Biyani's Think Tank

Concept based notes

Medical Biotechnology

[B.Sc. Biotech Part-III]

[Paper BT-IV]

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

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Dr. B.L. Sharma

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Syllabus

MEDICAL BIOTECHNOLOGY

BT 703

Note : Question No. 1 shall consisit of wuestions requiring short answers and shall cover entire paper . The paper is divided into four sections. Student are required to attempt five questions in all, selecting not more than one question from each section. All question carry equal marks.

Section -A

General Introduction to Biomedical Engineering. Application of Engineering in medicine, Electrical Potentials in the human body. Neuromuscular system: neurons, synapases and muscles, electrical properties of nerves and muscles, problems and diagnostics. Cardiovascular system : anatomy & physiology of heart, ECG and the cardiac cycle, problems and solulations to electrical problems in the heart, blood and vascular modeling, haemodynamics, vascular disease management, Skeletal System (Including Prosthetics). Biomaterials and Implantable sensors, testing of Biomaterials in vitro and in vivo.

Excretory, system (including Dialysis) : renal anatomy & physiology, the nephron, dialysis machines & mass transport. Medical Imagining: X-rays, design considerations of X-ray tubes, medical Image Processing - projections, 3D-2D, sclice identification, CAT, NMR, MRI,PET/ SPECT. Cellilar engineering and genetic engineering - Ethical consideration in Medical research.

Section -B

Innate and Acquired Immunity.

Antigens : types of antigen specificty, haptens, antibody structure and functions MHC, Complement System.

Cell mediated cytotoxicity : Origin, maturation and characterization of TY- lymphocytes, Monocytes & Macrophages, Mechanisn of T cell and NK cell mediated lysis, ADCC, macrophage mediated cytotoxicity, lymphokines the product of T cell activation.

Humoral immune response : Origin maturation and characterization of B-lymphocytes, Activation and proliferation of B- cells, Formation of plasmablast, Plasma cells and memory cells, Interaction of B and T cells.

Section -C

- Hypersensitivity, Monoclonal antibodies and its applications.

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-Radioimmunoassay, enzyme - linked immunosorbant assay, immunoblotting, immunofluorescence and flowcytometry.\

- Characteristics of infectious diseases, Herd immunity.

- Disease cycle (Source of disease, reservoir, carriers).

- Transmission of pathogens (Air Borne. contect transmission and vector transmission) .

Section -D

Bacterial Diseases : Epidemiology, Pathogenicity, Laboratory Diagnosis, Prevention & control of the following diseases : Anthrax, Tuberculosis, Typhoid, Whooping cough, Tetanus, Diphtheria Lerprosy.

General Account of viral & protozoan diseases : Chickenpox, AIDS and Malaria, Leishmniasis, Brief account of sexually transmitted diseases.

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Content

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Section –A

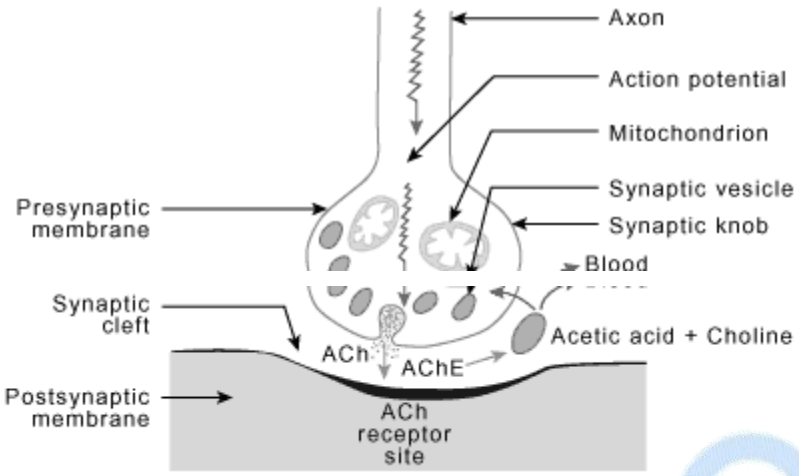
General Introduction to Biomedical Engineering & Excretory, system

Q.1 Write short notes on:

Q.1 (a) Synapses

Ans. Neurons communicate across junctions called synapses. A synapse consists of a swollen end at the nerve fibre called a synaptic knob lying in a close proximity to the membrane of a dendrite. The synaptic knob contains many mitochondria and small synaptic vesicles which contain neurotransmitter responsible for transmission of impulse across the synapse. The membrane of synaptic knob is thickened and called presynaptic membrane. The membrane of dendrite is also thickened and called postsynaptic membrane. These membranes are separated by synaptic cleft. The postsynaptic membrane contains receptor sites, channels and pores. The two main neurotransmitters are acetylcholine and noradrenaline. A charge which exists across the cell surface membranes of all cells is usually negative in a neuron with respect to outside. The membrane is said to be polarized. Depolarization of the presynaptic membrane takes place when an impulse arrives at a synaptic junction. The permeability of the membrane increases for Ca++.

As Ca++ ions enter the synaptic cleft it causes the release of neurotransmitter from synaptic vesicles present in synaptic cleft. The neurotransmitter is released by the process of exocytosis.The neurotransmitter diffuses across the synaptic cleft and gets attached to the receptors present on the membrane of the dendrite of next neuron. A cytoplasm in the axon, axoplasm, has high concentration of Na+ and K+ ions.



Transmission of an impulse at a synapse

This results in ionic flow through channels. The Na+ ions enter the dendrite while K+ ions leave the dendrite. This causes depolarization of postsynaptic membrane and initiates a new action potential.
The new action potential passes impulse along new neuron.

Q.1 (b) Blood and vascular model and remodeling

Ans. Blood is the fluid medium for transport in animals.
Blood consists of two constituents:

1. Plasma, and
2. Blood cells.

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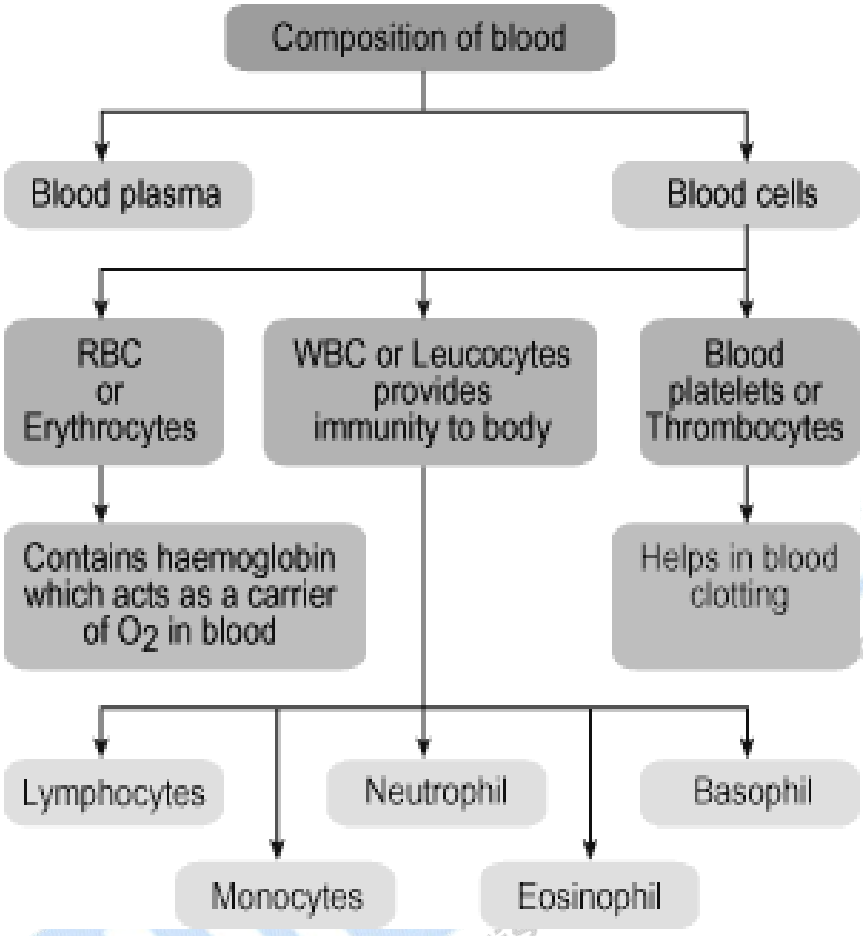

Plasma:

Plasma is a straw-coloured fluid containing 90 per cent water, 7 per cent plasma proteins (albumin, globulin, fibrinogen) and 3 per cent nutrients (glucose, amino acids, fatty acids), inorganic salts (Na, K, Ca, Mg), organic compounds (enzymes, hormones, antibodies, heparin), gases (oxygen, carbon dioxide, nitrogen) and wastes (urea, uric acid).

Blood cells:

Blood cells are of three types:

1. RBC or erythrocyte: Transport of oxygen and carbon dioxide.
2. WBC or leucocyte: Engulfs bacteria, Allergic responses and anti-histamine properties. Produces histamine and heparin.

3. Platelet or thrombocyte: Blood clotting

Major cardiovascular diseases such as hypertension, diabetes and atherosclerosis have a strong structural component. These structural alterations have detrimental hemodynamic consequences through their effects on the mechanical properties of the vessel wall. For instance, resistance artery remodeling contributes to elevated peripheral resistance in essential hypertension, conduit artery fibrosis contributes to systolic hypertension in the elderly, and inadequate expansion of the intact media beneath an arterial lesion contributes to the formation of a flow limiting stenosis.

That vascular structural changes adversely affect hemodynamics is in sharp contrast to the remarkable capacity of especially muscular arteries of healthy young individuals to adjust their lumen diameter and wall mass in response to acute and chronic changes in blood flow and blood pressure. This adaptive and compensatory remodeling operates during the entire development and maturation of the vascular system, and can be observed in selected situations in the adult such as the female reproductive system and the establishment of collateral circulations. It has been proposed that the endothelium is the main shearsensor and that the cytoskeleton plays in general a key role in biological responses to mechanical factors.

