calcification:

# **Dystrophic Calcification:**

\*When the deposition occurs in dead or dying tissues

\*it occurs with normal serum levels of calcium and normal calcium metabolism

# **Metastatic Calcification:**

The deposition of calcium salts in normal tissues

It almost always reflects some derangement in calcium metabolism and increased levels of calcium (hypercalcemia)

#### **Dystrophic Calcification**:

Dystrophic calcification is encountered in areas of necrosis of any type.

◆ It is certain in the atheromas of advanced atherosclerosis, associated with intimal injury in the aorta and large arteries

• Although dystrophic calcification may be an incidental finding indicating insignificant past cell injury, it may also be a cause of organ dysfunction.

♦ For example,

Dystrophic calcification of the aortic valves is an important cause of aortic stenosis in the elderly

#### Morphology of Dystrophic calcification

•Calcium salts are grossly seen as fine white granules or clumps, often felt as gritty deposits.

♦ Histologically, calcification appears as intracellular and/or extracellular basophilic deposits.

♦ In time, heterotopic bone may be formed in the focus of calcification.

#### **Metastatic Calcification**

• Metastatic calcification can occur in normal tissues whenever there is hypercalcemia.

Main Causes:

1.Increased secretion of parathyroid hormone

2.Destruction of bone due to the effects of accelerated turnover (e.g., Paget disease),

immobilization, or tumors (multiple myeloma, leukemia, or diffuse skeletal metastases)

#### Morphology of Metastatic calcification

• Metastatic calcification can occur widely throughout the body but principally affects the interstitial tissues of the vasculature, kidneys, lungs, and gastric mucosa.

♦ The calcium deposits morphologically resemble those described in dystrophic calcification

#### Video Content / Details of website for further learning (if any):

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**LECTURE HANDOUTS** 

II/IV

L - 5

Course Name with Code	: 16BMD11 &PATHOLOGY &MICROBIOLOGY	
Course Teacher	: Ms.A.Anjali	
Unit	: I	Date of Lecture:

Topic of Lecture: cellular adaptations of growth and differentiation,

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

Adaptations of Cellular Growth and Differentiation:

Adaptations are reversible changes in the size, number, phenotype, metabolic activity, or functions of cells in response to changes in their environment. Such adaptations may take several distinct forms.

#### HYPERTROPHY:

Hypertrophy refers to an increase in the size of cells, resulting in an increase in the size of the organ. The hypertrophied organ has no new cells, just larger cells. The increased size of the cells is due to the synthesis of more structural components of the cells. Cells capable of division may respond to stress by undergoing both hyperplasia (described below) and hypertrophy, whereas in nondividing cells (e.g., myocardial fibers) increased tissue mass is due to hypertrophy. In many organs hypertrophy and hyperplasia may coexist and contribute to increased size. Hypertrophy can be physiologic or pathologic and is caused by increased functional demand or by stimulation by hormones and growth factors. The striated muscle cells in the heart and skeletal muscles have only a limited capacity for division, and respond to increased metabolic demands mainly by undergoing hypertrophy. The most common stimulus for hypertrophy of muscle is increased workload. For example, the bulging muscles of

bodybuilders engaged in "pumping iron" result from an increase in size of the individual muscle fibers in response to increased demand. In the heart, the stimulus for hypertrophy is usually chronic hemodynamic overload, resulting from either hypertension or faulty valves. In both tissue types the muscle cells synthesize more proteins and the number of myofilaments increases. This increases the amount of force each myocyte can generate, and thus increases the strength and work capacity of the muscle as a whole.

## HYPERPLASIA:

Hyperplasia is an increase in the number of cells in an organ or tissue, usually resulting in increased mass of the organ or tissue. Although hyperplasia and hypertrophy are distinct processes, frequently they occur together, and they may be triggered by the same external stimulus. Hyperplasia takes place if the cell population is capable of dividing, and thus increasing the number of cells. Hyperplasia can be physiologic or pathologic.

ATROPHY:

Atrophy is reduced size of an organ or tissue resulting from a decrease in cell size and number. Atrophy can be physiologic or pathologic. Physiologic atrophy is common during normal development. Some embryonic structures, such as the notochord and thyroglossal duct, undergo atrophy during fetal development. The uterus decreases in size shortly after parturition, and this is a form of physiologic atrophy.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

## Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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**LECTURE HANDOUTS** 

BME		II/IV
Course Name with Code	: 16BMD11 &PATHOLOGY &MICRO	BIOLOGY
Course Teacher	: Ms.A.Anjali	
Unit	: I Da	ate of Lecture:

Topic of Lecture: Inflammation and Repair including fracture healing,

## **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

## Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

• Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. • Causes of inflammation – Infective agents like bacteria, viruses and their toxins, fungi, parasites. – Immunological agents like cell-mediated and antigen-antibody reactions. – Physical agents like heat, cold, radiation, mechanical trauma. – Chemical agents like organic and inorganic poisons. – Inert materials such as foreign bodies. Signs of inflammation • Signs of inflammation Five cardinal signs of inflammation as • rubor (redness) • tumor (swelling) • calor (heat) • dolor (pain) • functio laesa (loss of function)

Types of inflammation • Depending upon the defense capacity of the host and duration of response, inflammation can be classified as – acute – chronic Acute inflammation • Causes of acute inflammation: Infection, trauma, physical and chemical agents, necrosis, foreign bodies, and immune reactions. • Stages of acute inflammation: – Vasodilation – Increased vascular permeability – Movement of white blood cells from blood vessels into soft tissue at the site of inflammation Acute inflammation • Stages of acute inflammation: – Vasodilation: Vasodilation occurs through release of mediators [include histamine, prostacyclin (PGI2), and nitric oxide (NO)] from cells. Vasodilation increases the hydrostatic pressure by causing slowing (sludging) of blood flow. Sludging of blood also causes margination of leukocytes along the wall of the blood vessel

Chronic inflammation • Prolonged inflammation consisting of active inflammation and tissue destruction and repair, all occurring simultaneously. • Causes of Chronic inflammation: – Chronic inflammation following acute inflammation – Recurrent attacks of acute inflammation – Chronic inflammation starting

Healing of Tissues • Healing is the body response to injury in an attempt to restore normal structure and function. Healing involves 2 distinct processes: – Regeneration: healing takes place by proliferation of parenchymal cells and usually results in complete restoration of the original tissues – Repair: healing takes place by proliferation of connective tissue elements resulting in fibrosis and scarring

Regeneration: – Some parenchymal cells are short-lived while others have a longer lifespan. – In order to maintain proper structure of tissues, these cells are under the constant regulatory control of their cell cycle.

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LECTURE HANDOUTS

L - 7
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BME		II/IV
Course Name with Code	: 16BMD11 &PATHOL	OGY &MICROBIOLOGY
Course Teacher	: Ms.A.Anjali	
Unit	: I	Date of Lecture:

**Topic of Lecture: Neoplasia, Classification, Benign and Malignant tumours** 

#### Introduction:

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

Neoplasia is new, uncontrolled growth of cells that is not under physiologic control. A "tumor" or "mass lesion" is simply a "growth" or "enlargement" which may not be neoplastic (such as a granuloma). The term "cancer" implies malignancy, but neoplasms can be subclassified as either benign or malignant.

A tumor develops when cells reproduce too quickly.Tumors can vary in size from a tiny nodule to a large mass, depending on the type, and they can appear almost anywhere on the body.

Benign:

Most benign tumors are not harmful, and they are unlikely to affect other parts of the body.However, they can cause pain or other problems if they press against nerves or blood vessels or if they trigger the overproduction of hormones, as in the endocrine system.Examples of benign tumors include:AdenomasAdenomas develop in glandular epithelial tissue, which is the thin membrane that covers glands, organs, and other structures in the body.

Examples include:

- I. polyps in the colon
- II. fibroadenomas, a common form of benign breast tumor
- III. hepatic adenomas, which occur on the liver
- IV. Adenomas do not start as cancer. However, some can change and become adenocarcinomas, which are cancerous.

Malignant

Malignant tumors are cancerous. They develop when cells grow uncontrollably. If the cells continue to grow and spread, the disease can become life threatening.

Malignant tumors can grow quickly and spread to other parts of the body in a process called metastasis.

The cancer cells that move to other parts of the body are the same as the original ones, but they have the ability to invade other organs. If lung cancer spreads to the liver, for example, the cancer cells in the liver are still lung cancer cells.

Different types of malignant tumor originate in different types of cell.

## Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

**Important Books/Journals for further learning including the page nos.:** Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseasesl",7th Edition,WB Saunders Co 2005

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**LECTURE HANDOUTS** 

II/IV

L - 8

Course Name with Code: 16BMD11 &PATHOLOGY &MICROBIOLOGYCourse Teacher: Ms.A.AnjaliUnit: IDate of Lecture:

**Topic of Lecture: carcinogenesis, spread of tumours** 

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

Carcinogenesis, also called oncogenesis or tumorigenesis, is the formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by changes at the cellular, genetic, and epigenetic levels and abnormal cell division.

Fibroids

Fibroids, or fibromas, are benign tumors that can grow on the fibrous or connective tissue of any organ.

Uterine fibroids are common and can cause:

vaginal bleeding

pelvic pain or discomfort

urinary incontinence

They can be "soft" or "hard," depending on the proportion of fibers to cells.

There are many types of fibroma, including:

angiofibromas, which can appear as small red bumps on the face

dermatofibromas, which appear on the skin, often on the lower legs

Examples include:

Carcinoma: These tumors form from epithelial cells, which are present in the skin and the tissue that covers or lines the body's organs. Carcinomas can occur in the stomach, prostate, pancreas, lung, liver, colon, or breast. They are a common type of malignant tumor.

Sarcoma: These tumors start in connective tissue, such as cartilage, bones, fat, and nerves. They originate in the cells outside the bone marrow. Most sarcomas are malignant.

Germ cell tumor: These tumors develop in the cells that produce sperm and eggs. They usually occur in the ovaries or testicles, but they may also appear in the brain, abdomen, or chest.

Blastoma: These tumors form from embryonic tissue or developing cells. Blastomas are much more common in children than in adults. They can lead to tumors in the brain, eye, or nervous system

Video Content / Details of website for further learning (if any):

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**LECTURE HANDOUTS** 



II/IV

L - 9

Course Name with Code	: 16BMD11 &PATHOLOGY &MICROBIOLOGY		
Course Teacher	: Ms.A.Anjali		
Unit	: I	Date of Lecture:	

# Topic of Lecture: Autopsy and biopsy.

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

#### **Detailed content of the Lecture:**

An autopsy (post-mortem examination, obduction, necropsy, or autopsia cadaverum) is a surgical procedure that consists of a thorough <u>examination</u> of a <u>corpse</u> by <u>dissection</u> to determine the cause, mode, and manner of <u>death</u> or to evaluate any <u>disease</u> or <u>injury</u> that may be present for research or educational purposes. Autopsies are usually performed by a specialized medical doctor called a <u>pathologist</u>. In most cases, a <u>medical examiner</u> or <u>coroner</u> can determine cause of death and only a small portion of deaths require an autopsy.

PURPOSES:

Autopsies are performed for either legal or medical purposes. Autopsies can be performed when any of the following information is desired:

- Determine if death was natural or unnatural
- Injury source and extent on the corpse
- Manner of death must be determined
- Time since death
- Establish identity of deceased
- Retain relevant organs
- If infant, determine live birth and viability

For example, a forensic autopsy is carried out when the cause of death may be a criminal matter, while a clinical or academic autopsy is performed to find the medical cause of death and is used in cases of unknown

or uncertain death, or for research purposes. Autopsies can be further classified into cases where external examination suffices, and those where the body is dissected and internal examination is conducted. Permission from next of kin may be required for internal autopsy in some cases. Once an internal autopsy is complete the body is reconstituted by sewing it back together.

TYPES:

There are four main types of autopsies:

- *Medico-Legal Autopsy or Forensic* or *coroner's autopsies* seek to find the cause and manner of death and to identify the decedent. They are generally performed, as prescribed by applicable law, in cases of violent, suspicious or sudden deaths, deaths without medical assistance or during surgical procedures.
- *Clinical* or *Pathological autopsies* are performed to diagnose a particular disease or for research purposes. They aim to determine, clarify, or confirm medical diagnoses that remained unknown or unclear prior to the patient's death.
- Anatomical or academic autopsies are performed by students of anatomy for study purpose only.
- *Virtual* or *medical imaging autopsies* are performed utilizing imaging technology only, primarily magnetic resonance imaging (MRI) and computed tomography (CT).