Manipulation of vascular remodeling with the aim to improve cardiovascular function in cardiovascular diseases, requires detailed knowledge of the (ultra)structural basis of vessel wall mechanics, of the transition from acute vasomotor to chronic vascular structural responses, and of the signaling and paracrine mechanisms that can modulate the preexisting structure. Comparative studies of large conduit arteries, small muscular arteries and arterioles and attention for the endothelium, may be helpful in this respect.

Q.1 (c) Haemodynamics

Ans. Haemodynamics is the branch of science that describes how blood flows. The physical principles of haemodynamics is a specific example of the general principles of fluid dynamics and the equations of blood flow and general fluid flow are identical. Blood is a liquid tissue: it has both physical and

biological properties. For the purposes of haemodynamics it is the physical properties that are of specific interest. The two fundamental properties of a fluid are its density and its viscosity. The density is the mass per unit volume and blood is slightly denser than water. The viscosity is the resistance of the fluid to being moved. Blood is nearly four times more viscid than water.

In order for a fluid to move something must push it. This something is pressure. To be more accurate, the fluid will move from a region of higher pressure towards a region of lower pressure. The greater the difference in the pressure, the quicker the flow. The more viscid the fluid the more it will resist the movement and the more pressure difference will be required to achieve the same flow.

The relationship between the pressure difference, the flow, the viscosity of the fluid and the area of the tube was determined experimentally by J.L.M.Poiseuille in the mid 1800's. Poiseuille found that:

The flow was proportional to the pressure difference between the ends of the tube.

The flow was inversely proportional to the area of the tube squared.

This means that the bigger the tube and the greater the pressure difference the more blood can flow. It also means that the smaller the tube the more difficult it is to push a given flow of blood through.

In the body there is a network of blood vessels which are of two types. Arteries carry blood from the heart to all the organs of the body to keep them supplied with oxygen. Veins carry the blood back from the organs to the heart and the heart then pumps the blood through the blood vessels in the lungs. In the lungs the waste product from the body called carbon dioxide is released from the blood and replaced with oxygen from the air. The blood goes around and around this loop hundreds of times a day, delivering

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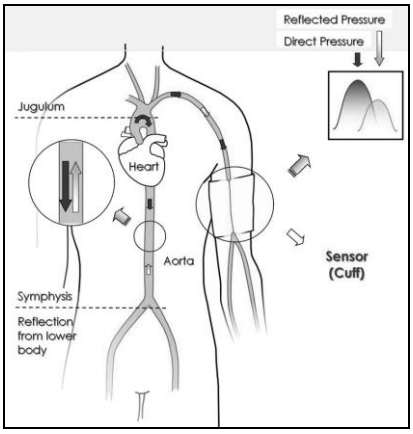
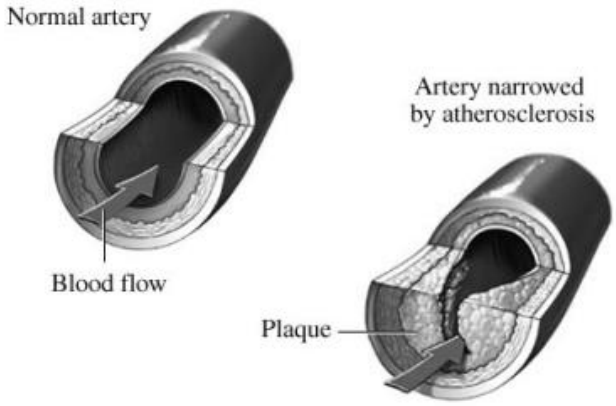


oxygen to the body and removing the waste carbon dioxide.

When you walk the muscles in the body move the legs and also prevent you from falling over. When muscles contract they need more oxygen and this oxygen has to be delivered to the muscles by the blood. So, as you walk, or do any form of muscular exertion, the blood flow to the muscles increases. In fact the muscles control this change in flow themselves by altering the size of the small arteries inside the muscles. To increase the flow they allow the artery to widen and this allows a greater flow. The increase in flow means that the blood pressure in the arteries will tend to fall. The body detects this fall in pressure and makes the heart beat faster to compensate. So, as you exercise, the blood flow through the arteries to the muscles increases, the heart beats faster but the blood pressure stays about the same. Normal arteries are large enough to handle the extra blood flow that is needed during exercise and the muscles are not starved of oxygen. Blood flow to the legs can easily increase by ten fold during exercise!

If an artery is narrowed (stenosis), then the blood must squeeze through the narrow part and as it does so it has to move more quickly. When the blood

squirts from the narrowing it forms a jet that catches up with the slower



moving

blood in the normal artery beyond the narrowing. The mixing of the jet and the slower moving blood downstream causes the blood to swirl (spin). Swirling flow is not as efficient as a smooth flow and a greater pressure is needed to force the blood to flow through a narrowing. In other words, for a given pressure difference, the flow of blood is less if it is swirling than if it is moving smoothly.

Q.1 (d) Prosthetics

Ans. P**rosthesis,** in medicine is an artificial extension that replaces a missing body part. It is part of the field of biomechatronics, the science of fusing mechanical devices with human muscle, skeleton, and nervous systems to assist or enhance motor control lost by trauma, disease, or defect. Prostheses are typically used to replace parts lost by injury (traumatic) or missing from birth (congenital) or to supplement defective body parts. Inside the body, artificial heart valves are in common use with artificial hearts and lungs seeing less common use but under active technology development. Other medical devices and aids that can be considered prosthetics include artificial eyes, palatal obturator, gastric bands, and dentures.

Lower extremity prosthetics describes artificially replaced limbs located at the hip level or lower. The two main subcategories of lower extremity prosthetic devices are 1.trans-tibial (any amputation transecting the tibia bone or a congenital anomaly resulting in a tibial deficiency) and 2.trans-femural (any amputation transecting the femur bone or a congenital anomaly resulting in a femural deficiency).

In order for a robotic prosthetic limb to work, it must have several components to integrate it into the body's function: Biosensors detect signals from the user's nervous or muscular systems. It then relays this information to a controller located inside the device, and processes feedback from the limb and actuator (e.g., position, force) and sends it to the controller.

With advances in modern technology, cosmesis, the creation of lifelike limbs made from silicone or PVC has been made possible. Such prosthetics, such as artificial hands, can now be made to mimic the appearance of real hands, complete with freckles, veins, hair, fingerprints and even tattoos. Another option is the custom-made silicone cover, which can be made to match a person's skin tone but not details such as freckles or wrinkles. Cosmeses are attached to the body in any number of ways, using an adhesive, suction, form-fitting, stretchable skin, or a skin sleeve.

Unlike neuromotor prostheses, neurocognitive prostheses would sense or modulate neural function in order to physically reconstitute or augment cognitive processes such as executive function, attention, language, and memory. No neurocognitive prostheses are currently available but the development of implantable neurocognitive brain-computer interfaces has been proposed to help treat conditions such as stroke, traumatic brain injury, cerebral palsy, autism, and Alzheimer's disease.

Q.1 (e) NMR

Ans. NMR (Nuclear magnetic resonance) is a property that magnetic nuclei have in a magnetic field and applied electromagnetic (EM) pulse, which cause the nuclei to absorb energy from the EM pulse and radiate this energy back out. The energy radiated back out is at a specific resonance frequency which depends on the strength of the magnetic field and other factors. This allows the observation of specific quantum mechanical magnetic properties of an atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals and non-crystalline materials through NMR spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

Any nucleus that contains an odd number of protons and/or of neutrons has an intrinsic magnetic moment and angular momentum. The most commonly studied nuclei are ^{1}H (the most NMR-sensitive isotope after the radioactive ^{3}H) and ^{13}C, although nuclei from isotopes of many other elements (e.g. ^{2}H, ^{10}B, ^{11}B, ^{14}N, ^{15}N, ^{17}O, ^{19}F, ^{23}Na, ^{29}Si, ^{31}P, ^{35}Cl, ^{113}Cd, ^{195}Pt) are studied by high-field NMR spectroscopy as well.

A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field. It is this feature that is exploited in imaging techniques; if a sample is placed in a non-uniform magnetic field then the resonance frequencies of the sample's nuclei depend on where in the field they are located. Since the resolution of the imaging techniques depends on how big the gradient of the field is, many efforts are made to develop more powerful magnets, often using superconductors. The effectiveness of NMR can also be improved using hyperpolarization, and/or using two-dimensional, three-dimensional and higher dimension multi-frequency techniques.

The principle of NMR usually involves two sequential steps:

- The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field H_0.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field (H_0) and the nuclei of observation.

The two fields are usually chosen to be perpendicular to each other as this maximises the NMR signal strength. The resulting response by the total magnetization (M) of the nuclear spins is the phenomenon that is exploited in NMR spectroscopy and magnetic resonance imaging. Both use intense applied magnetic fields (H_0) in order to achieve dispersion and very high stability to deliver spectral resolution.

Q.2 Describe electrical problems in the heart.

Ans. **Arrhythmias that originate in the heart's upper chambers, the atria**
Atrial Fibrillation (AF or A Fib)

More than 2 million people in the United States have atrial fibrillation, making it a very common heart rhythm disorder. In A Fib, the heartbeat is irregular and rapid, sometimes beating as often as 300 times a minute, about four times faster than normal. Although it isn't life threatening, A Fib can lead to other rhythm problems, chronic fatigue and congestive heart failure. Chances of having a stroke are five times higher for those with A Fib.

Atrial Flutter (AFL)

Atrial flutter is similar to A Fib because it too is characterized by a rapid heartbeat. Instead of many disorganized signals, however, AFL is caused by a single electrical wave that circulates very rapidly in the atrium, about 300 times a minute, leading to a very fast, steady heartbeat.

Sick Sinus Syndrome (SSS)

SSS is not a specific disease, but a group of signs or symptoms that indicate the heart's natural electrical pacemaker, the sinoatrial node, is not functioning properly. In SSS, the heart rate can switch back and forth between a slow rate (bradycardia) and a fast rate (tachycardia). A permanent pacemaker, sometimes in combination with medication, is the primary treatment.