A biopsy is a medical test commonly performed by a surgeon, interventional radiologist, or an interventional cardiologist involving extraction of sample cells or tissues for examination to determine the presence or extent of a disease. The tissue is generally examined under a microscope by a pathologist, and can also be analyzed chemically. When an entire lump or suspicious area is removed, the procedure is called an excisional biopsy. An incisional biopsy or core biopsy samples a portion of the abnormal tissue without attempting to remove the entire lesion or tumor. When a sample of tissue or fluid is removed with a needle in such a way that cells are removed without preserving the histological architecture of the tissue cells, the procedure is called a needle aspiration biopsy. Biopsies are most commonly performed for insight into possible cancerous and inflammatory conditions.

Video Content / Detail	s of website for furth	er learning (if any):	
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	-	CTURE HANDOUTS	L - 10
BME			II/IV
ırse Name with Code	: 16BMD11&P	ATHOLOGY AND MICROBIOLOGY	
ırse Teacher	: Ms.A.Anjali		
t	: II	Date of Lecture:	
Topic of Lecture: Edema, Hyperemia,I	schemia,		
Introduction :			
Analyze struct	ural and functional asp	bects of living organisms.	
-	nctions of blood		

#### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

## **Detailed content of the Lecture:**

Edema" is the medical term for swelling. Body parts swell from injury or inflammation. It can affect a small area or the entire body. Medications, pregnancy, infections, and many other medical problems can cause edema. Edema happens when your small blood vessels leak fluid into nearby tissues.

Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair.

**Ischemia** or ischaemia is a restriction in blood supply to tissues, causing a shortage of oxygen that is needed for cellular metabolism (to keep tissue alive). **Ischemia** is generally caused by problems with blood vessels, with resultant damage to or dysfunction of tissue.

## Video Content / Details of website for further learning (if any):

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BME		II/IV
arse Name with Code	: 16BMD11&P	ATHOLOGY AND MICROBIOLOGY
ırse Teacher	: Ms.A.Anjali	
lt	: П	Date of Lecture:
Topic of Lecture: n	ormal hemostasis	

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of blood

## Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

#### **Detailed content of the Lecture:**

Hemostasis or haemostasis is a process to prevent and stop bleeding, meaning to keep blood within a damaged blood vessel (the opposite of hemostasis is hemorrhage). It is the first stage of wound healing. This involves coagulation, blood changing from a liquid to a gel.

Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair.

Ischemia or ischaemia is a restriction in blood supply to tissues, causing a shortage of oxygen that is needed for cellular metabolism (to keep tissue alive). Ischemia is generally caused by problems with blood vessels, with resultant damage to or dysfunction of tissue.

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Inse Teacher : Ms.A.Anjali   t : II   Date of Lecture:     Topic of Lecture: thrombosis     Introduction :   • Analyze structural and functional aspects of living organisms.   • Explain the functions of blood   Prerequisite knowledge for Complete understanding and learning of Topic:					
(An Autonomous Institution)   (Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University)   Rasipuram - 637 408, Namakkal Dist., Tamil Nadu   L - 12     BME   II/IV   rse Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY ruse Teacher : Ms.A.Anjali t : II Date of Lecture:   Topic of Lecture: thrombosis   Introduction :   • Analyze structural and functional aspects of living organisms.   • Explain the functions of blood   Prerequisite knowledge for Complete understanding and learning of Topic:					
(An Autonomous Institution)   (Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University)   Rasipuram - 637 408, Namakkal Dist., Tamil Nadu   L - 12     BME   II/IV   rse Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY ruse Teacher : Ms.A.Anjali t : II Date of Lecture:   Topic of Lecture: thrombosis   Introduction :   • Analyze structural and functional aspects of living organisms.   • Explain the functions of blood   Prerequisite knowledge for Complete understanding and learning of Topic:					
Inse Name with Code       : 16BMD11&PATHOLOGY AND MICROBIOLOGY         Inse Teacher       : Ms.A.Anjali         t       : II         Date of Lecture:         Topic of Lecture: thrombosis         Introduction :         • Analyze structural and functional aspects of living organisms.         • Explain the functions of blood         Prerequisite knowledge for Complete understanding and learning of Topic:	DESIGNING HORE PUPULE Estd. 2000	(An Auto (Approved by AICTE, New De Anr Rasipuram - 637 408	nomous Institution) lhi, Accredited by NAA na University) 5, Namakkal Dist., Tamil	C & Affiliated t	0
Inse Teacher : Ms.A.Anjali   t : II     Topic of Lecture: thrombosis     Introduction :   • Analyze structural and functional aspects of living organisms.   • Explain the functions of blood   Prerequisite knowledge for Complete understanding and learning of Topic:	BME				II/IV
t : II Date of Lecture: Topic of Lecture: thrombosis Introduction : • Analyze structural and functional aspects of living organisms. • Explain the functions of blood Prerequisite knowledge for Complete understanding and learning of Topic:	ırse Name wi	th Code : 16BMD11&PA	ATHOLOGY AND MIC	CROBIOLOGY	
Topic of Lecture: thrombosis         Introduction :       • Analyze structural and functional aspects of living organisms.         • Explain the functions of blood         Prerequisite knowledge for Complete understanding and learning of Topic:	ırse Teacher	: Ms.A.Anjali			
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<ul> <li>Analyze structural and functional aspects of living organisms.</li> <li>Explain the functions of blood</li> </ul> Prerequisite knowledge for Complete understanding and learning of Topic:	Topic of Le	cture: thrombosis			
	• Anal	yze structural and functional aspe	ects of living organisms.		
Anatomy and Human Physiology			erstanding and learning	of Topic:	

Fundamentals of biochemistry

#### **Detailed content of the Lecture:**

Thrombosis is the formation of a blood clot, known as a thrombus, within a blood vessel. It prevents blood from flowing normally through the circulatory system.

#### Mechanism:

The main causes of thrombosis are given in Virchow's triad which lists thrombophilia, endothelial cell injury, and disturbed blood flow.

## Hypercoagulability

Hypercoagulability or thrombophilia, is caused by, for example, genetic deficiencies or autoimmune disorders. Recent studies indicate that white blood cells play a pivotal role in deep vein thrombosis, mediating numerous pro-thrombotic actions.

Endothelial cell injury

Any inflammatory process, such as trauma, surgery or infection, can cause damage to the endothelial lining of the vessel's wall. The main mechanism is exposure of tissue factor to the blood coagulation system.Inflammatory and other stimuli (such as hypercholesterolemia) can lead to changes in gene expression in endothelium producing to a pro-thrombotic state. When this occurs, endothelial cells downregulate substances such as thrombomodulin, which is a key modulator of thrombin activity.

Disturbed blood flow

Causes of disturbed blood flow include stagnation of blood flow past the point of injury, or venous stasis which may occur in heart failure, or after long periods of sedentary behaviour, such as sitting on a long airplane flight. Also, atrial fibrillation, causes stagnant blood in the left atrium (LA), or left atrial appendage (LAA), and can lead to a thromboembolism.

Fibrinolysis is the physiological breakdown of blood clots by enzymes such as plasmin.

#### Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

#### Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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ırse Name with C	ode : 16BMD11&PATH	IOLOGY AND MIC	ROBIOLOGY	
ırse Teacher	: Ms.A.Anjali			
t	: 11	I	Date of Lecture	:
Topic of Lectur	e: disseminated intravascular o	coagulation		
	structural and functional aspects the functions of blood	of living organisms.		
Prerequisite kn	owledge for Complete understa	nding and learning o	of Topic:	
Anatomy and Hu Fundamentals of	uman Physiology f biochemistry			

#### **Detailed content of the Lecture:**

Disseminated intravascular coagulation is a condition in which small blood clots develop throughout the bloodstream, blocking small blood vessels. The increased clotting depletes the platelets and clotting factors needed to control bleeding, causing excessive bleeding

- There are a number of possible causes, including infection and surgery.
- Excessive clotting is followed by excessive bleeding.
- The amount of clotting factors in the blood is measured.
- The underlying disorder is treated.

Disseminated intravascular coagulation (DIC) begins with excessive clotting. The excessive clotting is usually stimulated by a substance that enters the blood as part of a disease (such as an infection or certain cancers) or as a complication of childbirth, retention of a dead fetus, or surgery. People who have a severe head injury or who have tissue damage caused by shock, burns, frostbite, other injuries, or even a bite by a poisonous snake are also at risk. As the clotting factors and platelets (cell fragments that circulate in the bloodstream and help blood clot) are depleted, excessive bleeding occurs.

DIC may develop

- Suddenly
- Slowly

Slowly developing disseminated intravascular coagulation typically results from cancer, aneurysms, or cavernous hemangiomas (collection of dilated blood vessels).

# SYMPTOMS:

DIC that develops suddenly usually causes bleeding, which may be very severe. If the condition follows surgery or childbirth, bleeding may be uncontrollable. Bleeding may occur at the site of an intravenous injection or in the brain, digestive tract, skin, muscles, or cavities of the body. If DIC develops more slowly, as in people with cancer, then clots in veins (deep venous thrombosis) are more common than bleeding. If clots form in veins (usually in the legs), the person may have swelling, redness, or pain in the area. However, sometimes no symptoms develop. A clot that forms in a vein may break free and travel (becoming an embolus) to the lungs. Clots in the lungs may make people short of breath.

# DIAGNOSIS

# Blood tests

Blood tests may show that the number of platelets in a blood sample has dropped (platelets are used up when blood clots) and that the blood is taking a long time to clot. The diagnosis of DIC is

confirmed if test results show abnormally increased quantities of plasma D-dimer (a substance that blood clots release when they break down; more D-dimer indicates that more clots are being produced than usual) and often a low or decreasing level of fibrinogen (a protein that is consumed when blood clots).

Treatment:

• Treatment of the underlying disorder

The underlying disorder must be identified and corrected, whether it is an obstetric problem, an infection, or a cancer. The clotting problems subside when the cause is corrected.

DIC that develops suddenly is life threatening and is treated as an emergency. Platelets and clotting factors are transfused to replace those depleted and to stop bleeding. Heparin may be used to slow the clotting in people who have more chronic, milder DIC in which clotting is more of a problem than bleeding.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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rse Name with Co	de : 16BM	D11&PATHOLOGY AND MICROBIOLOG	GY
rse Teacher	: Ms.A.	Anjali	
	: 11	Date of Lect	ure:
Topic of Lecture	embolism, inf	arction, shock	
-	tructural and functi e functions of bloo	onal aspects of living organisms.	
Prerequisite kno	wledge for Compl	ete understanding and learning of Topic:	
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Detailed content	of the Lecture:		
area of the body,	which can effective	ve surgical technique. The purpose is to prevent ely shrink a tumor or block an aneurysm. The pr an interventional radiologist in an interventiona	ocedure is carried
		due to inadequate blood supply to the affected a mechanical compression, or vasoconstriction. T	-

is referred to as an **infarct**.

**Shock** is the state of insufficient blood flow to the tissues of the body as a result of problems with the circulatory system. Initial symptoms of **shock** may include weakness, fast heart rate, fast breathing, sweating, anxiety, and increased thirst.

Causes: <u>Low volume</u>: Bleeding, vomiting, pan.

Symptoms: Initial: Weakness, fast heart rate, fast .

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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Topic of Lecture	e: Chronic venous con	gestion.		
Introduction :				
•	structural and functional as he functions of blood	pects of living organisms.		
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Prerequisite kno	owledge for Complete un	derstanding and learning	of Topic:	
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Increased volum resulting from in failure. There ma	e of blood in a particula	r tissue is known as cong ue. It may occur due to any of obstruction.		
	n volume of blood in parti- skin during exercise or at t	cular tissue but is an active he site of inflammation.	process, resultir	ng from arteriolar

## Chronic venous congestion in lungs

## Gross

Color of the lungs will be dusky reddish blue. Lungs are heavy and wet. Cut sections show hemorrhagic areas due to abundant extravasation from alveolar capillaries.

# Microscopy

Engorged alveolar capillaries seen in alveolar septa. There will be intra-alveolar hemorrhages, alveolar septa and spaces contain numerous heart failure cells. There is thickening or fibrosis of alveolar septa.

## Chronic venous congestion in liver

## Gross

Liver is larger and wet. Cut section shows congested red centers of hepatic lobules surrounded by pale coloured unaffected peripheral areas. This appearance is known as nut meg liver. In a long standing case of chronic venous congestion, there will be increased fibrosis, decreased liver size and parenchyma is divided into lobules and condition is known as cirrhosis of liver.

## Microscopy

Central vein and sinusoids are distended with RBCs. Some areas of hemorrhage are present. When these RBCs are phagocytosed by macrophages, they are called siderophages. Central lobular necrosis due to central vein congestion is seen. Fatty change is due to hypoxia in peripheral hepatocytes is also seen.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

#### Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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rse Name with Co		OLOGY AND MICROBIOLO	JGY
rse Teacher	: Ms.A.Anjali		
	: 11	Date of Lee	cture:
Topic of Lectury	e: Hematological disorders		
Introduction :			
•	structural and functional aspects o	f living organisms.	
• Explain t	he functions of blood		
Prerequisite know	owledge for Complete understa	nding and learning of Topic:	
Anatomy and Hu Fundamentals of			
Detailed content			
Hematologic di	seases are disorders which prir	narily affect the blood & bl	ood-forming organs.
Hematologic dise	eases include rare genetic disorde	ers, anemia, HIV, sickle cell dise	ease & complications
from chemothera	py or transfusions.		
Hematological r	<u>nalignancies</u>		
• <u>Lymphor</u>	nas		
	odgkin's disease		
0 <u>N</u>	on-Hodgkin's lymphoma {include	es the next five entries}	

- Burkitt's lymphoma
- <u>Anaplastic large cell lymphoma</u>
- <u>Splenic marginal zone lymphoma</u>
- Hepatosplenic T-cell lymphoma
- Angioimmunoblastic T-cell lymphoma (AILT)
- <u>Myelomas</u>
  - o <u>Multiple myeloma</u>
  - <u>Waldenström macroglobulinemia</u>
  - o <u>Plasmacytoma</u>
- <u>Leukemias</u> increased WBC
  - <u>Acute lymphocytic leukemia</u> (ALL)
  - <u>Chronic lymphocytic leukemia</u> (CLL){now included in theCLL/SCLL type NHL}
  - <u>Acute myelogenous leukemia</u> (AML)
  - <u>Acute megakaryoblastic leukemia</u> (AMKL), a sub-type of acute myelogenous leukemia
  - <u>Chronic Idiopathic Myelofibrosis</u> (MF)
  - <u>Chronic myelogenous leukemia</u> (CML)
  - <u>T-cell prolymphocytic leukemia</u> (T-PLL)
  - <u>B-cell prolymphocytic leukemia</u> (B-PLL)
  - <u>Chronic neutrophilic leukemia</u> (CNL)
  - <u>Hairy cell leukemia</u> (HCL)
  - o <u>T-cell large granular lymphocyte leukemia</u> (T-LGL)
  - o Aggressive NK-cell leukemia

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# **Important Books/Journals for further learning including the page nos.:**

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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BME				II/IV
rse Name with	Code : 16BMD11&PA7	THOLOGY AND MIC	ROBIOLOGY	
rse Teacher	: Ms.A.Anjali			
t	: II	]	Date of Lecture	e:
Topic of Lect	cure: Bleeding disorders			
-	: ze structural and functional aspect n the functions of blood	ts of living organisms.		
Prerequisite	knowledge for Complete unders	standing and learning o	of Topic:	
Fundamentals	Human Physiology of biochemistry ent of the Lecture:			
are characterit bleeding is sp defects in bloo body produce	rders are a group of disorders that zed by extended bleeding after inj ontaneous, without a known or id od components such as platelets a s 13 clotting factors. If any of the e or severe bleeding disorder can	ury, surgery, trauma or i entifiable cause. Improp nd/or clotting proteins, a m are defective or deficie	menstruation. S er clotting can b also called clotti	ometimes the be caused by ang factors. The
conditions as	g disorders, such as hemophilia, c anemia, cirrhosis of the liver, HIV rtain medications that thin the blo	/, leukemia and vitamin	K deficiency. T	hey also can

#### **Symptoms**

Symptoms of a bleeding disorder include:

- Bleeding into joints, muscles and soft tissues
- Excessive bruising
- Prolonged, heavy menstrual periods (menorrhagia)
- Unexplained nosebleeds
- Extended bleeding after minor cuts, blood draws or vaccinations, minor surgery or dental procedures

#### Treatment

Treatment for bleeding disorders varies, depending on the condition and its severity. For some bleeding disorders, there are clotting factor concentrates that can be infused prophylactically or on-demand at home, to prevent or treat bleeds. For other bleeding disorders, there are topical products, nasal sprays and fresh frozen plasma, which is administered in a hospital setting.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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urse Name with	Code : 16BMI	D11&PATHOLOGY AND MIC	CROBIOLOGY	
irse Teacher	: Ms.A.A	Anjali		
t	: П		Date of Lecture	<b>5.</b>
Topic of Lectr	ure: Leukaemias, Lyn	nphomas, Haemorrhage		
•		onal aspects of living organisms.		
Prerequisite k	nowledge for Comple	ete understanding and learning	of Topic:	
•	Human Physiology of biochemistry			
	ent of the Lecture:	y, including the blood. Leukemia	and lymphoma	are both forms of
blood cancer.		is that leukemia affects the b		
-	are some similarities atment, and survival rat	s between the two types of <u>ca</u> te are different.	<u>uncer</u> , their caus	ses and origins,
In this arti	1 . 1	comprehensive look at the	similarities a	and differences

Leukemia and lymphoma are two types of cancer that affect the blood. Both cancers typically affect the white blood cells.

Leukemia occurs when the <u>bone marrow</u> produces too many abnormal white blood cells. It is typically a slow-growing cancer, though there are cases where it progresses faster.

If a person has leukemia, their abnormal white blood cells do not die off in a normal cycle. Instead, the white blood cells multiply rapidly, eventually leaving less room for red blood cells required to carry oxygen through the body.

There are four main types of leukemia, classified according to their rate of growth and where the cancer originated in the body.

The types of leukemia include:

- acute lymphocytic leukemia
- chronic lymphocytic leukemia
- <u>acute myeloid leukemia</u>
- chronic myeloid leukemia

#### Lymphoma

Lymphoma starts in the immune system and affects the lymph nodes and lymphocytes, which are a type of white blood cell. There are two main types of lymphocyte, B cells and T cells.

The two main types of lymphoma are:

- 1. Hodgkin lymphoma, which involves a specific type of abnormal B cell called a Reed-Sterberg cell. This type is less common.
- 2. Non-Hodgkin lymphoma, which can start in either B cells or T cells.

Bleeding, also known as a hemorrhage or haemorrhage, is blood escaping from the circulatory system from damaged blood vessels.Bleeding can occur internally, or externally either through a natural opening such as the mouth, nose, ear, urethra, vagina or anus, or through a wound in the skin. Hypovolemia is a massive decrease in blood volume, and death by excessive loss of blood is referred to as exsanguination. Typically, a healthy person can endure a loss of 10–15% of the total blood volume without serious medical difficulties (by comparison, blood donation typically takes 8–10% of the donor's blood volume). The stopping or controlling of bleeding is called hemostasis and is an important part of both first aid and surgery.

#### Blood loss classes

Hemorrhaging is broken down into four classes by the American College of Surgeons' <u>advanced</u> trauma life support (ATLS).

- **Class I Hemorrhage** involves up to 15% of blood volume. There is typically no change in vital signs and <u>fluid resuscitation</u> is not usually necessary.
- **Class II Hemorrhage** involves 15-30% of total blood volume. A patient is often <u>tachycardic</u> (rapid heart beat) with a reduction in the difference between the <u>systolic</u> and <u>diastolic</u> blood pressures. The body attempts to compensate with <u>peripheral</u>
<u>vasoconstriction</u>. Skin may start to look pale and be cool to the touch. The patient may exhibit slight changes in behavior. Volume resuscitation with crystalloids (<u>Saline solution</u> or <u>Lactated</u> <u>Ringer's solution</u>) is all that is typically required. <u>Blood transfusion</u> is not usually required.

- **Class III Hemorrhage** involves loss of 30-40% of circulating blood volume. The patient's <u>blood pressure</u> drops, the <u>heart rate</u> increases, peripheral hypoperfusion (<u>shock</u>) with diminished <u>capillary refill</u> occurs, and the mental status worsens. Fluid resuscitation with crystalloid and blood transfusion are usually necessary.
- **Class IV Hemorrhage** involves loss of >40% of circulating blood volume. The limit of the body's compensation is reached and aggressive resuscitation is required to prevent death. This system is basically the same as used in the staging of hypovolemic shock.

Individuals in excellent physical and <u>cardiovascular</u> shape may have more effective compensatory mechanisms before experiencing cardiovascular collapse. These patients may look deceptively stable, with minimal derangements in vital signs, while having poor peripheral perfusion. Elderly patients or those with chronic medical conditions may have less tolerance to blood loss, less ability to compensate, and may take medications such as betablockers that can potentially blunt the cardiovascular response

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

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**Course Teacher** 

	<b>JTHAYAMMAL ENGINE</b> (An Autonomous Institu roved by AICTE, New Delhi, Accredited by NAAC Rasipuram - 637 408, Namakkal Di	ution) C & Affiliated to Anna University) Dist., Tamil Nadu
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Course Name with	Code : 16BMD11&PATHOLO	OGY AND MICROBIOLOGY
<b>Course Faculty</b>	: Ms.A.Anjali	
Unit	: 3	Date of Lecture:
Topic of Lecture:	Structure of Bacteria and Virus	
•	ructural and functional aspects of living of functions of cells	organisms.
Prerequisite know	vledge for Complete understanding an	nd learning of Topic:
Anatomy and Hum Fundamentals of bi		

# **Detailed content of the Lecture:**

Although bacteria and viruses both are very small to be seen without a microscope, there are many differences between Bacteria and Viruses.

Some of the Differences Between Bacteria and Viruses are as follows:

S.N.	Characteristics	Bacteria	Viruses
1	Size	Larger (1000 nm)	Smaller (20-400 nm)
2	Cell Wall	Peptidoglycan or Lipopolysaccharide	No cell wall. Protein coat present instead.
3	Ribosomes	Present	Absent
4	Number of cells	One cell (Unicellular)	No cells
5	Living/Non-Living	Living organisms	Between living and non- living things.
6	DNA and RNA	DNA and RNA floating freely in cytoplasm.	DNA or RNA enclosed inside a coat of protein.
7	Infection	Localized	Systemic
8	Reproduce	Able to reproduce by itself	Need a living cell to reproduce
9	Reproduction	Fission- a form of asexual reproduction	Invades a host cell and takes over the cell causing it to make copies of the viral DNA/RNA. Destroys the host cell releasing new viruses.
10	Duration of illness	A bacterial illness commonly will last longer than 10 days.	Most viral illnesses last 2 to 10 days.
11	Under Microscope	Visible under Light Microscope.	Visible only under Electron Microscope.
12	Benefits	Some bacteria are beneficial (Normal Flora)	Viruses are not beneficial. However, a particular virus may be able to destroy brain tumors. Viruses can be useful in genetic engineering.
13	Treatment	Antibiotics	Virus does not respond to antibiotics.
14	Examples	Staphylococcus aureus, Vibrio cholerae, etc	HIV, Hepatitis A virus, Rhino Virus, etc

15	Diseases/Infections	Food poisoning, gastritis and ulcers, meningitis, pneumonia, etc	AIDS, common cold, influenza, chickenpox, etc	
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# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

**Important Books/Journals for further learning including the page nos.:** 

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Faculty** 

Verified by HOD



# **MUTHAYAMMAL ENGINEERING COLLEGE**



(An Autonomous Institution)

(Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University) Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

# LECTURE HANDOUTS

Course Name with Code	: 16BMD11&PATHOLOGY AN	ND MICROBIOLOGY
Course Faculty	: Ms.A.Anjali	
Unit	: 3	Date of Lecture:

# Topic of Lecture: Routes of infection and spread

### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

Detailed content of the Lecture:

### **Route Definitions**

Aerosol: Pathogenic agents contained in aerosol droplets are passed from one animal to another, or between animals and humans. Most pathogenic agents do not survive for extended periods of time within the aerosol droplets and close proximity of infected and susceptible animals is required for transmission

**Direct contact:** A susceptible animal becomes exposed through physical contact when the agent from an infected animal or the environment enters open wounds, mucous membranes, or the skin through blood, saliva, nose-to-nose, rubbing, or biting another animal. Some disease agents can spread between animals of different species, as well as to humans.