Sinus Tachycardia :

A harmless rhythm, sinus tachycardia is a normal increase in heart rate that happens with fever, excitement and exercise. It does not require treatment except in rare cases when an underlying problem, such as anemia or hyperthyroidism, should be treated.

Arrhythmias that originate in the heart's lower chambers, the ventricles

Ventricular Tachycardia (VT) :

Characterized by a very fast heart rate,VT usually is seen in the setting of other serious heart disease. Occasionally, it occurs in people with normal hearts. It usually requires prompt treatment, sometimes with medication. Sometimes it is treated with radiofrequency ablation or surgery.Often people with VT are protected by implantation of a defibrillator. Because VT can lead to ventricular fibrillation (next item) it is considered a serious condition that warrants aggressive monitoring and treatment.

Ventricular Fibrillation (VF) :

Sudden cardiac death, caused by ventricular fibrillation, poses the greatest threat and accounts for half of all cardiac deaths. In VF, the heartbeat is rapid and chaotic,which causes the lower heart chambers, or ventricles, to go into a spasm. Sometimes, however, a heart attack can lead to VF. VF is abrupt and happens without any warning and it halts all heart functioning.The lack of oxygen throughout the body, and especially to the brain, is deadly.Also known as cardiac arrest, sudden cardiac death is due to an electrical circuitry problem. It is not a the same as a heart attack, or myocardial infarction,which is a circulatory problem caused by clogged blood vessels that cut off the supply of blood to the heart.

Although CPR can provide some benefit, the only truly effective VF treatment is defibrillation, which relies on paddles or electrodes to "shock" the heart back to normal rhythm. Without treatment, loss of consciousness comes in seconds, and death is inevitable.

Other :

Premature Contractions

Extra, early or "skipped" beats are the most frequent cause of irregular heart rhythms.These can start in the upper or lower chambers of the heart

Long QT Syndrome (LQTS)

Long QT Syndrome is a disorder of the electrical system. It can be inherited, acquired after taking certain medications, or caused by a combination of heredity and medications. People with LQTS are susceptible to ventricular fibrillation.

Heart Block :

When electrical impulses generated in the upper chambers of the heart are not properly transmitted to the lower chambers,Heart Block happens.The heart then beats too slowly,reducing the oxygen that gets to the body and brain.

Syncope (Fainting) :

Fainting, or feeling as if one might faint, can be caused by serious heart rhythm disorders and needs to be evaluated carefully. Sometimes the cause is not heart related, for instance when low blood sugar is to blame, but still can

be dangerous.No matter what the cause, fainting can be dangerous simply because of the potential for injuries from falling.

Q.3 Give detailed account of biomaterials and implantable sensors?

Ans. A biomaterial is "any substance (other than drugs) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body". Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. Host Response is the response of the host organism (local and systemic) to the implanted material or device.

Some Commonly Used Biomaterials

Material	Applications
Silicone rubber	Catheters, tubing
Dacron	Vascular grafts
Cellulose	Dialysis membranes
Poly(methyl methacrylate)	Intraocular lenses, bone cement
Polyurethanes	Catheters, pacemaker leads
Hydogels	Opthalmological devices, Drug Delivery
Stainless steel	Orthopedic devices, stents
Titanium	Orthopedic and dental devices
Alumina	Orthopedic and dental devices
Hydroxyapatite	Orthopedic and dental devices
Collagen (reprocessed)	Opthalmologic applications, wound dressings

A Little History on Biomaterials

- Romans, Chinese, and Aztecs used gold in dentistry over 2000 years ago, Cu not good.
- Ivory & wood teeth
- Aseptic surgery 1860 (Lister)
- Bone plates 1900, joints 1930

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- Turn of the century, synthetic plastics came into use
  - WWII, shards of PMMA unintentionally got lodged into eyes of aviators
  - Parachute cloth used for vascular prosthesis
- 1960- Polyethylene and stainless steel being used for hip implants

Uses of Biomaterials :

- Replace diseased part – dialysis
- Assist in healing – sutures
- Improve function – contacts
- Correct function – spinal rods
- Correct cosmetic – nose, ear
- Aid dx – probe
- Aid tx – catheter
- Replace rotten – amalgam
- Replace dead - skin

First Generation Implants :

- "ad hoc" implants
- specified by physicians using common and borrowed materials
- most successes were accidental rather than by design

Examples — First Generation Implants

- gold fillings, wooden teeth, PMMA dental prosthesis
- steel, gold, ivory, etc., bone plates
- glass eyes and other body parts
- dacron and parachute cloth vascular implants

Second generation implants :

- engineered implants using common and borrowed materials
- developed through collaborations of physicians and engineers
- built on first generation experiences

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- used advances in materials science (from other fields)

Examples — Second generation implants

- titanium alloy dental and orthopaedic implants
- cobalt-chromium-molybdinum orthopaedic implants
- UHMW polyethylene bearing surfaces for total joint replacements
- heart valves and pacemakers

Third generation implants :

- bioengineered implants using bioengineered materials
- few examples on the market
- some modified and new polymeric devices
- many under development

Example - Third generation implants

- tissue engineered implants designed to regrow rather than replace tissues
- Integra LifeSciences artificial skin
- Genzyme cartilage cell procedure
- some resorbable bone repair cements
- genetically engineered "biological" components (Genetics Institute and Creative Biomolecules BMPs)

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component.

It consists of 3 parts:

- the *sensitive biological element* (biological material (eg. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc), a biologically derived material or biomimic) The sensitive elements can be created by biological engineering.
- the *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified;

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- associated electronics or signal processors that is primarily responsible for the display of the results in a user-friendly way.

The most widespread example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. Recently, arrays of many different detector molecules have been applied in so called electronic nose devices, where the pattern of response from the detectors is used to fingerprint a substance.

A canary in a cage, as used by miners to warn of gas could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at a much lower level than us to warn us of their presence. Such devices can be used both in environmental monitoring and in water treatment facilities.

Optical biosensors based on the phenomenon of surface plasmon resonance are evanescent wave techniques. This utilises a property shown of gold and other materials; specifically that a thin layer of gold on a high refractive index glass surface can absorb laser light, producing electron waves (surface plasmons) on the gold surface. This occurs only at a specific angle and wavelength of incident light and is highly dependent on the surface of the gold, such that binding of a target analyte to a receptor on the gold surface produces a measurable signal.

Other optical biosensors are mainly based on changes in absorbance or fluorescence of an appropriate indicator compound. A widely used research tool, the micro-array, is basically a biosensor.

Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons (such enzymes are rightly called redox enzymes). The sensor substrate usually contains three electrodes, a reference electrode, an active electrode and a sink electrode. An auxiliary electrode (or counter electrode) may also be present as an ion source. The target analyte is involved in the reaction that takes place on the active electrode surface, and the ions produced create a potential which is subtracted from that of the reference electrode to give a signal. We can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the potential can be measured at zero current (this gives a logarithmic response). Note that potential of the working or active electrode is space charge sensitive and this is often used.

A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks (see later), show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme.
3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects.
5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
6. There should be a market for the biosensor.

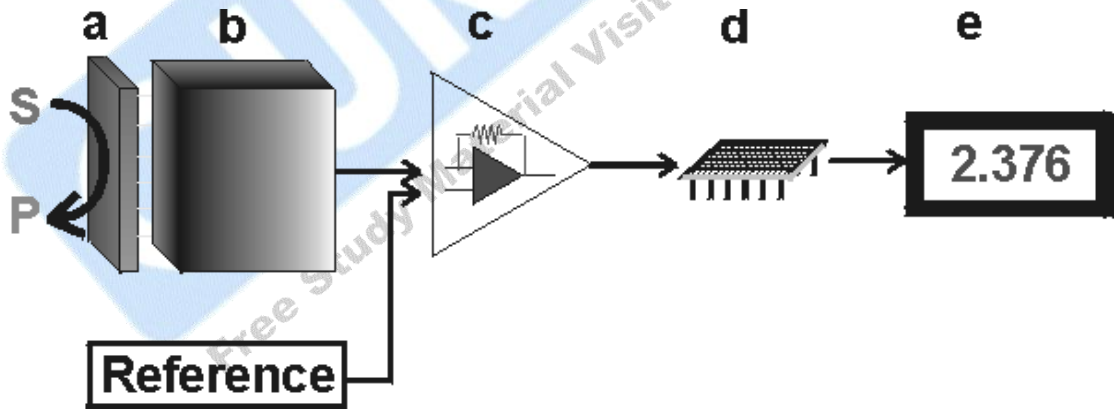

Figure: Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

The key part of a biosensor is the transducer (shown as the 'black box' in Figure) which makes use of a physical change accompanying the reaction. This may be

1. the heat output (or absorbed) by the reaction (calorimetric biosensors),
2. changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),
3. movement of electrons produced in a redox reaction (amperometric biosensors),
4. light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or
5. effects due to the mass of the reactants or products (piezo-electric biosensors).

There are three so-called 'generations' of biosensors; First generation biosensors where the normal product of the reaction diffuses to the transducer and causes the electrical response, second generation biosensors which involve specific 'mediators' between the reaction and the transducer in order to generate improved response, and third generation biosensors where the reaction itself causes the response and no product or mediator diffusion is directly involved.

The electrical signal from the transducer is often low and superimposed upon a relatively high and noisy (i.e. containing a high frequency signal component of an apparently random nature, due to electrical interference or generated within the electronic components of the transducer) baseline. The signal processing normally involves subtracting a 'reference' baseline signal, derived from a similar transducer without any biocatalytic membrane, from the sample signal, amplifying the resultant signal difference and electronically filtering (smoothing) out the unwanted signal noise. The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration. The analogue signal produced at this stage may be output directly but is usually converted to a digital signal and passed to a microprocessor stage where the data is processed, converted to concentration units and output to a display device or data store.