• Subtype: **Reproductive** Diseases spread through venereal contact (from animal-toanimal through coitus) and in-utero (from dam to offspring during gestation).

**Oral:** Consumption of pathogenic agents in contaminated feed, water or licking/chewing on contaminated environmental objects. Feed and water contaminated with feces or urine are frequently the cause of oral transmission of disease agents. Contaminated environmental objects could include equipment, feed bunks, water troughs, fencing, salt and mineral blocks, and other items an animal may lick or chew.

**Fomite:** A contaminated inanimate object transmits a disease agent from one susceptible animal to another. It involves a secondary route of transmission (direct contact or oral) for the pathogen to enter the host. Examples include contaminated shovels, clothing, bowls/buckets, brushes, tack, and clippers.

• Subtype: **Traffic** Vehicle, trailer, or human causes the spread of a pathogenic agent through contaminated tires, wheel wells, undercarriage, clothing, or shoes/boots by spreading organic material to another location.

Vector-borne: An insect acquires a pathogen from one animal and transmits it to another either mechanically or biologically. Mechanical transmission: disease agent does not replicate or develop

in/on the vector; it is simply transported by the vector from one animal to another (e.g., flies). Biological transmission: vector takes up the agent, usually through a blood meal from an infected animal, replicates and/or develops it, and then regurgitates the pathogen onto or injects it into a susceptible animal. Fleas, ticks, and mosquitoes are common biological vectors of disease.

**Zoonotic:** Diseases transmitted between animals and humans. Human exposure occurs through one of the previously listed five main routes of transmission (aerosol, direct contact, fomite, oral, and vector-borne). It is a separate route of transmission due to its importance.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Faculty** 



# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

Only a minority of bacteria species cause disease in humans; and many species colonize in the human body to create an ecosystem known as microbiota. Bacterial flora is endogenous bacteria, which is defined as bacteria that naturally reside in a closed system. Disease can occur when microbes included in normal bacteria flora enter a sterile area of the body such as the brain or muscle. This is considered an endogenous infection. A prime example of this is when the residential bacterium E. coli of the GI tract enters the urinary tract. This causes a urinary tract infection. Infections caused by exogenous bacteria occurs when microbes that are noncommensal enter a host. These microbes can enter a host via inhalation of aerosolized bacteria, ingestion of contaminated or ill-prepared foods, sexual activity, or the direct contact of a wound with the bacteria.

Diseases Caused by Exogenous Bacteria

# Waterborne and Foodborne

Microbial ecosystems in aquatic environments depend on a variety of factors including pH, temperature, and light exposure. Exogenous bacteria supported in specific aquatic environments can enter an host via consumption. Additionally, exogenous bacteria can enter a secondary host through an intermediate host such as insects and parasites. Exogenous bacteria can also enter an enclosed ecosystem via ingestion of contaminated food.Food-borne diseases such as *Salmonella* poisoning are transmitted by food not properly cooked or by individuals infected with the pathogen.

### Salmonella enterocolitis

One of the most common food-borne illnesses, *Salmonella* poisoning is caused by ingestion of unsanitary conditions during food preparation. *Salmonella* can also be transmitted to humans via reptiles like turtles and iguanas, which are known carriers of pathogen. Symptoms include chills, diarrhea and fever.

### Cholera

*Cholera* is a waterborne infection caused by the bacterium *Vibrio chloerae*, and is transmitted via food or water that is contaminated with fecal matter. *Vibrio chloerae* releases a toxin that induces an increased amount of water in the small intestines. Symptoms primarily observed include, watery diarrhea and vomiting that can cause dehydration and death if not treated. An estimated 3-5 million cases of *Cholera* occur yearly around the world. The exogenous bacteria derived infection is primarilyfound in Africa, Asia, as well as Central and South America.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# **Important Books/Journals for further learning including the page nos.:**

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Bacteria are microscopic, single-celled organisms that thrive in diverse environments. These organisms can live in soil, the ocean and inside the human gut.

### Structure

Bacteria (singular: bacterium) are classified as prokaryotes, which are single-celled organisms with a simple internal structure that lacks a nucleus, and contains <u>DNA</u> that either floats freely in a twisted, thread-like mass called the nucleoid, or in separate, circular pieces called plasmids. <u>Ribosomes</u> are the spherical units in the bacterial cell where proteins are assembled from individual amino acids using the information encoded in ribosomal RNA.

Bacterial cells are generally surrounded by two protective coverings: an outer cell wall and an inner cell membrane. Certain bacteria, like the <u>mycoplasmas</u>, do not have a cell wall at all. Some bacteria may even have a third, outermost protective layer called the capsule. Whip-like extensions often cover the surfaces of bacteria — long ones called flagella or short ones called pili — that help bacteria to move around and attach to a host.

A virus is a small <u>infectious agent</u> that <u>replicates</u> only inside the living <u>cells</u> of an <u>organism</u>. Viruses can infect all types of <u>life forms</u>, from animals and plants to <u>microorganisms</u>, including <u>bacteria</u> and <u>archaea</u>.

# Structure

Viruses display a wide diversity of shapes and sizes, called '<u>morphologies</u>'. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 <u>nanometres</u>.

Some <u>filoviruses</u> have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with an <u>optical microscope</u>, so scanning and transmission <u>electron</u> <u>microscopes</u> are used to visualise them.

To increase the contrast between viruses and the background, electron-dense "stains" are used. These are solutions of <u>salts</u> of heavy metals, such as <u>tungsten</u>, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured.

<u>Negative staining</u> overcomes this problem by staining the background only.

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a <u>capsid</u>.

These are formed from identical protein subunits called <u>capsomeres</u>. Viruses can have a <u>lipid</u> "envelope" derived from the host <u>cell membrane</u>.

The capsid is made from proteins encoded by the viral <u>genome</u> and its shape serves as the basis for morphological distinction.

Virally-coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome.

Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as <u>nucleoproteins</u>, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through <u>atomic force microscopy</u>. In general, there are four main morphological virus types:

### Helical

These viruses are composed of a single type of capsomere stacked around a central axis to form a <u>helical</u> structure, which may have a central cavity, or tube.

# Icosahedral

Most animal viruses are icosahedral or near-spherical with chiral <u>icosahedral symmetry</u>. A <u>regular icosahedron</u> is the optimum way of forming a closed shell from identical sub-units.

# Video Content / Details of website for further learning (if any):

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LECTURE HANDOUTS

BME		II/IV
Course Name with Code	: 16BMD11&PATHO	LOGY AND MICROBIOLOGY
Course Faculty	: Ms.A.Anjali	
J <b>nit</b>	: 3	Date of Lecture:

# **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells •

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

# **Principle:**

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1). The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.



Fig 1: Absorbance reading of bacterial suspension

The growth curve has four distinct phases (Fig 2)

# 1. Lag phase

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism.

When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase.

Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent.

# 2. Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate.

The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is  $2^0$ ,  $2^1$ ,  $2^2$ ,  $2^3$ ...... $2^n$ , n is the number of generations) This will result in a balanced growth.

The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms.

*E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes.

# 3. Stationary phase

As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This result in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium.

This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised.

If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual.

# 4. Decline or Death phase

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce.

Individual bacteria begin to die due to the unfavourable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores.



# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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# LECTURE HANDOUTS



Course Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY

Course Faculty	: Ms.A.Anjali	
Unit	: 3	Date of Lecture:

**Topic of Lecture:** culture media and its types

### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

### Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology

Fundamentals of biochemistry

### **Detailed content of the Lecture:**

Types of culture media used in microbiology Media are of different types on consistency and chemical composition. A. On Consistency: 1. Solid Media. Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is : (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent. 2. Liquid Media. It is used for profuse growth, e.g. blood culture in liquid media. Mixed organisms cannot be separated. B. On Chemical Composition : 1. Routine Laboratory Media 2. Synthetic Media. These are chemically defined media prepared from pure chemical substances. It is used in research work. ROUTINE LABORATORY MEDIA These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1. BASAL MEDIA. Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

 ENRICHED MEDIA. The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.
 SELECTIVE MEDIA. These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. INDICATOR (DIFFERENTIAL) MEDIA. An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

5. TRANSPORT MEDIA. These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. STORAGE MEDIA. Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth

# Video Content / Details of website for further learning (if any):

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Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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	LECTURE HANDOUTS	L - 25
BME		II/IV
Course Name wit	th Code : 16BMD11&PATHOLOGY AND MICROBIOLO	GY
<b>Course Faculty</b>	: Ms.A.Anjali	
Unit	: 3 Date of Lectr	ure:
Topic of Lecture	e: culture techniques and observation of culture	
•	structural and functional aspects of living organisms.	

• Explain the functions of cells

# **Detailed content of the Lecture:**

A **microbiological culture**, or **microbial culture**, is a method of multiplying <u>microbial organisms</u> by letting them reproduce in predetermined <u>culture medium</u> under controlled laboratory conditions. Microbial cultures are foundational and basic diagnostic methods used as a research tool in <u>molecular</u> <u>biology</u>.

Microbial cultures are used to determine the type of organism, its abundance in the sample being tested, or both. It is one of the primary <u>diagnostic</u> methods of <u>microbiology</u> and used as a tool to determine the cause of <u>infectious disease</u> by letting the agent multiply in a predetermined medium. For example, a <u>throat culture</u> is taken by scraping the lining of tissue in the back of the throat and blotting the sample into a medium to be able to screen for harmful microorganisms, such as <u>Streptococcus</u> <u>pyogenes</u>, the causative agent of strep throat.<sup>[1]</sup> Furthermore, the term culture is more generally used informally to refer to "selectively growing" a specific kind of microorganism in the lab.

It is often essential to isolate a pure culture of microorganisms. A pure (or *axenic*) culture is a population of <u>cells</u> or <u>multicellular organisms</u> growing in the absence of other <u>species</u> or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic <u>clones</u> of one another. For the purpose of gelling the microbial culture, the medium of agarose gel (<u>agar</u>) is used. Agar is a gelatinous substance derived from <u>seaweed</u>. A cheap substitute for agar is <u>guar gum</u>, which can be used for the isolation and maintenance of <u>thermophiles</u>.

# **Bacterial culture**

There are several types of bacterial culture methods that are selected based on the agent being cultured and the downstream use.

# **Broth cultures**

One method of bacterial culture is liquid culture, in which the desired bacteria are suspended in a liquid nutrient medium, such as <u>Luria Broth</u>, in an upright flask. This allows a scientist to grow up large amounts of bacteria for a variety of downstream applications.

Liquid cultures are ideal for preparation of an antimicrobial assay in which the experimenter inoculates liquid broth with bacteria and lets it grow overnight (they may use a shaker for uniform growth). Then they would take aliquots of the sample to test for the antimicrobial activity of a specific drug or protein (antimicrobial peptides).

As an alternative, the microbiologist may decide to use static liquid cultures. These cultures are not shaken and they provide the microbes with an oxygen gradient.

# Video Content / Details of website for further learning (if any):

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# LECTURE HANDOUTS



BME

# II/IV

**Date of Lecture:** 

**Course Name with Code** 

: 16BMD11&PATHOLOGY AND MICROBIOLOGY

**Course Faculty** 

: Ms.A.Anjali

Unit

Topic of Lecture: Disease caused by bacteria, fungi

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 3

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

Infections in specific tissue

Bacterial pathogens often cause infection in specific areas of the body. Others are generalists.

- Bacterial vaginosis is caused by bacteria that change the vaginal microbiota caused by an overgrowth of bacteria that crowd out the Lactobacilli species that maintain healthy vaginal microbial populations.
- Other non-bacterial vaginal infections include: yeast infection (candidiasis), Trichomonas vaginalis (trichomoniasis).
- Bacterial meningitis is a bacterial inflammation of the meninges, that is, the protective membranes covering the brain and spinal cord.
- Bacterial pneumonia is a bacterial infection of the lungs.

• Urinary tract infection is predominantly caused by bacteria. Symptoms include the strong and frequent sensation or urge to urinate, pain during urination, and urine that is cloudy.[10] The main causal agent is Escherichia coli. Urine is typically sterile but contains a variety of salts, and waste products.[11] Bacteria can ascend into the bladder or kidney and causing cystitis and nephritis.

• Bacterial gastroenteritis is caused by enteric, pathogenic bacteria. These pathogenic species are usually distinct from the usually harmless bacteria of the normal gut flora. But a different strain of the same species may be pathogenic. The distinction is sometimes difficult as in the case of Escherichia.

• Bacterial skin infections include:

o Impetigo is a highly contagious bacterial skin infection commonly seen in children. It is caused

by Staphylococcus aureus, and Streptococcus pyogenes.

• Erysipelas is an acute streptococcus bacterial infection of the deeper skin layers that spreads via with lymphatic system.

Most common fungal diseases

Fungal nail infections Common infections of the fingernails or toenails.

Vaginal candidiasis Caused by the yeast Candida, also called a "vaginal yeast infection."

Ringworm A common fungal skin infection that often looks like a circular rash.

Candid infections of the mouth, throat, and esophagus Caused by the yeast Candida, also called "thrush.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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II/IV

**Course Name with Code** 

: 16BMD11&PATHOLOGY AND MICROBIOLOGY

Unit

**Date of Lecture:** 

**Topic of Lecture:** protozoal, virus and helminthes.

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 3

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

Protozoa are broken down into different classes: Sporozoa (intracellular parasites), flagellates (which possess tail-like structures that flap around for movement), amoeba (which move using temporary cell body projections called pseudopods), and ciliates (which move by beating multiple hair-like structures called cilia).

What Diseases Do They Cause?

Common infectious diseases caused by protozoans include <u>malaria</u>, <u>giardia</u>, and <u>toxoplasmosis</u>. These infections are found in very different parts of the body — <u>malaria infections start in the blood</u>, giardia starts in the gut, and toxoplasmosis can be found in lymph nodes, the eye, and also (worrisomely) the brain. Likewise, sleeping sickness is caused by a protozoan infection, as is dysentery due to *Entamoeba histolytica*.

Human African trypanosomiasis is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. The former causes most cases (about 98 percent) but both are spread by tsetse fly bites.<sup>2</sup>

Entamoeba histolytica can cause diarrhea and GI upset. It can, in fact, cause amoebic dysentery in severe cases, as well as asymptomatic cases for others. It can also travel through the walls of the intestines and go into the bloodstream and on to other organs, like the liver, where it can create liver abscesses.

# Treatment

Treatment options just depend on what protozoa are infecting you. Some are a lot more successful than others. Malaria is a common illness worldwide that has <u>straightforward treatment</u>, though the treatment depends on what type of malaria (Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax). Treatment also depends on whether there is resistance (P. falciparum especially has grown resistant over the last few decades to some important drugs)

Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socioeconomic development, and poverty. Chronic illness, <u>malnutrition</u>, and <u>anemia</u> are further examples of secondary effects.

Soil-transmitted helminthiases are responsible for parasitic infections in as much as a quarter of the human population worldwide. One well-known example of soil-transmitted helminthiases is <u>ascariasis</u>.

Not all viral diseases are contagious. This means they aren't always spread from person to person. But

many of them are. Common examples of contagious viral diseases include the flu, the common cold, HIV, and herpes.

Other types of viral diseases spread through other means, such as the bite of an infected insect.

Respiratory viral diseases

Respiratory viral diseases are contagious and commonly affect the upper or lower parts of your respiratory tract.

Common symptoms of a respiratory viral disease include:

- runny or stuffy nose
- coughing or sneezing
- fever
- body aches

# Examples

Examples of respiratory diseases include:

- <u>flu</u>
- <u>common cold</u>
- <u>respiratory syncytial virus infection</u>
- adenovirus infection

# Video Content / Details of website for further learning (if any):

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# LECTURE HANDOUTS

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BME
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II/IV

**Date of Lecture:** 

Course Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY

**Course Faculty** 

: Ms.A.Anjali

Unit

Topic of Lecture: Light microscope

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 4

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

Detailed content of the Lecture:

A light microscope (LM) is an instrument that uses visible light and magnifying lenses to examine small objects not visible to the naked eye, or in finer detail than the naked eye allows. Magnification, however, is not the most important issue in microscopy. Mere magnification without added detail is scientifically useless, just as endlessly enlarging a small photograph may not reveal any more detail, but only larger blurs. The usefulness of any microscope is that it produces better resolution than the eye. Resolution is the ability to distinguish two objects as separate entities, rather than seeing them blurred together as a single smudge. The history of microscopy has revolved largely around technological advances that have produced better resolution.

**Tissue Preparation** 

The advancement of light microscopy also required methods for preserving plant and animal tissues and making their cellular details more visible, methods collectively called histotechnique (from *histo*, meaning "tissue"). In brief, classical histotechnique involves preserving a specimen in a fixative, such as formalin, to prevent decay; embedding it in a block of paraffin and slicing it very thinly with an instrument called a microtome; removing the paraffin with a solvent; and then staining the tissue, usually with two or more dyes. The slices of tissue, called histological sections, are typically thinner than a single cell. The colors of a prepared tissue are not natural colors, but they make the tissue's structural details more visible. A widely used stain combination called hematoxylin and eosin, for example, typically colors cell nuclei violet and the cytoplasm pink.

# Varieties of Light Microscopes

Most compound microscopes today have an illuminator built into the base. A condenser located below the stage has lenses that focus the light on the specimen and a diaphragm that regulates contrast. After passing through the specimen on the stage, the light enters an objective lens. Most light microscopes have three or four objective lenses on a rotating turret. These lenses magnify the image by 4x to 100x. The light then passes up the body tube to an ocular lens that magnifies the image another 10x to 15x. Research-grade microscopes and the better student microscopes have a pair of ocular lenses so that one can view the specimen with both eyes at once.

There are many varieties of compound light microscopes for special purposes. For viewing tissue cultures covered with liquid media, biologists can use an inverted light microscope in which the culture is illuminated from above and the objective lenses are positioned below the specimen. The phase contrast microscope can be used to enhance contrast in living specimens, thus avoiding the use of lethal fixatives and stains. The polarizing light microscope is used for analyzing crystals and **minerals**, among other things. The fluorescence microscope is used to examine structures that bind special fluorescent dyes. It can be used, for example, to identify where a dyetagged **hormone** binds to its target cell.

Compound light microscopes achieve useful magnifications up to 1200x and resolutions down to about 0.25 micrometers. That is, two objects in a cell can be as close as 0.25 micrometers and still detected as separate entities. Such resolution is good enough to see most bacteria and some **mitochondria** and microvilli.

These microscopes generally require thin, transparent, relatively small specimens. They also require that the user adjust to the phenomenon of optical inversion; if a specimen is moved to the left, it appears under the microscope to move right; when moved up, it appears to move down; and vice versa. The stereomicroscope works at much lower magnification and resolution, but has several advantages: (1) it has two lens systems that view the specimen from slightly different angles, thus giving the specimen a stereoscopic (three-dimensional) appearance; (2) it can use either transmitted or reflected light; and with reflected light, it can be used to view opaque specimens such as rocks, fossils, insects, electronic circuit boards, and so forth; (3) it has a much greater working distance between the specimen and objective lens, allowing for the examination of relatively large objects and for easier manipulation of objects under the microscope; (4) the working distance enables relatively easy dissection of specimens such as insects, allowing hands and instruments to reach the working space while one looks through the microscope; and (5) it does not produce optical inversion; that is, movements to the right appear to go to the right, making dissection and other manipulations much easier.

The utility of light microscopy is governed by its use of visible light, which limits resolution. The shorter the wavelength of the illumination, the better the resolution. Electron beams have shorter wavelengths than photons. The invention of the electron microscope in the late 1930s and its refinement over the next half century permitted vastly improved visualization of cell and tissue fine structure.

# Video Content / Details of website for further learning (if any):

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# LECTURE HANDOUTS

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II/IV

**Date of Lecture:** 

# BME

**Course Faculty** 

**Course Name with Code** 

: 16BMD11&PATHOLOGY AND MICROBIOLO	OGY
: Ms.A.Anjali	

Unit

Topic of Lecture: bright field, dark field

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 4

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic: Anatomy and Human Physiology Fundamentals of biochemistry Detailed content of the Lecture: Bright Field and Dark Field Lighting Understanding the "W": • Reflected: light is the same angle as the source • Bright field: light is reflected into the camera • Dark field: light is reflected away from the camera • Dark field: light is reflected away from the camera • Dark field • Dark field • Dark field • Dark field • Field of View

# **Bright Field Lighting:**

- Good for high contrast but specular reflections on shiny or reflective surfaces
- Rule of thumb: Light should be twice the field of view at the camera lens
- Avoid "hot spots": Diffused light source provides even illumination in the brightfield



# Dark Field Lighting:

- Diffused light is reflected into the camera; specular light is reflected away
- Light source is outside the "W"



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# LECTURE HANDOUTS

Course Name with Code	: 16BMD11&PATHOLOGY AND MICROBIOLOGY

<b>Course Faculty</b>	: Ms.A.Anjali	
Unit	: 4	Date of Lecture:

**Topic of Lecture:** phase contrast, fluorescence

### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

The basic principle to making phase changes visible in phase-contrast microscopy is to separate the illuminating (background) light from the specimen-scattered light (which makes up the foreground details) and to manipulate these differently.

The ring-shaped illuminating light (green) that passes the <u>condenser</u> annulus is focused on the specimen by the condenser. Some of the illuminating light is <u>scattered</u> by the specimen (yellow). The remaining light is unaffected by the specimen and forms the background light (red). When observing an unstained biological specimen, the scattered light is weak and typically <u>phase-shifted</u> by  $-90^{\circ}$  (due to both the typical thickness of specimens and the refractive index difference between biological tissue and the surrounding medium) relative to the background light. This leads to the foreground (blue vector) and background (red vector) having nearly the same intensity, resulting in low <u>image contrast</u>.

In a phase-contrast microscope, image contrast is increased in two ways: by generating constructive interference between scattered and background light rays in regions of the field of view that contain the specimen, and by reducing the amount of background light that reaches the image plane. First, the background light is phase-shifted by  $-90^{\circ}$  by passing it through a phase-shift ring, which eliminates the phase difference between the background and the scattered light rays.

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# **Important Books/Journals for further learning including the page nos.:**

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# **MUTHAYAMMAL ENGINEERING COLLEGE**

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L - 31

(Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University) Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

**LECTURE HANDOUTS** 

BME		II/IV
Course Name with Code	: 16BMD11&PATHOLOGY AND MICROBIOLOGY	
Course Faculty	: Ms.A.Anjali	
Unit	: 4	Date of Lecture:
Topic of Lecture: Electron n	nicroscope -TEM	

Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

### **Detailed content of the Lecture:**

**Transmission electron microscopy** (**TEM**, an abbreviation which can also stand for the instrument, a **transmission electron microscope**) is a <u>microscopy</u> technique in which a beam of <u>electrons</u> is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and <u>focused</u> onto an imaging device, such as a <u>fluorescent</u> screen, a layer of <u>photographic film</u>, or a sensor such as a scintillator attached to a <u>charge-coupled device</u>.

Transmission electron microscopes capable of imaging significantly are at а higher resolution than light microscopes, owing to the smaller de Broglie wavelength of electrons. This enables the instrument to capture fine detail-even as small as a single column of atoms, which is thousands of times smaller than a resolvable object seen in a light microscope. Transmission electron microscopy is a major analytical method in the physical, chemical and biological sciences. TEMs find application in cancer research, virology, and materials science as well as pollution, nanotechnology and semiconductor research, but also in other fields such as paleontology and palynology.