There are many potential [application of biosensors] of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are given below:

- Glucose monitoring in diabetes patients <-- historical market driver

- Other medical health related targets
- Environmental applications e.g. the detection of pesticides and river water contaminants
- Remote sensing of airborne bacteria e.g. in counter-bioterrorist activities
- Detection of pathogens
- Determining levels of toxic substances before and after bioremediation
- Detection and determining of organophosphate
- Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey.
- Drug discovery and evaluation of biological activity of new compounds.
- Protein engineering in biosensors
- Detection of toxic metabolites such as mycotoxins

Q.4 Give a detailed account on the medical imagining system?

Ans. Medical imaging is the technique and process used to create images of the human body for clinical purposes. As a discipline and in its widest sense, it is part of biological imaging and incorporates radiology, nuclear medicine, investigative radiological sciences, endoscopy, thermography, medical photography and microscopy. Measurement and recording techniques which are not primarily designed to produce images, such as electroencephalography (EEG), magnetoencephalography (MEG), Electrocardiography (EKG) and others, but which produce data susceptible to be represented as maps, can be seen as forms of medical imaging. Imaging technology includes:

Electron microscopy :

The electron microscope can magnify very small details with high resolving power due to the use of electrons as the source of illumination, magnifying at levels up to 2,000,000 times. Electron microscopy is employed in anatomic pathology to identify organelles within the cells. Its usefulness has been

greatly reduced by immunhistochemistry but it is still irreplaceable for the diagnosis of kidney disease, identification of immotile cilia syndrome and many other tasks

Radiographic :

Two forms of radiographic images are in use in medical imaging; projection radiography and fluoroscopy.

- *Fluoroscopy* produces real-time images of internal structures of the body in a similar fashion to radiography, but employs a constant input of x-rays, at a lower dose rate. Contrast media, such as barium, iodine, and air are used to visualize internal organs as they work. An image receptor is required to convert the radiation into an image after it has passed through the area of interest. Early on this was a fluorescing screen, which gave way to an Image Amplifier (IA) which was a large vacuum tube that had the receiving end coated with cesium iodide and a mirror at the opposite end. Eventually the mirror was replaced with a TV camera.
- *Projectional radiographs*, more commonly known as x-rays, are often used to determine the type and extent of a fracture as well as for detecting pathological changes in the lungs. With the use of radio-opaque contrast media, such as barium, they can also be used to visualize the structure of the stomach and intestines - this can help diagnose ulcers or certain types of colon cancer.

Magnetic resonance imaging (MRI) :

A magnetic resonance imaging instrument or "nuclear magnetic resonance (NMR) imaging" scanner as it was originally known, uses powerful magnets to polarise and excite hydrogen nuclei (single proton) in water molecules in human tissue, producing a detectable signal which is spatially encoded, resulting in images of the body. MRI uses three electromagnetic fields: a very strong static magnetic field to polarize the hydrogen nuclei, called the static field; a weaker time-varying field(s) for spatial encoding, called the gradient field(s); and a weak radio-frequency (RF) field for manipulation of the hydrogen nuclei to produce measurable signals, collected through an RF antenna.

Like CT, MRI traditionally creates a two dimensional image of a thin "slice" of the body and is therefore considered a tomographic imaging technique. Modern MRI instruments are capable of producing images in the form of 3D blocks, which may be considered a generalisation of the single-slice, tomographic, concept. Unlike CT, MRI does not involve the use of ionizing radiation and is therefore not associated with the same health hazards.

Nuclear medicine :

Nuclear medicine encompasses both diagnostic imaging and treatment of disease and may also be referred to as molecular medicine or molecular imaging & therapeutics. Nuclear medicine uses certain properties of isotopes and the energetic particles emitted from radioactive material to diagnose or treat various pathology. Different from the typical concept of anatomic radiology, nuclear medicine enables assessment of physiology. This function-based approach to medical evaluation has useful applications in most subspecialties, notably oncology, neurology, and cardiology.

- *Gamma cameras* are used in nuclear medicine to detect regions of biologic activity that may be associated with disease. Isotopes are often preferentially absorbed by biologically active tissue in the body, and can be used to identify tumors or fracture points in bone. Images are acquired after collimated photons are detected by a crystal. Gamma cameras can have a variable number of detector heads with two being the most common configuration. 2D planar images can be acquired of the body or multiple time-capture images can be combined into a dynamic sequence cine of a physiologic process over time. A 3D tomographic technique known as SPECT uses gamma camera data from many projections and can be reconstructed in different planes.
- *Positron emission tomography* (PET) uses coincidence detection to image functional processes. PET images can be viewed in comparison to computed tomography scans to determine an anatomic correlate. Modern scanners combine PET with a CT, or even MRI, to optimize the image reconstruction involved with positron imaging. This is performed on the same equipment without physically moving the patient off of the gantry.
- Nuclear medicine therapy includes treatment with unsealed radioactive material in various forms, including free beta radiation emitting isotope, bound to antibody (radioimmunotherapy), and directly administered, as in resin microsphere therapy.

Photoacoustic imaging :

Photoacoustic imaging is a recently developed hybrid biomedical imaging modality based on the photoacoustic effect. It combines the advantages of optical absorption contrast with ultrasonic spatial resolution for deep imaging in (optical) diffusive or quasi-diffusive regime. Recent studies have shown that photoacoustic imaging can be used in vivo for tumor angiogenesis monitoring, blood oxygenation mapping, functional brain imaging, and skin melanoma detection, etc.

Breast Thermography :

Digital Infrared Imaging Thermography is based on the principle that metabolic activity and vascular circulation in both pre-cancerous tissue and the area surrounding a developing breast cancer is almost always higher than in normal breast tissue. Cancerous tumors require an ever-increasing supply of nutrients and therefore increase circulation to their cells by holding open existing blood vessels, opening dormant vessels, and creating new ones (neoangiogenesis). This process frequently results in an increase in regional surface temperatures of the breast.

Tomography :

Tomography is the method of imaging a single plane, or slice, of an object resulting in a tomogram. There are several forms of tomography:

- Linear tomography: This is the most basic form of tomography. The X-ray tube moved from point "A" to point "B" above the patient, while the cassette holder (or "bucky") moves simultaneously under the patient from point "B" to point "A." No longer carried out and replaced by computed tomography.
- Poly tomography: This was a complex form of tomography. With this technique, a number of geometrical movements were programmed, such as hypocycloidic, circular, figure 8, and elliptical. Philips Medical Systems produced one such device called the 'Polytome.' This unit was still in use into the 1990s, as its resulting images for small or difficult physiology, such as the inner ear, was still difficult to image with CTs at that time. As the resolution of CTs got better, this procedure was taken over by the CT.
- Zonography: This is a variant of linear tomography, where a limited arc of movement is used. It is still used in some centres for visualising the kidney during an intravenous urogram (IVU).

- Orthopantomography (OPT or OPG): The only common tomographic examination in use. This makes use of a complex movement to allow the radiographic examination of the mandible, as if it were a flat bone.
- Computed Tomography (CT), or Computed Axial Tomography (CAT): A CT scan, also known as a CAT scan, is a helical tomography (latest generation), which traditionally produces a 2D image of the structures in a thin section of the body. It uses X-rays. It has a greater ionizing radiation dose burden than projection radiography; repeated scans must be limited to avoid health effects.

Ultrasound :

Medical ultrasonography uses high frequency broadband sound waves in the megahertz range that are reflected by tissue to varying degrees to produce images. This is commonly associated with imaging the fetus in pregnant women. Other important uses include imaging the abdominal organs, heart, breast, muscles, tendons, arteries and veins. While it may provide less anatomical detail than techniques such as CT or MRI, it has several advantages which make it ideal in numerous situations, in particular that it studies the function of moving structures in real-time, emits no ionizing radiation, and contains speckle that can be used in elastography. It is very safe to use and does not appear to cause any adverse effects, although information on this is not well documented.

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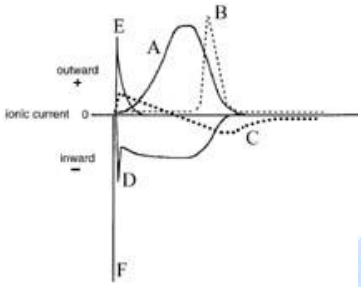
Multiple Choice Questions

1. Blood is transported to capillaries in the myocardium by
   A. Pulmonary arteries
   B. The coronary sinus
   C. The fossa ovalis
   D. Coronary arteries
   E. Coronary Veins

2. Which of the following is NOT an inward current?
   A. I Na

B. I Ca
C. I f
D. I **Na-K (pump)**

3. In the image, which curve represents the IK1?
   A. A
   B. B
   C. C
   D. D
   E. E



4. Which of the following is usually the dominant pacemaker and fires the fastest?
   A. **SA node.**
   B. AV node
   C. His bundle.
   D. Purkinje fibers.

5. What is the skeletal system?
   A. All the bones in the body
   B. All the muscles and tendons
   C. All the body's organs, both soft and hard tissue
   D. All the bones in the body and the tissues that connect them

6. Which of the following statement is INCORRECT?
   A. Bone is where most blood cells are made.
   B. Bone serves as a storehouse for various minerals

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C. Bone is a dry and non-living supporting structure
D. Bone protects and supports the body and its organs

7. Which bone protects the brain?

A. Calcium
B. The cranium
C. The cerebrum
D. The cerebellum

8. **The correct order for the basic features of a mass spectrometer is...**

A. acceleration, deflection, detection, ionization
B. ionisation, acceleration, deflection, detection
C.acceleration, ionisation, deflection, detection
D. acceleration, deflection, ionisation, detection

9. Which of the following is found in the center of each sarcomere?
A. Z Discs
B. I Bands
C. H Band
D. M Bands

10. Which type of glial cell is responsible for glucose uptake from the blood?
A. Ependyma
B. Oligodendrocytes
C. Microglia
D. Astrocytes

11. Which of the following statements is FALSE?
A. Only certain axons are myelinated
B. Oligodendrocytes are found only in the CNS
C. Both oligodendrocytes and schwann cells are involved in forming

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myelin sheaths around axons

D. **Myelin sheaths are predominantly carbohydrate-based**

12. Which of the following statements is FALSE

A. Cardiomyocytes are smaller than skeletal muscle fibres

B. Action potentials last longer in cardiomyocytes than in skeletal muscle

C. Cardiomyocytes usually have only one nucleus, whereas skeletal muscle fibres are multinucleated

E. Cardiomyocytes are not striated

□□□

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Section –B

Antigens ,Cell mediated cytotoxicity & Humoral immune response

Q.1 Write short notes on:

(a) Major histocompatibility complex

Ans. The major histocompatibility complex (MHC) is a large genomic region found in most vertebrates. It is the most gene-dense region of the mammalian genome and plays an important role in the immune system. The proteins encoded by the MHC are expressed on the surface of cells in all jawed vertebrates, and display both *self* antigens (peptide fragments from the cell itself) and *nonself* antigens (e.g., fragments of invading microorganisms) to a type of white blood cell called a T cell that has the capacity to kill or co-ordinate the killing of pathogens and infected cells.