TEM instruments boast an enormous array of operating modes including conventional imaging, scanning TEM imaging (STEM), diffraction, spectroscopy, and combinations of these. Even within conventional imaging, there are many fundamentally different ways that contrast is produced, called "image contrast mechanisms." Contrast can arise from position-to-position differences in the thickness or density ("mass-thickness contrast"), atomic number ("Z contrast," referring to the common abbreviation Z for atomic number), crystal structure or orientation ("crystallographic contrast" or "diffraction contrast"), the slight quantum-mechanical phase shifts that individual atoms produce in electrons that pass through them ("phase contrast"), the energy lost by electrons on passing through the sample ("spectrum imaging") and more. Each mechanism tells the user a different kind of information, depending not only on the contrast mechanism but on how the microscope is used—the settings of lenses, apertures, and detectors. What this means is that a TEM is capable of returning an extraordinary variety of nanometer- and atomic-resolution information, in ideal cases revealing not only where all the atoms are but what kinds of atoms they are and how they are bonded to each other. For this reason TEM is regarded as an essential tool for nanoscience in both biological and materials fields.

The first TEM was demonstrated by <u>Max Knoll</u> and <u>Ernst Ruska</u> in 1931, with this group developing the first TEM with resolution greater than that of light in 1933 and the first commercial TEM in 1939. In 1986, Ruska was awarded the Nobel Prize in physics for the development of transmission electron microscopy.

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**Important Books/Journals for further learning including the page nos.:** 



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# LECTURE HANDOUTS

: 16BMD11&PATHOLOGY AND MICROBIOLOGY



L - 32

BME

II/IV

**Date of Lecture:** 

Course Name with Code

**Course Faculty** 

: Ms.A.Anjali

Unit

**Topic of Lecture: SEM** 

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 4

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

### **Detailed content of the Lecture:**

A scanning electron microscope (SEM) is a type of <u>electron microscope</u> that produces images of a sample by scanning the surface with a focused beam of <u>electrons</u>. The electrons interact with <u>atoms</u> in the sample, producing various signals that contain information about the surface <u>topography</u> and composition of the sample. The electron beam is scanned in a <u>raster scan</u> pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. In the most common SEM mode, <u>secondary electrons</u> emitted by atoms excited by the electron beam are detected using a secondary electron detector (<u>Everhart-Thornley detector</u>). The number of secondary electrons that can be detected, and thus the signal intensity, depends, among other things, on specimen topography. SEM can achieve resolution better than 1 nanometer.

Specimens are observed in high vacuum in conventional SEM, or in low vacuum or wet conditions in variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instruments.<sup>[1]</sup>

The signals used by a scanning electron microscope to produce an image result from interactions of the electron beam with atoms at various depths within the sample. Various types of signals are produced including <u>secondary electrons</u> (SE), reflected or <u>back-scattered electrons</u> (BSE), characteristic X-rays and light (<u>cathodoluminescence</u>) (CL), absorbed current (specimen current) and transmitted electrons. Secondary electron detectors are standard equipment in all SEMs, but it is rare for a single machine to have detectors for all other possible signals.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

**Important Books/Journals for further learning including the page nos.:** 

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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# **LECTURE HANDOUTS**



L - 33

BME

**Course Name with Code** 

: 16BMD11&PATHOLOGY AND MICROBIOLOGY

**Course Teacher** 

: Ms.A.Anjali

# Topic of Lecture: Preparation of samples for electron microscope

# **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

### **Detailed content of the Lecture:**

Specimen preparation for scanning electron microscopy (SEM): topography imaging

# **STEP 1: PRIMARY FIXATION WITH ALDEHYDES (PROTEINS)**

Proteins are crosslinked by glutaraldehyde and formaldehyde to stabilise the ultrastructure before further processing.

# **STEP 2: SECONDARY FIXATION WITH OSMIUM TETROXIDE (LIPIDS)**

Blipid membranes are fixed to prevent their extraction by solvents during dehydration. The black osmium precipitate which is formed during this process increases sample conductivity and minimizes image distortions resulting from charging.

# **STEP 3: DEHYDRATION SERIES WITH SOLVENT (ETHANOL OR ACETONE)**

A fixed specimen is dehydrated by incubation in a series of ethanol or acetone solutions. Solvent concentration is increased gradually so that water is removed gently, without causing specimen shrinkage.

# **STEP 4: DRYING**

Allowing acetone or ethanol to simply evaporate from sample surface would create artefacts as these solvents have relatively high surface tension and would create micro-ripping of the surface upon leaving. To prevent this, dehydration solvents are replaced either with Hexamethyldisilazane (HMDS) or liquid CO<sub>2</sub>. HMDS can be used in cell preparations and after a short (3 minute) incubation it is removed and excess is left to evaporate. Liquid CO<sub>2</sub> on the other hand is applied to tissues in a critical point drier where it is brought to a critical temperature and pressure point at which it vaporises.

# **STEP 5: MOUNTING ON A STUB**

The specimen is mounted on a metal stub using a sticky carbon disc which increases conductivity. Silver-containing glue can additionally be applied for even more conductivity.

# **STEP 6: SPUTTER COATING WITH CONDUCTVE MATERIAL**

To prevent charge buildup on specimen surface, it is coated with a conductive material, most commonly gold. The metal is applied in a controlled manner in a sputter coater. It is critical that the coating is thick enough to prevent charging (typically around 10 nm) but not thick enough to obscure specimen surface details.



Whole insect heads mounted on a stub and coated with gold



Compound eye surface imaged in the SEM

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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**LECTURE HANDOUTS** 

BME
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L - 34

<b>Course Name with Code</b>	: 16BMD11&PATHOLOGY AND MICROBIOLOGY

**Course Faculty** 

: Ms.A.Anjali

Unit

Date of Lecture:

**Topic of Lecture:** Staining methods

# **Introduction :**

• Analyze structural and functional aspects of living organisms.

: 4

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry Detailed content of the Lecture:

Detailed content of the Lectu

Staining methods

In their natural state, most of the cells and microorganisms that we observe under the microscope lack color and contrast. This makes it difficult, if not impossible, to detect important cellular structures and their distinguishing characteristics without artificially treating specimens. We have already alluded to certain techniques involving stains and fluorescent dyes, and in this section we will discuss specific techniques for sample preparation in greater detail. Indeed, numerous methods have been developed to identify specific microbes, cellular structures, DNA sequences, or indicators of infection in tissue samples, under the microscope. Here, we will focus on the most clinically relevant techniques.

# **Preparing Specimens for Light Microscopy**

In clinical settings, light microscopes are the most commonly used microscopes. There are two basic types of preparation used to view specimens with a light microscope: wet mounts and fixed specimens.

The simplest type of preparation is the **wet mount**, in which the specimen is placed on the slide in a drop of liquid. Some specimens, such as a drop of urine, are already in a liquid form and can be deposited on the slide using a dropper. Solid specimens, such as a skin scraping, can be placed on the slide before adding a drop of liquid to prepare the wet mount. Sometimes the liquid used is simply water, but often stains are added to enhance contrast. Once the liquid has been added to the slide, a

coverslip is placed on top and the specimen is ready for examination under the microscope.

The second method of preparing specimens for light microscopy is **fixation**. The "fixing" of a sample refers to the process of attaching cells to a slide. Fixation is often achieved either by heating (**heat fixing**) or chemically treating the specimen. In addition to attaching the specimen to the slide, fixation also kills microorganisms in the specimen, stopping their movement and metabolism while preserving the integrity of their cellular components for observation.

To heat-fix a sample, a thin layer of the specimen is spread on the slide (called a **smear**), and the slide is then briefly heated over a heat source (Figure 1b). **Chemical fixatives** are often preferable to heat for tissue specimens. Chemical agents such as acetic acid, ethanol, methanol, formaldehyde (formalin), and glutaraldehyde can denature proteins, stop biochemical reactions, and stabilize cell structures in tissue samples (Figure 1c).



Figure 1. (a) A specimen can be heat-fixed by using a slide warmer like this one. (b) Another method for heat-fixing a specimen is to hold a slide with a smear over a microincinerator. (c) This tissue sample is being fixed in a solution of formalin (also known as formaldehyde). Chemical fixation kills microorganisms in the specimen, stopping degradation of the tissues and preserving their structure so that they can be examined later under the microscope. In addition to fixation, **staining** is almost always applied to color certain features of a specimen before examining it under a light microscope. Stains, or dyes, contain salts made up of a positive ion and a negative ion. Depending on the type of dye, the positive or the negative ion may be the chromophore (the colored ion); the other, uncolored ion is called the counterion. If the chromophore is the positively charged ion, the stain is classified as a **basic dye**; if the negative ion is the chromophore, the stain is considered an **acidic dye**.

Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed, which determine how the dye will interact with the specimen. In most cases, it is preferable to use a **positive stain**, a dye that will be absorbed by the cells or organisms being observed, adding color to objects of interest to make them stand out against the background. However, there are scenarios in which it is advantageous to use a **negative stain**, which is absorbed by the background but not by the cells or organisms in the specimen. Negative staining produces an outline or silhouette of the organisms against a colorful background.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# Important Books/Journals for further learning including the page nos.:

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# **MUTHAYAMMAL ENGINEERING COLLEGE**

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L - 35

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**LECTURE HANDOUTS** 

BME		II/IV
Course Name with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY		
Course Faculty	: Ms.A.Anjali	
	_	
Unit	: 4	Date of Lecture:
Unit Topic of Lecture: simple, gra		Date of Lecture:

• Explain the functions of cells

Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

### **Detailed content of the Lecture:**

In simple staining, the bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. Basic stains with a positively charged chromogen are preferred because bacterial nucleic acids and certain cell wall components carry a negative charge that strongly attracts and binds to the cationic chromogen. The purpose of simple staining is to elucidate the morphology and arrangement of bacterial cells. The most commonly used basic stains are methylene blue, crystal violet, and carbol fuchsin.

Gram Staining

The **Gram stain procedure** is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian **Gram** in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques. The steps of the Gram stain procedure are as follows:

- 1. First, **crystal violet**, a **primary stain**, is applied to a heat-fixed smear, giving all of the cells a purple color.
- 2. Next, **Gram's iodine**, a **mordant**, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
- 3. Next, a **decolorizing agent** is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
- 4. Finally, a secondary **counter stain**, usually **safranin**, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.

Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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# LECTURE HANDOUTS



BME		II/IV
Course Name with Code	: 16BMD11&PATHOLOGY AND MICROBIOLOGY	
Course Faculty	: Ms.A.Anjali	
Unit	: 4	Date of Lecture:

# **Topic of Lecture:** AFB staining

#### **Introduction :**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

Anatomy and Human Physiology Fundamentals of biochemistry

# **Detailed content of the Lecture:**

Acid-Fast Stains

Acid-fast staining is another commonly used, differential staining technique that can be an important diagnostic tool. An **acid-fast stain** is able to differentiate two types of gram-positive cells: those that have waxy mycolic acids in their cell walls, and those that do not. Two different methods for acid-fast staining are the **Ziehl-Neelsen technique** and the **Kinyoun technique**. Both use **carbolfuchsin** as the primary stain. The waxy, acid-fast cells retain the carbolfuchsin even after a decolorizing agent (an acid-alcohol solution) is applied. A secondary counterstain, methylene blue, is then applied, which renders non–acid-fast cells blue.

The fundamental difference between the two carbolfuchsin-based methods is whether heat is used during the primary staining process. The Ziehl-Neelsen method uses heat to infuse the carbolfuchsin into the acid-fast cells, whereas the Kinyoun method does not use heat. Both techniques are important diagnostic tools because a number of specific diseases are caused by **acid-fast bacteria** (AFB). If AFB are present in a tissue sample, their red or pink color can be seen clearly against the blue background of the surrounding tissue cells .

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# **Important Books/Journals for further learning including the page nos.:**

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005



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BME			II/IV
Course Nam	e with Code :	16BMD11&PATHOLOGY AND	MICROBIOLOGY
Course Teac	her :	Ms.A.Anjali	
Unit	:	5	Date of Lecture:
Introductio • Anal • Expl Prerequisite ( Max. Four Anatomy an	ain the functions of cell e knowledge for Comp important topics) d Human Physiology	tional aspects of living organisms.	of Topic:
	ls of biochemistry ntent of the Lecture:		
Immunity is response ge There are tw artificial. Naturally ac	the state of protection nerated by immunization wo ways to acquire act quired active immunity	on against infectious disease confe on or previous infection, or by or ive resistance against invading mic r occurs when the person is exposed	ther non-immunological factors. crobes: active natural and active to a live pathogen, develops the
the body's s B-cells in the immune res	kin, mucous membrand e body produce antibo ponse generated agains	result of the primary immune resp es, or other primary defenses, it in dies that help to fight against the i st the pathogen takes days or week ection, for example with hepatitis	teracts with the immune system. nvading microbes. The adaptive ks to develop but may be long-



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MUTHAYAMMAL ENGINEERING COLLEGE (An Autonomous Institution)



L - 2

II/IV

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**LECTURE HANDOUTS** 

Course Name with Code	: 16BMD11&PATHOLOGY AND MICROBIOLOGY
Course Teacher	: Ms.A.Anjali

Unit

Date of Lecture:

Topic of Lecture: types of Hypersensitivity

#### **Introduction : (Maximum 5 sentences)**

• Analyze structural and functional aspects of living organisms.

: 5

• Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

#### ( Max. Four important topics)

Anatomy and Human Physiology

Fundamentals of biochemistry

**Detailed content of the Lecture:** 

# **Types of Hypersensitivity Reactions**

The response of the host to the presence of foreign substances can trigger four types of hypersensitivity reactions:

Immediate Cytotoxic Immune complex Cell-mediated

# Type I: Immediate Hypersensitivity (Anaphylactic Reaction)

These allergic reactions are systemic or localized, as in allergic dermatitis (e.g., hives, wheal and erythema reactions). The reaction is the result of an antigen cross-linking with membrane-bound IgE antibody of a mast cell or basophil. Histamine, serotonin, bradykinin, and lipid mediators (e.g., platelet activating factor, prostaglandins, and leukotrienes) are released during the anaphylactic reaction. These released substances have the potential to cause tissue damage.

# Type II: Cytotoxic Reaction (Antibody-dependent)

In a cytotoxic reaction, the antibody reacts directly with the antigen that is bound to the cell membrane to induce cell lysis through complement activation. These antigens may be intrinsic or "self" as in autoimmune reactions or extrinsic or "non-self." Cytotoxic reactions are mediated by IgG and IgM. Examples of cytotoxic reaction are the Rh incompatibility of a newborn, blood transfusion reactions, and autoimmune diseases like Pemphigus Vulgaris, Bullous Pemphigoid, autoimmune hemolytic anemia and Goodpasture's syndrome to name a few.

# **Type III: Immune Complex Reaction**

IgG and IgM bind antigen, forming antigen-antibody (immune) complexes. These activate complement, which results in PMN chemotaxis and activation. PMNs then release tissue damaging enzymes. Tissue damage present in autoimmune diseases (e.g., systemic lupus erythematosus), and chronic infectious diseases (e.g., leprosy) can be attributed, in part, to immune complex reactions. **Type IV: Cell-Mediated (Delayed Hypersensitivity)** 

Cell-mediated reactions are initiated by T-lymphocytes and mediated by effector T-cells and macrophages. This response involves the interaction of antigens with the surface of lymphocytes. Sensitized lymphocytes can produce cytokines, which are biologically active substances that affect the functions of other cells. This type of reaction takes 48-72 hours, or longer, after contact with the antigen to fully develop. Many chronic infectious diseases, including tuberculosis and fungal infections, exhibit delayed hypersensitivity.

Evidence suggests that hypersensitivity reactions, particularly Type III and IV, may be involved in the

pathogenesis of periodontal disease.

Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

**Important Books/Journals for further learning including the page nos.:** 

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 



#### **Detailed content of the Lecture:**

**Cell-mediated immunity** is an immune response that does not involve antibodies. Rather, cell mediated immunity is the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to antigen. Historically, the immune system was separated into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or <u>serum</u>) and **cellular immunity**, for which the protective function against different pathogens. Naive T cells, which are mature T cells that have yet to encounter an <u>antigen</u>, are converted into activated effector T cells after encountering <u>antigen-presenting cells</u> (APCs). These APCs, such as <u>macrophages</u>, <u>dendritic cells</u>, and <u>B cells</u> in some circumstances, load antigenic peptides onto the <u>MHC</u> of the cell, in turn presenting the peptide to receptors on T cells. The most important of these APCs are highly specialized dendritic cells; conceivably operating solely to ingest and present antigens. [1]

Activated Effector T cells can be placed into three functioning classes, detecting <u>peptide</u> antigens originating from various types of <u>pathogen</u>: The first class being 1) <u>Cytotoxic T cells</u>, which kill infected target cells by <u>apoptosis</u> without using cytokines, 2) <u>TH1 cells</u>, which primarily function to activate macrophages, and 3) <u>TH2 cells</u>, which primarily function to stimulate <u>B cells</u> into producing <u>antibodies</u>.<sup>[1]</sup>

The <u>innate immune system</u> and the <u>adaptive immune system</u> each comprise both <u>humoral</u> and cellmediated components.

Cellular immunity protects the body through:

- T-cell mediated immunity or <u>T-cell immunity</u>: activating antigen-specific <u>cytotoxic T cells</u> that are able to induce <u>apoptosis</u> in body cells displaying <u>epitopes</u> of foreign antigen on their surface, such as <u>virus</u>-infected cells, cells with intracellular bacteria, and cancer cells displaying <u>tumor</u> antigens;
- <u>Macrophage</u> and <u>natural killer cell</u> action: enabling the destruction of pathogens via recognition and secretion of cytotoxic granules (for natural killer cells)<sup>[2]</sup> and phagocytosis (for macrophages)<sup>[3]</sup>; and
- Stimulating cells to secrete a variety of <u>cytokines</u> that influence the function of other cells involved in adaptive immune responses and innate immune responses.<sup>[2][3]</sup>

Cell-mediated immunity is directed primarily at microbes that survive in <u>phagocytes</u> and <u>microbes</u> that infect non-phagocytic cells. It is most effective in removing virus-infected cells, but also participates in defending against <u>fungi</u>, <u>protozoans</u>, <u>cancers</u>, and intracellular bacteria. It also plays a major role in <u>transplant rejection</u>.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# **Important Books/Journals for further learning including the page nos.:**

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

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**LECTURE HANDOUTS** 



II/IV

**Date of Lecture:** 

L - 4

BME

Course Name with Code: 16BMD11&PATHOLOGY AND MICROBIOLOGYCourse Teacher: Ms.A.Anjali

Unit

: 5

**Topic of Lecture:** opsonization, phagocytosis, inflammation

# **Introduction : (Maximum 5 sentences)**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

**Prerequisite knowledge for Complete understanding and learning of Topic:** (Max. Four important topics)

Anatomy and Human Physiology

Fundamentals of biochemistry

**Detailed content of the Lecture:** 

**Antibody opsonization** is a process by which a pathogen is marked for ingestion and destruction by <u>phagocytes<sup>[1]</sup></u>.



Antibodies (A) and pathogens (B) free roam in the blood. 2) The antibodies bind to pathogens, and can do so in different formations such as: opsonization (2a), neutralisation (2b), and agglutination (2c).
A phagocyte (C) approaches the pathogen, and Fc region (D) of the antibody binds to one of the Fc receptors (E) on the phagocyte. 4) Phagocytosis occurs as the pathogen is ingested.

Given normal inflammatory circumstances, microbial <u>pathogen-associated molecular</u> <u>patterns</u> (PAMPs) bind with the endocytic <u>pattern recognition receptors</u> (PRRs) of <u>phagocytes</u>, which mediates <u>neutrophil</u> mediation or <u>macrophage phagocytosis</u>. As well as endocytic PRRs, phagocytes furthermore express <u>opsonin</u> receptors such as <u>Fc receptor</u> and <u>complement receptor 1</u> (CR1). Should the microbe be coated with opsonising antibodies or <u>C3b</u> complement, the co-stimulation of endocytic PRR and opsonin receptor increases the efficacy of the phagocytic process, enhancing the <u>lysosomal</u> elimination of the infective agent. This mechanism of antibody-mediated increase in phagocytic efficacy is named opsonization.

Opsonization involves the binding of an <u>opsonin</u> (e.g., <u>antibody</u>) to an epitope on a pathogen.<sup>[2]</sup> After opsonin binds to the membrane, <u>phagocytes</u> are attracted to the pathogen. The Fab portion of the antibody binds to the antigen, whereas the Fc portion of the antibody binds to an <u>Fc receptor</u> on the phagocyte, facilitating phagocytosis.<sup>[3]</sup> The core receptor + opsonin complex also creates byproducts like <u>C3b</u> and <u>C4b</u> which are important components for the efficient function of the <u>complement</u> system. These components are deposited on the cell surface of the pathogen and aid in its destruction.<sup>[4]</sup>

The cell can also be destroyed by a process called <u>antibody-dependent cell-mediated cytotoxicity</u>, in which the pathogen does not need to be phagocytozed to be destroyed. During this process, the pathogen is opsonized and bound with the antibody <u>IgG</u> via its Fab domain. This allows the antibody binding of an immune effector cell via its Fc domain. Antibody-dependent cell-mediated inherent mediation then triggers a release of lysis products from the bound immune effector cell (monocytes, neutrophils, eosinophils and NK cells). Lack of mediation can cause inflammation of surrounding tissues and damage to healthy cells.

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

# Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 



Secondary immunodeficiency

The immune system is composed of a variety of cells, especially white blood cells, and proteins, for which one of the principal functions is microbial defense. A deficit in the immune system can therefore lead to unusually severe or uncommon recurrent infections. Immune deficits (immunodeficiency) may be primary or secondary. Secondary immune deficiencies or acquired deficiencies, more frequent than primary immune deficiencies, are problems of the immune system that are not genetic and which are caused by external factors.

An example of a secondary immune deficiency: AIDS

The most well-known example of a secondary immune deficiency is the immunodeficiency caused by the **h**uman **i**mmunodeficiency **v**irus, or HIV. HIV attacks certain cells in the immune system and prevents them from carrying out their proper functions against microbes. When the immune system is sufficiently weakened, infected people catch atypical and severe infections. This is then called

the Acquired Immunodeficiency Syndrome, or AIDS. AIDS at this time is often treated by a specialized multidisciplinary team.

What are other causes of secondary immunodeficiency?

Other causes of secondary immunodeficiency include: severe malnutrition, certain chronic diseases such as diabetes, immunosuppressive medication or chemotherapy, certain cancers such as leukemia, and the absence of the spleen (sometimes the spleen must be removed because of trauma, for example).

How are secondary immunodeficiencies treated?

The treatment depends on the severity in the deficiency of the immune system. Treatment of the underlying cause often leads to improvement of the condition. Administration of immunoglobulins and antibioprophylaxis may also be useful in some cases.