The MHC region is divided into three subgroups, class I, class II, and class III.

Name	Function	Expression
MHC class I	Encodes heterodimeric peptide-binding proteins, as well as antigen-processing molecules such as TAP and Tapasin.	All nucleated cells. MHC class I proteins contain an α chain & β2-micro-globulin(not part of the MHC). They present antigen fragments to cytotoxic T-cells via the CD8 receptor on the cytotoxic T-cells and also bind inhibitory receptors on NK cells.
MHC class II	Encodes heterodimeric peptide-binding proteins and proteins that modulate antigen loading onto MHC class II proteins in the	On most immune system cells, specifically on antigen-presenting cells. MHC class II proteins contain α & β chains and they present antigen fragments to T-helper cells by binding

	lysosomal compartment such as MHC II DM, MHC II DQ, MHC II DR, and MHC II DP.	to the CD4 receptor on the T-helper cells.
MHC class III region	Encodes for other immune components, such as complement components (e.g., C2, C4, factor B) and some that encode cytokines (e.g., TNF-α) and also hsp.	Variable

Class III has a function very different from that of class I and class II, but, since it has a locus between the other two (on chromosome 6 in humans), they are frequently discussed together.

The MHC proteins act as "signposts" that display fragmented pieces of an *antigen* on the host cell's surface. These antigens may be *self* or *nonself*. If they are *nonself*, there are two ways by which the foreign protein can be processed and recognized as being "nonself".

Cells constantly process endogenous proteins and present them within the context of MHC I. Immune effector cells are trained not to react to self peptides within MHC, and as such are able to recognize when foreign peptides are being presented during an infection/cancer.

The classical MHC molecules have a vital role in the complex immunological dialogue that must occur between T cells and other cells of the body. At maturity, MHC molecules are anchored in the cell membrane, where they display short polypeptides to T cells, via the T cell receptors (TCR). The polypeptides may be "self," that is, originating from a protein created by the organism itself, or they may be foreign ("nonself"), originating from bacteria, viruses, pollen, and so on. The overarching design of the MHC-TCR interaction is that T cells should ignore self-peptides while reacting appropriately to the foreign peptides.

The immune system has *another* and equally important method for identifying an antigen: B cells with their membrane-bound antibodies, also known as B cell receptors (BCR). However, whereas the BCRs of B cells can bind to antigens without much outside help, the TCRs require "presentation"

of the antigen through the help of MHC. For most of the time, however, MHC are kept busy presenting self-peptides, which T cells should appropriately ignore. A full-force immune response usually requires the activation of B cells via BCRs *and* T cells via the MHC-TCR interaction. This duplicity creates a system of "checks and balances" and underscores the immune system's potential for running amok and causing harm to the body (see autoimmune disorders).

MHC molecules retrieve polypeptides from the interior of the cell they are part of and display them on the cell's surface for recognition by T cells. However, MHC class I and MHC class II differ significantly in the method of peptide presentation.

Q.1 (b) Lymphokines

Ans. Lymphokines are a subset of cytokines that are produced by a type of immune cell known as a lymphocyte. They are typically produced by T cells to direct the immune system response by signaling between its cells. Lymphokines have many roles, including the attraction of other immune cells, like macrophages and other lymphocytes, to an infected site and their subsequent activation prepare them to attack the invaders. Circulating lymphocytes can detect a very small concentration of lymphokine and then move up the concentration gradient towards where the immune response is required.

Q.1 (c) Haptens

Ans. Haptens are small molecules that can elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself. Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody.

The concept of haptens emerged from the work of Karl Landsteiner who also pioneered the use of synthetic haptens to study immunochemical phenomena.

A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinone, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure only causes sensitization, in which there is a proliferation of effector T-cells. After a second exposure later, the proliferated T cells can become activated, generating an immune reaction, producing the typical blisters of poison ivy exposure.

Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs causes autoimmune hemolytic anemia.

(d) Macrophages

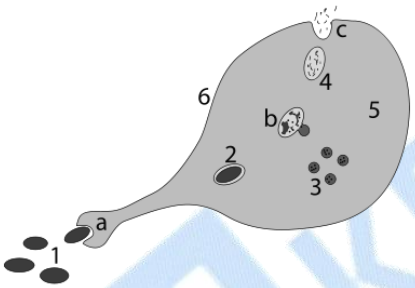
Ans. Macrophages (makros "large" + phagein "eat") are white blood cells within tissues, produced by the division of monocytes. Monocytes and macrophages are phagocytes, acting in both non-specific defense or innate immunity as well as to help initiate specific defense mechanisms of vertebrate animals. Their role is to phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or as mobile cells, and to stimulate lymphocytes and other immune cells to respond to the pathogen. They can be identified by specific expression of a number of proteins. They move by action of Amoeboid movement.

When a monocyte enters damaged tissue through the endothelium of a blood vessel, it undergoes a series of changes to become a macrophage. Monocytes are attracted to a damaged site by chemical substances through chemotaxis, triggered by a range of stimuli including damaged cells, pathogens and cytokines released by macrophages already at the site. Unlike short-lived

neutrophils, macrophages survive longer in the body up to a maximum of several months.

Steps of a macrophage ingesting a pathogen:
a. Ingestion through phagocytosis, a phagosome is formed
b. The fusion of lysosomes with the phagosome creates a phagolysosome; the pathogen is broken down by enzymes

c. Waste material is expelled or assimilated



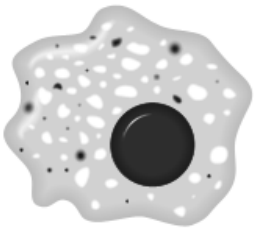
Parts: 1. Pathogens 2. Phagosome 3. Lysosomes 4. Waste material 5. Cytoplasm 6. Cell membrane

One important role of the macrophage is the removal of necrotic cellular debris in the lungs. Removing dead cell material is important in chronic inflammation, as the early stages of inflammation are dominated by neutrophil granulocytes, which are ingested by macrophages if they come of age.

The removal of necrotic tissue is, to a greater extent, handled by fixed macrophages, which will stay at strategic locations such as the lungs, liver, neural tissue, bone, spleen and connective tissue, ingesting foreign materials such as pathogens, recruiting additional macrophages if needed.

When a macrophage ingests a pathogen, the pathogen becomes trapped in a phagosome, which then fuses with a lysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. However, some bacteria, such as Mycobacterium tuberculosis, have become resistant to these methods of digestion. Macrophages can digest more than 100 bacteria before they finally die due to their own digestive compounds.

A majority of macrophages are stationed at strategic points where microbial invasion or accumulation of dust is likely to occur. These are called fixed macrophages.



Macrophage

Each type of macrophage, determined by its location, has a specific name:

Name of cell	**Location**
Dust cells/Alveolar macrophages	pulmonary alveolus of lungs
Histiocytes	connective tissue
Kupffer cells	liver
Microglia	neural tissue
Epithelioid cells	granulomas
Osteoclasts	bone
Sinusoidal lining cells	spleen
Mesangial cells	kidney

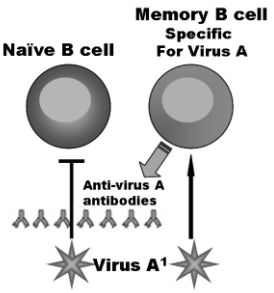
Investigations concerning Kupffer cells are hampered because in humans Kupffer cells are only accessible for immunohistochemical analysis from biopsies or autopsies.

Q.1 (e) Memory cells

Ans. Memory cell may refer to:

- Memory B cell, an antibody producing cell
- Memory T cell, an infection fighting cell

Memory B cells are a B cell sub-type that are formed following primary infection.



In wake of first infection involving a particular antigen, the responding naïve (ones which have never been exposed to the antigen) cells proliferate to produce a colony of cells, most of which differentiate into the plasma cells, also called effector B cells and clear away with the resolution of infection, and the rest persist as the memory cells that can survive for years, or even a lifetime.

Antibody molecules present on a clone of B cells have a unique paratope (the sequence of amino acids that binds to the epitope on an antigen).

And, each time these cells are induced to proliferate due to an infection, the genetic region coding for the paratope undergoes spontaneous mutations with a frequency of about 1 in every 1600 cell-divisions.

All these events occur in the highly "eventful" germinal centers of lymphoid follicles, within the lymph nodes. Some of the resulting paratopes have a better affinity for the antigen (actually, the epitope) and are more likely to proliferate than the others.

Memory T cells are a specific type of infection-fighting T cell that can recognize foreign invaders such as bacteria or viruses, that were encountered during a prior infection or vaccination. At a second encounter with the invader, memory T cells can reproduce to mount a faster and stronger immune response than the first time the immune system responded to the invader. This behaviour is utilized in T lymphocyte proliferation assays, which can reveal exposure to specific antigens.

Q.2 (f) T-lymphocytes

Ans. 'T cells' belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. They can be distinguished from

other lymphocyte types, such as B cells and natural killer cells by the presence of a special receptor on their cell surface called *T cell receptors* (TCR). The abbreviation *T*, in *T cell*, stands for thymus, since this is the principal organ responsible for the T cell's maturation. The types are as follow:

Helper :

T cells which express the CD4 protein on their surface are called T helper cells because they assist other leukocytes (e.g. macrophages, B cells, cytotoxic T cells) in immunological processes. There is also a sub-population of CD4 cells which suppress the activity of B cells via the production of gamma interferon and equally a sub-population of CD4 cells may suppress cell mediated immune responses via the production IL-4 and IL-10. Helper T cells are presented peptide antigens associated with MHC class II on the surface of Antigen Presenting Cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the immune response.

Cytotoxic :

Cytotoxic T cells (T_C cells) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as $CD8^+$ T cells (associated with MHC class I), since they express the CD8 glycoprotein at their surface. IL-10, adenosine and other molecules secreted by T regulatory cells.

Memory :

Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T cells comprise two subtypes: central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells). Memory cells may be either CD4+ or CD8+.

Regulatory :

Regulatory T cells (T_{reg} cells), formerly known as **suppressor T cells**, are crucial for the maintenance of immunological tolerance. Their major role is to

shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus.

Natural killer :

Natural killer T cells (NKT cells) are a special kind of lymphocyte that bridges the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigen presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. Once activated, these cells can perform functions ascribed to both T_h and T_c cells (i.e., cytokine production and release of cytolytic/cell killing molecules). They are also able to recognize and eliminate some tumor cells and cells infected with herpes viruses.

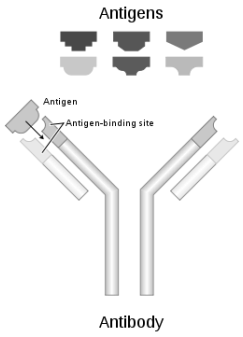
Gamma Delta T cells (γδ) :

γδ T cells (gamma delta T cells) represent a small subset of T cells that possess a distinct T cell receptor (TCR) on their surface. A majority of T cells have a TCR composed of two glycoprotein chains called α- and β- TCR chains. However, in γδ T cells, the TCR is made up of one γ-chain and one δ-chain. This group of T cells is much less common (5% of total T cells) than the αβ T cells. The antigenic molecules that activate γδ T cells are still widely unknown. However, γδ T cells are not MHC restricted and seem to be able to recognise whole proteins rather than requiring peptides to be presented by MHC molecules on antigen presenting cells. Human Vγ9/Vδ2 T cells, which constitute the major γδ T cell population in peripheral blood, are unique in that they specifically and rapidly respond to a small non-peptidic microbial metabolite, an isopentenyl pyrophosphate precursor.

Q.3 Describe the structure and functions of antibodies?

Ans. Antibodies (also known as immunoglobulins, abbreviated Ig) are gamma globulin proteins that are found in blood or other bodily fluids of vertebrates and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. They are typically made of basic structural units—each with two large heavy chains and two small light chains—to form, for example, monomers with one unit, dimers with two units or pentamers with five units. Antibodies are produced by a kind of white blood cell called a

plasma cell. Five different antibody isotypes are known in mammals, which perform different roles, and help direct the appropriate immune response for each different type of foreign object they encounter.



Although the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures or antigen binding sites, to exist. This region is known as the hypervariable region. Each of these variants can bind to a different target, known as an antigen. The unique part of the antigen recognized by an antibody is called an epitope. These epitopes bind with their antibody in a highly specific interaction, called induced fit, that allows antibodies to identify and bind only their unique antigen. Recognition of an antigen by an antibody *tags* it for attack by other parts of the immune system.

The large and diverse population of antibodies is generated by random combinations of a set of gene segments that encode different antigen binding sites (or *paratopes*), followed by random mutations in this area of the antibody gene, which create further diversity. Production of antibodies is the main function of the humoral immune system.

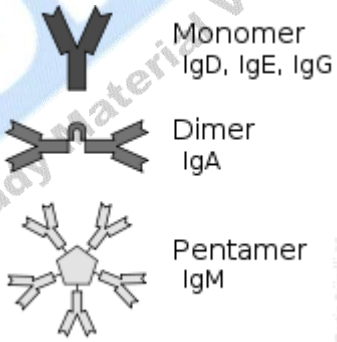
Forms:

Surface immunoglobulin (Ig) is attached to the membrane of the effector B cells by its transmembrane region, while antibodies are the secreted form of Ig and lack the trans membrane region so that antibodies can be secreted into the bloodstream and body cavities. As a result, surface Ig and antibodies are identical except for the transmembrane regions.

The membrane-bound form of an antibody may be called a *surface immunoglobulin* (sIg) or a *membrane immunoglobulin* (mIg). It is part of the *B cell receptor* (BCR), which allows a B cell to detect when a specific antigen is present in the body and triggers B cell activation.

Isotypes:

Name	Description
IgA	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.
IgD	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.
IgE	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms.
IgG	In its four forms, provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to fetus.
IgM	Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG.



Antibodies can come in different varieties known as isotypes or classes. In placental mammals there are five antibody isotypes known as IgA, IgD, IgE, IgG and IgM. They are each named with an "Ig" prefix that stands for immunoglobulin, another name for antibody, and differ in their biological properties, functional locations and ability to deal with different antigens.

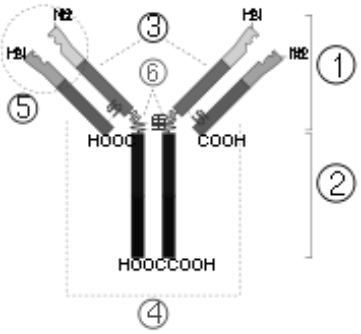
The antibody isotype of a B cell changes during cell development and activation. Immature B cells, which have never been exposed to an antigen,

are known as naïve B cells and express only the IgM isotype in a cell surface bound form. B cells begin to express both IgM and IgD when they reach maturity—the co-expression of both these immunoglobulin isotypes renders the B cell 'mature' and ready to respond to antigen.[14] B cell activation follows engagement of the cell bound antibody molecule with an antigen, causing the cell to divide and differentiate into an antibody producing cell called a plasma cell. In this activated form, the B cell starts to produce antibody in a secreted form rather than a membrane-bound form.

Antibodies are heavy (~150kDa) globular plasma proteins. They have sugar chains added to some of their amino acid residues. In other words, antibodies are *glycoproteins*. The basic functional unit of each antibody is an immunoglobulin (Ig) monomer (containing only one Ig unit); secreted antibodies can also be dimeric with two Ig units as with IgA, tetrameric with four Ig units like immunoglobulin domains make up the two heavy chains (red and blue) and the two light chains (green and yellow) of an antibody.

The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical *heavy chains* and two identical *light chains* connected by disulfide bonds. Each chain is composed of structural domains called Ig domains. These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function. They have a characteristic immunoglobulin fold in which two beta sheets create a “sandwich” shape, held together by interactions between conserved cysteines and other charged amino acids.

There are five types of mammalian Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ. The type of heavy chain present defines the *class* of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and composition; α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids.



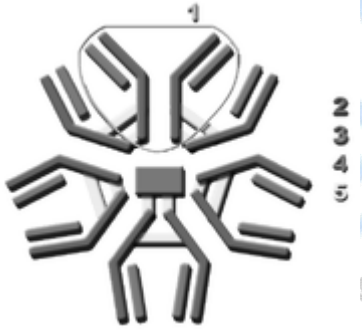
1. Fab region
2. Fc region
3. Heavy chain with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4. Light chain with one variable (V_L) and one constant (C_L) domain
5. Antigen binding site (paratope)
6. Hinge regions.

Each heavy chain has two regions, the *constant region* and the *variable region*. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of *three* tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of *four* immunoglobulin domains. The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

In mammals there are two types of Immunoglobulin light chain, which are called lambda (λ) and kappa (κ). A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217 amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals. Other types of light chains, such as the iota (ι) chain, are found in lower vertebrates like Chondrichthyes and Teleostei.

Activated B cells differentiate into either antibody-producing cells called plasma cells that secrete soluble antibody or memory cells that survive in the body for years afterward in order to allow the immune system to remember an antigen and respond faster upon future exposures.

Since antibodies exist freely in the bloodstream, they are said to be part of the humoral immune system. Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen (an example is a virus capsid protein fragment). Antibodies contribute to immunity in three ways: they prevent pathogens from entering or damaging cells by binding to them; they stimulate removal of pathogens by macrophages and other cells by coating the pathogen; and they trigger destruction of pathogens by stimulating other immune responses such as the complement pathway.



The secreted mammalian IgM has five Ig units. Each Ig unit (labeled 1) has two epitope binding Fab regions, so IgM is capable of binding up to 10 epitopes.

Q.4 Differentiate CMI and HMI. Describe in detail about antibody mediated immunity?

Ans. Humoral system defends against bacteria and viruses present in body fluids

- Fluids: blood, lymph, and interstitial fluid
- Antibodies, *secreted by B cells* and dissolved in the blood, are carried in lymph and blood to sites of infections

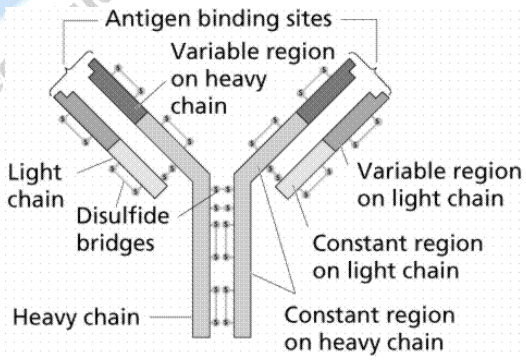
- Cell-mediated immunity
  - T-cells circulate in blood and lymph
  - *Attack body cells* that have been infected

The antibody mediated immunity is the aspect of immunity that is mediated by secreted antibodies produced in the cells of the B lymphocyte lineage. It is also known as humoral immunity. B Cells transform into plasma cells which secrete antibodies. Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which flags them for destruction. Humoral immunity is so named because it involves substances found in the humours, or body fluids.

The study of the molecular and cellular components that comprise the immune system, including their function and interaction, is the central science of immunology. The immune system is divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, each of which contains humoral and cellular components.