Video Content / Details of website for further learning (if any):

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DESIGNING INFORMATION	MUTHAYAMMAL ENGINEERING COLLEGE (An Autonomous Institution) (Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University) Rasipuram - 637 408, Namakkal Dist., Tamil Nadu LECTURE HANDOUTS	L-6
BME		II/IV
Course Name	e with Code : 16BMD11&PATHOLOGY AND MICROBIOLOGY	 Z

**Course Teacher** 

: Ms.A.Anjali

Topic of Lecture: Auto-immune disorders: Basic concepts and classification

#### Introduction : (Maximum 5 sentences)

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

**Prerequisite knowledge for Complete understanding and learning of Topic:** (Max. Four important topics)

Anatomy and Human Physiology

Fundamentals of biochemistry

# **Detailed content of the Lecture:**

An **autoimmune disease** is a condition arising from an abnormal <u>immune response</u> to a normal body part.<sup>[1]</sup> There are at least 80 types of autoimmune diseases.<sup>[1]</sup> Nearly any body part can be involved.<sup>[3]</sup> Common symptoms include low grade <u>fever</u> and <u>feeling tired</u>.<sup>[1]</sup> Often symptoms come and go.<sup>[1]</sup>

The cause is generally unknown.<sup>[3]</sup> Some autoimmune diseases such as <u>lupus</u> run in families, and certain cases may be triggered by <u>infections</u> or other environmental factors.<sup>[1]</sup> Some common diseases that are generally considered autoimmune include <u>celiac disease</u>, <u>diabetes mellitus type 1</u>, <u>Graves'</u> <u>disease</u>, <u>inflammatory bowel disease</u>, <u>multiple sclerosis</u>, <u>psoriasis</u>, <u>rheumatoid arthritis</u>, and <u>systemic lupus erythematosus</u>.<sup>[11][4]</sup> The diagnosis can be difficult to determine.<sup>[1]</sup>

Treatment depends on the type and severity of the condition.<sup>[1]</sup> <u>Nonsteroidal anti-inflammatory</u> <u>drugs</u> (NSAIDs) and <u>immunosuppressants</u> are often used.<sup>[1]</sup> <u>Intravenous immunoglobulin</u> may also occasionally be used.<sup>[2]</sup> While treatment usually improves symptoms, they do not typically cure the disease

The human immune system typically produces both <u>T cells</u> and <u>B cells</u> that are capable of being reactive with self-antigens, but these self-reactive cells are usually either killed prior to becoming active within the immune system, placed into a state of anergy (silently removed from their role within the immune system due to over-activation), or removed from their role within the immune system by regulatory cells. When any one of these mechanisms fail, it is possible to have a reservoir of self-reactive cells that become functional within the immune system. The mechanisms of preventing self-reactive T cells from being created takes place through negative selection process within the <u>thymus</u> as the T cell is developing into a mature immune cell.

Some infections, such as <u>Campylobacter jejuni</u>, have <u>antigens</u> that are similar (but not identical) to our own self-molecules. In this case, a normal immune response to *C. jejuni* can result in the production of antibodies that also react to a lesser degree with gangliosides of myelin sheath surrounding peripheral nerves' axons (i.e., <u>Guillain–Barré</u>). A major understanding of the underlying pathophysiology of autoimmune diseases has been the application of genome wide association scans that have identified a degree of genetic sharing among the autoimmune diseases.<sup>[10]</sup>

<u>Autoimmunity</u>, on the other hand, is the presence of self-reactive immune response (e.g., autoantibodies, self-reactive T cells), with or without damage or pathology resulting from it.<sup>[11]</sup> This may be restricted to certain <u>organs</u> (e.g. in <u>autoimmune thyroiditis</u>) or involve a particular tissue in different places (e.g. <u>Goodpasture's disease</u> which may affect the <u>basement membrane</u> in both the <u>lung</u> and the <u>kidney</u>).

# Video Content / Details of website for further learning (if any):

• https://nptel.ac.in/courses/102106025/36

Important Books/Journals for further learning including the page nos.:

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<u>nodes</u>, <u>feeling tired</u>, and a red <u>rash</u> which is most commonly on the face.<sup>[1]</sup> Often there are periods of illness, called <u>flares</u>, and periods of <u>remission</u> during which there are few symptoms.<sup>[1]</sup>

The cause of SLE is not clear.<sup>[11]</sup> It is thought to involve <u>genetics</u> together with <u>environmental</u> <u>factors</u>.<sup>[4]</sup> Among <u>identical twins</u>, if one is affected there is a 24% chance the other one will be as well.<sup>[11]</sup> <u>Female sex hormones</u>, sunlight, smoking, <u>vitamin D deficiency</u>, and certain infections, are also believed to increase the risk.<sup>[4]</sup> The mechanism involves an immune response by <u>autoantibodies</u> against a person's own tissues.<sup>[11]</sup> These are most commonly <u>anti-nuclear antibodies</u> and they result in <u>inflammation</u>.<sup>[11]</sup> Diagnosis can be difficult and is based on a combination of symptoms and laboratory tests.<sup>[11]</sup> There are a number of other kinds of <u>lupus erythematosus</u> including <u>discoid lupus</u> erythematosus, neonatal lupus, and subacute cutaneous lupus erythematosus.<sup>[11]</sup>

ThereisnocureforSLE.[1]Treatmentsmayinclude NSAIDs, corticosteroids, immunosuppressants, hydroxychloroquine,

and <u>methotrexate</u>.<sup>[1]</sup> Although corticosteroids are rapidly effective, long term use results in side effects.<sup>[5]</sup> <u>Alternative medicine</u> has not been shown to affect the disease.<sup>[1]</sup> Life expectancy is lower among people with SLE.<sup>[6]</sup> SLE significantly increases the risk of <u>cardiovascular disease</u> with this being the most common cause of death.<sup>[4]</sup> With modern treatment about 80% of those affected survive more than 15 years.<sup>[3]</sup> Women with lupus have pregnancies that are higher risk but are mostly successful.<sup>[1]</sup>

# Laboratory tests

<u>Antinuclear antibody</u> (ANA) testing and anti-extractable nuclear antigen (<u>anti-ENA</u>) form the mainstay of <u>serologic</u> testing for SLE. If ANA is negative the disease can be ruled out.<sup>[71]</sup>

detect Several techniques are used ANAs. The widely used to most is indirect immunofluorescence (IF). The pattern of fluorescence suggests the type of antibody present in the people's serum. Direct immunofluorescence can detect deposits of immunoglobulins and complement proteins in the people's skin. When skin not exposed to the sun is tested, a positive direct IF (the so-called lupus band test) is an evidence of systemic lupus erythematosus.<sup>[72]</sup>

# Video Content / Details of website for further learning (if any):

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# MUTHAYAMMAL ENGINEERING COLLEGE (An Autonomous Institution)



L - 8

(Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University)

Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

**LECTURE HANDOUTS** 

BME			II/IV
Course Name w	vith Code	: 16BMD11&PATHOLOGY AND	MICROBIOLOGY
Course Teacher	c	: Ms.A.Anjali	
Unit		: 5	Date of Lecture:

Topic of Lecture: immunological techniques: immune diffusion, immuno electrophoresis

#### Introduction : (Maximum 5 sentences)

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic: ( Max. Four important topics)

Anatomy and Human Physiology

Fundamentals of biochemistry

**Detailed content of the Lecture:** 

**Immunoelectrophoresis** is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies, reacting with the proteins to be separated or characterized. The methods were developed and used extensively during the second half of the 20th century. In somewhat chronological order: Immunoelectrophoretic analysis (onedimensional immunoelectrophoresis ad modum Grabar), crossed immunoelectrophoresis (twodimensional immunoelectrophoresis ad Freeman quantitative modum Clarke and or ad modum Laurell), rocket-immunoelectrophoresis (one-dimensional quantitative immunoelectrophoresis ad modum Laurell), fused rocket immunoelectrophoresis ad modum Svendsen and Harboe, affinity immunoelectrophoresis ad modum Bøg-Hansen.

<u>Agarose</u> as 1% gel slabs of about 1 mm thickness buffered at high <u>pH</u> (around 8.6) is traditionally preferred for the electrophoresis as well as the reaction with antibodies. The agarose was chosen as the gel matrix because it has large pores allowing free passage and separation of proteins, but provides an anchor for the immunoprecipitates of protein and specific antibodies. The high pH was chosen because antibodies are practically immobile at high pH. An electrophoresis equipment with a horizontal cooling plate was normally recommended for the electrophoresis.

Immunoprecipitates may be seen in the wet agarose gel, but are stained with protein stains like <u>Coomassie Brilliant Blue</u> in the dried gel. In contrast to SDS-<u>gel electrophoresis</u>, the electrophoresis in agarose allows native conditions, preserving the native structure and activities of the proteins under investigation, therefore immunoelectrophoresis allows characterization of <u>enzyme</u> activities and ligand binding etc. in addition to electrophoretic separation.

The **immunoelectrophoretic** analysis *ad* modum Grabar is the classical of method immunoelectrophoresis. Proteins are separated by electrophoresis, then antibodies are applied in a trough next to the separated proteins and immunoprecipitates are formed after a period of diffusion of the separated proteins and antibodies against each other. The introduction of the immunoelectrophoretic analysis gave a great boost to protein chemistry, some of the very first results were the resolution of proteins in biological fluids and biological extracts. Among the important observations made were the great number of different proteins in serum, the existence of several immunoglobulin classes and their electrophoretic heterogeneity.

**Rocket immunoelectrophoresis** is one-dimensional quantitative immunoelectrophoresis. The method has been used for quantitation of human serum proteins before automated methods became available.

**Fused rocket immunoelectrophoresis** is a modification of one-dimensional quantitative immunoelectrophorsis used for detailed measurement of proteins in fractions from protein separation experiments.

# Video Content / Details of website for further learning (if any):

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Important Books/Journals for further learning including the page nos.:

Ramzi S Cotran, Vinay Kumar & Stanley L Robbins, "Pathologic Basis of Diseases",7th Edition,WB Saunders Co 2005

**Course Teacher** 

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DESIGNING YOUR FUTURE Estd. 2000	l by AICTE, New Delhi, Accredited by N Anna University) Rasipuram - 637 408, Namakkal Dist., T LECTURE HANDOUT	Camil Nadu
BME		II/IV
Course Name with Code	e : 16BMD11&PATHOLOGY A	ND MICROBIOLOGY
<b>Course Teacher</b>	: Ms.A.Anjali	
Unit	: 5	Date of Lecture:
<b>Topic of Lecture:</b> RIA a	nd ELISA, monoclonal antibodies.	

#### **Introduction : (Maximum 5 sentences)**

- Analyze structural and functional aspects of living organisms.
- Explain the functions of cells

# Prerequisite knowledge for Complete understanding and learning of Topic:

( **Max. Four important topics**) Anatomy and Human Physiology

Fundamentals of biochemistry

# **Detailed content of the Lecture:**

A **radioimmunoassay** (**RIA**) is an <u>immunoassay</u> that uses <u>radiolabeled</u> molecules in a stepwise formation of <u>immune complexes</u>. A RIA is a very sensitive <u>in vitro</u> assay technique used to measure concentrations of substances, usually measuring <u>antigen</u> concentrations (for example, <u>hormone</u> levels in <u>blood</u>) by use of <u>antibodies</u>.

Although the RIA technique is extremely <u>sensitive</u> and extremely <u>specific</u>, requiring specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing, since radioactive substances are used.

In contrast, an <u>immunoradiometric assay</u> (IRMA) is an immunoassay that uses radiolabeled molecules but in an immediate rather than stepwise way.

A <u>radioallergosorbent test</u> (RAST) is an example of radioimmunoassay. It is used to detect the causative <u>allergen</u> for an <u>allergy</u>.

Classically, to perform a radioimmunoassay, a known quantity of an <u>antigen</u> is made <u>radioactive</u>, frequently by labeling it with gamma-radioactive <u>isotopes of iodine</u>, such as <u>125-I</u>, attached to <u>tyrosine</u>. This radiolabeled antigen is then mixed with a known amount of <u>antibody</u> for that antigen, and as a result, the two specifically bind to one another. Then, a sample of <u>serum</u> from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the <u>concentration</u> of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free(unbound) antigen remaining in the <u>supernatant</u> is measured using a <u>gamma counter</u>.

This method can be used for any biological molecule in principle and is not restricted to serum antigens, nor is it required to use the indirect method of measuring the free antigen instead of directly measuring the captured antigen. For example, if it is undesirable or not possible to radiolabel the antigen or target molecule of interest, a RIA can be done if two different antibodies that recognize the target are available and the target is large enough (e.g., a protein) to present multiple <u>epitopes</u> to the antibodies. One antibody would be radiolabeled as above while the other would remain unmodified. The RIA would begin with the "cold" unlabeled antibody being allowed to interact and bind to the target molecule in solution. Preferably, this unlabeled antibody is immobilized in some way, such as coupled to an <u>agarose</u> bead, coated to a surface, etc. Next, the "hot" radiolabeled antibody is allowed to interact amount of radioactive antibody bound is measured and the amount of target molecule quantified by comparing it to a reference amount assayed at the same time. This method is similar in principle to the non-radioactive sandwich <u>ELISA</u> method.

# Video Content / Details of website for further learning (if any):

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