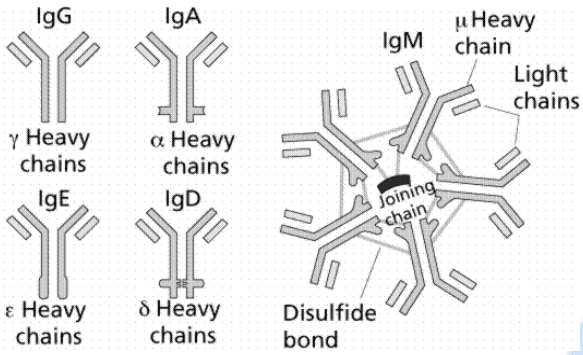
Humoral immunity refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibody, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

Immunoglobulins are glycoproteins in the immunoglobulin superfamily that function as antibodies. The terms *antibody* and *immunoglobulin* are often used interchangeably. They are found in the blood and tissue fluids, as well as many secretions. In structure, they are large Y-shaped globular proteins.

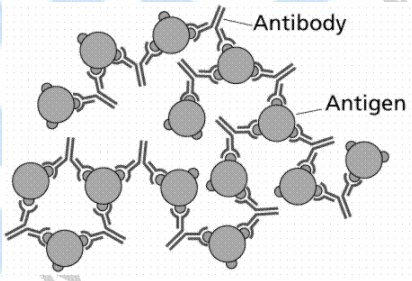


In mammals there are five types of antibody: IgA, IgD, IgE, IgG, and IgM. Each immunoglobulin class differs in its biological properties and has evolved to deal with different antigens. Antibodies are synthesized and

secreted by plasma cells that are derived from the B cells of the immune system.



An antibody is used by the acquired immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target. By binding their specific antigens, antibodies can cause agglutination and precipitation of antibody-antigen products, prime for phagocytosis by macrophages and other cells, block viral receptors, and stimulate other immune responses, such as the complement pathway.



Naive B cells can be activated in a T-cell dependent or independent manner, but two signals are always required to initiate activation.

B cell activation depends on one of three mechanisms: *Type 1 T cell-independent* (polyclonal) activation, *Type 2 T cell-independent* activation (in which macrophages present several of the same antigen in a way that causes cross-linking of antibodies on the surface of B cells), and, T cell-dependent activation. During T cell-dependent activation, an antigen presenting cell (APC) presents a processed antigen to a helper T (T_h) cell, priming it. When a B cell processes and presents the *same* antigen to the *primed T_h cell*, the T cell releases cytokines that activate the B cell.

Multiple Choice Questions

1. Nonspecific host defences that exist prior to exposure to an antigen is called
   A. Acquired immunity
   B. Innate immunity
   C. Adaptive immunity
   D. All of these

2. Innate immunity is also called
   A. Familial
   B. Genetic
   C. Inborn
   D. All the these

3. The internal second line of defense involves all except
   A. Natural killer cells
   B. Complement System
   C. Interferons
   D. Antibodies

4. A Fab fragment:
   A. Is produced by pepsin treatment.
   B. Is produced by separation of heavy and light chains.
   C. Binds antigen.
   D. Lacks light chains.
   E. Has no interchain disulfide bonds

5. The complementarity determining regions:

   A. Are restricted to light chains.

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B. Are in the constant part of the Ig molecule
C. Bind to Fc receptors.
D. Are concerned in antigen recognition.
E. Occur at the C-terminal end of the Ig peptide chains.

6. When antigen reaches a lymph node in a primed animal:

A. There is an increase in the output of cells in the efferent lymphatics over the following 24 h.
B. There is a decrease in the output of cells in the efferent lymphatics over the following 24 h.
C. There is an immediate output of activated blast cells.
D. It is transported to the spleen.
E. It is all immediately destroyed by macrophages.

7. **TH lymphocytes activate macrophages, Tc cells, and B cells, but what activates TH cells?**
A. **macrophages and/or dendritic cells**
B. TH cells (i.e., self-activation)
C. IL-1 and IL-2
D. All of the above
8. **Professional antigen presenting cells have -- , whereas most other cells have --- .**
A.MHC I; MHC I and II
B.**MHC II; MHC I**
C.MHC I and II; MHC II
D.MHC I; MHC II

9. **The --- portion of an antibody molecule determines the antigen binding specificity, whereas the --- portion determines the class to which it belongs**.
A. heavy hypervariable, light constant
B. IgM, IgG
C. **light and heavy variable and hypervariable, heavy constant**
D. light hypervariable, heavy variable

10. Body's own cells are protected from membrane attack complex by a surface glycoprotein called

A. MHC
B. **DAF**
C. TCR
D. BCR

11. Classical pathway of complement system is involved in
A. Specific Defense
B. Adaptive immunity
C. **both a and b**
D. non-specific defense

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Section –C

Hypersensitivity, Monoclonal antibodies and its applications

Q.1 Write short notes on:

Q.1 (a) ELISA

Ans. ELISA (Enzyme-linked immunosorbent assay) is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal. Thus in the case of fluorescence ELISA, when light of the appropriate wavelength is shone upon the sample, any antigen/antibody complexes will fluoresce so that the amount of antigen in the sample can be inferred through the magnitude of the fluorescence.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bioconjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations. It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

The ELISA, or the enzyme immunoassay (EIA), was the first screening test widely used for HIV. It has a high sensitivity. The types are Indirect ELISA, Sandwich ELISA, Competitive ELISA and Reverse ELISA.

Q.1 (b) Floweytometry

Ans. Flow cytometry is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both research and clinical practice.

Modern flow cytometers are able to analyze several thousand particles every second, in "real time," and can actively separate and isolate particles having specified properties. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has 5 main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- an optical system - commonly used are lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488nm), red-HeNe (633nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals

- a detector and Analogue-to-Digital Conversion (ADC) system - which generates FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals.

The process of collecting data from samples using the flow cytometer is termed 'Acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (i.e. voltage, compensation, etc) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to insure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled wide-spread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently-labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors (the current record for a commercial instrument is 4 lasers and 18 fluorescence detectors). Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cells.

The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. In the field of molecular biology it is especially useful when used with fluorescence tagged antibodies. These specific antibodies (which number tens of thousands against several thousand genes) bind to antigens on the target cells and help to give information on specific characteristics of the cells being studied in the cytometer. It has broad application in medicine (especially in transplantation, hematology, tumor immunology and chemotherapy, genetics and sperm sorting for sex preselection). In marine biology, the auto-fluorescent properties of photosynthetic plankton can be exploited by flow cytometry in order to characterise abundance and community structure. In protein

engineering, flow cytometry is used in conjunction with yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties.

Q.1 (c) Herd immunity

Ans. Herd immunity (or community immunity) describes a type of immunity that occurs when the vaccination of a portion of the population (or herd) provides protection to unprotected individuals. Herd immunity theory proposes that, in diseases passed from person-to-person, it is more difficult to maintain a chain of infection when large numbers of a population are immune. The more immune individuals present in a population, the lower the likelihood that a susceptible person will come into contact with an infected individual.

The effectiveness of a vaccine depends on the immune system reaction which the patient develops. This involves the generation of long term memory B cells and T cells via adaptive immunity following innate immune responses. Sometimes the antigen contained in the vaccine doesn't trigger an immune response. In the latter case there is need for new and stronger vaccines.

Vaccination acts as a sort of "firebreak" in the spread of the disease, slowing or preventing further transmission of the disease to others. For example, if Person A had a disease and exposed Person B who was immune because of vaccination, Person B would not get ill and could not pass on the disease to Person C when he comes into contact with him. So even if Person C is not vaccinated, he indirectly gets protection from the disease. Hence herd immunity may be used to reduce spread of an illness and to protect a vulnerable, un-vaccinated subgroup. However because only a small fraction of the population (or herd) can be left un-vaccinated for this method to be effective, it is considered best left for those who cannot safely receive vaccines due a medical condition such as an immune disorder or for organ transplant recipients.

Estimated Herd Immunity thresholds for vaccine preventable diseases			
Disease	**Transmission**	**R_0**	**Herd immunity threshold**
Diphtheria	Saliva	6-7	85%
Measles	Airborne	12-18	83 - 94%
Mumps	Airborne droplet	4-7	75 - 86%
Pertussis	Airborne droplet	12-17	92 - 94%
Polio	Fecal-oral route	5-7	80 - 86%
Rubella	Airborne droplet	5-7	80 - 85%
Smallpox	Social contact	6-7	83 - 85%
^ - R0 is the basic reproduction number, or the average number of secondary infectious cases that are produced by a single index case in completely susceptible population.			

Although no vaccine offers 100% protection, the spread of disease from person to person is much higher in those who remain un-vaccinated. Virologists have found that when a certain percentage of a population is vaccinated, the spread of the disease is effectively stopped. This critical percentage, called the *herd immunity threshold*, depends on the disease, the vaccine, and the contact parameter for the population. It is the general aim of those involved in public health to establish herd immunity in most populations. However complications arise when wide spread vaccination is not possible, and when vaccines fail (the MMR vaccine controversy in the UK.) Herd immunity is only relevant for diseases that are contagious. It does not apply to diseases that are not contagious and caused only by environmental factors, such as tetanus. For instance, even if only one member of a population was not immune to tetanus, that person could get it through direct contact with the pathogen in the environment.

Herd immunity should not be confused with contact immunity, a related concept wherein a vaccinated individual can 'pass-on' the vaccine to another individual through contact.

Q.1 (d) Hypersensitivity

Ans. Hypersensitivity or hypersensitivity reaction refers to undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. The four-group classification was expounded by P. H. G. Gell and Robin Coombs in 1963. Coombs and Gell classification is as follow:

Comparison of hypersensitivity types

Type	Alternative names	Often mentioned disorders	Mediators
I	Allergy (immediate)	• Atopy • Anaphylaxis • Asthma	• IgE
II	Cytotoxic, antibody-dependent	• Autoimmune hemolytic anemia • Thrombocytopenia • Erythroblastosis fetalis • Goodpasture's syndrome	• IgM or IgG • (Complement)
III	Immune complex disease	• Serum sickness • Arthus reaction • Systemic lupus erythematosus (SLE)	• IgG • (Complement)
IV	Delayed-type hypersensitivity[3] (DTH), cell-mediated immune memory response, antibody-independent	• Contact dermatitis • Mantoux test • Chronic transplant rejection • Multiple sclerosis	• T-cells
V	Autoimmune disease	• Grave's disease • Myasthenia Gravis • Hashimoto's thyroiditis • Systemic lupus erythematosus	• IgM or IgG • (Complement)

Instead of binding to cell surface components, the antibodies recognize and bind to the cell surface receptors, which either prevents the intended ligand binding with the receptor or mimics the effects of the ligand, thus impairing cell signaling. Some clinical examples are Graves' disease, Myasthenia gravis .

Q.1 (e) Immunofluorescence

Ans. Immunofluorescence is the labeling of antibodies or antigens with fluorescent dyes. This technique is often used to visualize the subcellular distribution of biomolecules of interest. Immunofluorescent-labeled tissue sections or cultures are studied using a fluorescence microscope or by confocal microscopy.

Most commonly, immunofluorescence employs two sets of antibodies: a primary antibody is used against the antigen of interest; a subsequent, secondary ("indirect"), dye-coupled antibody is introduced that recognizes the primary antibody. Typically this is done by using antibodies made in different species. For example, a researcher might create antibodies in a goat that recognize several antigens, and then employ dye-coupled rabbit antibodies that recognize the goat antibody constant region (denoted rabbit anti-goat). This allows re-use of the difficult-to-make dye-coupled antibodies in multiple experiments.

In some cases, it is advantageous to use primary antibodies directly labelled with a fluorophore. This direct labelling decreases the number of steps in the staining procedure and, more importantly, often avoids cross-reactivity and high background problems. As with most fluorescence techniques, a significant problem with immunofluorescence is photobleaching. Loss of activity caused by photobleaching can be controlled by reducing the intensity or time-span of light exposure, by increasing the concentration of fluorophores, or by employing more robust fluorophores that are less prone to bleaching.

Many uses of immunofluorescence have been outmoded by the development of recombinant proteins containing fluorescent protein domains, e.g. green

fluorescent protein (GFP). Use of such "tagged" proteins allows much better localization and less disruption of protein function.

Q.1 (f) Immunoblotting

Ans. Immunoblot or western blot is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose), where they are probed (detected) using antibodies specific to the target protein.

There are now many reagent companies that specialize in providing antibodies (both monoclonal and polyclonal antibodies) against many thousands of different proteins. Commercial antibodies can be expensive, although the unbound antibody can be reused between experiments. This method is used in the fields of molecular biology, biochemistry, immunogenetics and other molecular biology disciplines. Other related techniques include using antibodies to detect proteins in tissues and cells by immunostaining and enzyme-linked immunosorbent assay (ELISA).

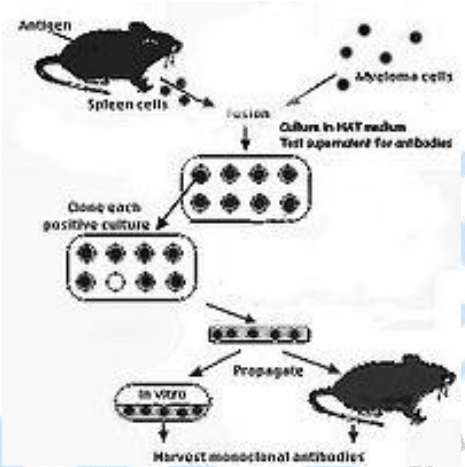
The name western blot was given to the technique by W. Neal Burnette and is a play on the name Southern blot, a technique for DNA detection developed earlier by Edwin Southern. Detection of RNA is termed northern blotting.

Medical diagnostic applications of the technique are:

- The confirmatory HIV test employs a Western blot to detect anti-HIV antibody in a human serum sample. Proteins from known HIV-infected cells are separated and blotted on a membrane as above. Then, the serum to be tested is applied in the primary antibody incubation step; free antibody is washed away, and a secondary anti-human antibody linked to an enzyme signal is added. The stained bands then indicate the proteins to which the patient's serum contains antibody.
- A Western blot is also used as the definitive test for Bovine spongiform encephalopathy (BSE, commonly referred to as 'mad cow disease').
- Some forms of Lyme disease testing employ Western blotting.

Q.2 What are monoclonal antibodies? Give a brief account of different applications of monoclonal antibodies.

Ans. Monoclonal antibodies (mAb or moAb) are monospecific antibodies that are identical because they are produced by one type of immune cell that are all clones of a single parent cell. Given almost any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine.



Production

Researchers looking at slides of cultures of cells that make monoclonal antibodies. These are grown in a lab and the researchers are analyzing the products to select the most promising of them. Monoclonal antibodies can be grown in unlimited quantities in the bottles shown in this picture. Technician hand-filling wells with a liquid for a research test. This test involves preparation of cultures in which hybrids are grown in large quantities to produce desired antibody. This is effected by fusing myeloma cell and mouse lymphocyte to form a hybrid cell (hybridoma).

Hybridoma cell production

Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen. However, recent advances have allowed the use of rabbit B-cells. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low so a selective medium

is used in which only fused cells can grow. This is because myeloma cells have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the salvage synthesis of nucleic acids. This enzyme enables cells to synthesize purines by the salvage pathway, here using an extracellular source of hypoxanthine as a precursor. Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an already existing biochemical pathway.

The selective culture medium is called HAT medium because it contains Hypoxanthine, Aminopterin, and Thymidine. This medium is selective for fused (hybridoma) cells. Unfused myeloma cells cannot grow because they lack HGPRT and thus cannot replicate their DNA. Unfused spleen cells cannot grow indefinitely because of their limited life span. Only fused hybrid cells, referred to as hybridomas, are able to grow indefinitely in the media because the spleen cell partner supplies HGPRT and the myeloma partner has traits that make it immortal (as it is a cancer cell).

This mixture of cells is then diluted and clones are grown from single parent cells on microtitre wells. The antibodies secreted by the different clones are then assayed for their ability to bind to the antigen or immuno-dot blot. The most productive and stable clone is then selected for future use.

The hybridomas can be grown indefinitely in a suitable cell culture media, or they can be injected in mice, they produce tumors containing an antibody-rich fluid called ascites fluid. The medium must be enriched during selection to further favour hybridoma growth. This can be achieved by the use of a layer of feeder fibrocyte cells or supplement medium such as briclone. Production in cell culture is usually preferred as the ascites technique is painful to the animal and if replacement techniques exist, this method is considered unethical.

Monoclonal antibodies

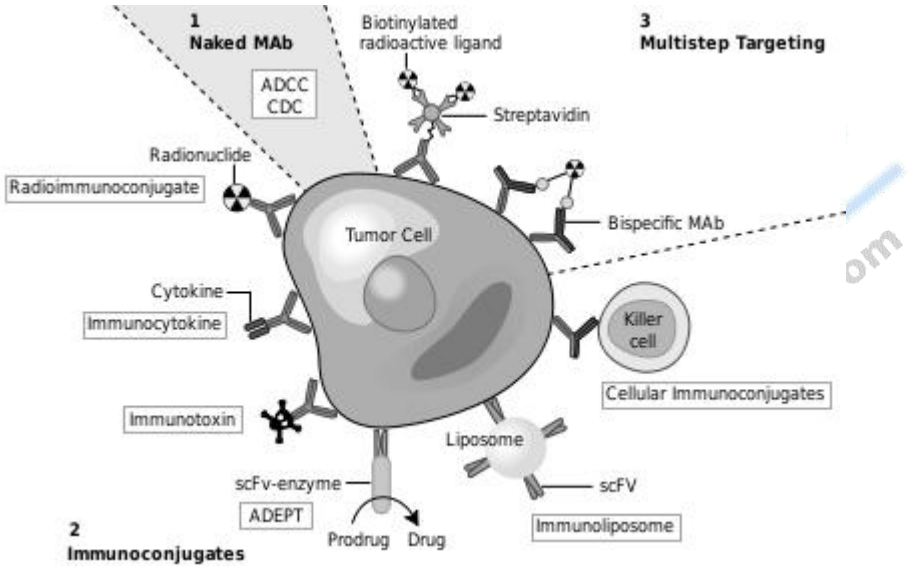
Type	Application	Mechanism	Mode
infliximab	• rheumatoid arthritis • Crohn's disease	inhibits TNF-α	chimeric
basiliximab	• Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	chimeric
bevacizumab	• Anti-angiogenic cancer therapy	inhibits VEGF	humanized
abciximab	• Prevent coagulation in coronary angioplasty	inhibits the receptor GpIIb/IIIa on platelets	chimeric
daclizumab	• Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	humanized
gemtuzumab	• relapsed acute myeloid leukaemia	targets an antigen on leukemia cells	humanized
alemtuzumab	• B cell leukemia	targets an antigen CD52 on T- and B-lymphocytes	humanized
rituximab	• non-Hodgkin's lymphoma	targets phosphoprotein CD20 on B lymphocytes	chimeric
palivizumab	• RSV infections in children	inhibits an RSV protein	humanized
trastuzumab	• anti-cancer therapy for a specific kind of breast cancer	targets the HER2/neu (erbB2) receptor	humanized
etanercept	• rheumatoid arthritis	contains TNF receptor	fusion protein
adalimumab	• rheumatoid arthritis • Crohn's disease	inhibits TNF-α	human
Nimotuzumab	• Approved in SCCHN, Glioma • Clinical trials for other indications underway	EGFR inhibitor	Humanized

Applications:

(i) Diagnostic tests: The Western blot test and immuno dot blot tests detect the protein on a membrane. They are also very useful in immunohistochemistry which

detect antigen in fixed tissue sections and immunofluorescence test which detect the substance in a frozen tissue section or in live cells.

(ii) Monoclonal antibodies for cancer treatment: That bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell. Such mAb could also be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate; it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell. In fact, every intact antibody can bind to cell receptors or other proteins with its Fc region.



(iii) Chimeric and humanized antibodies: Murine antibodies are very similar to human ones there are differences. The human immune system hence recognizes mouse antibodies as foreign, rapidly removing them from circulation and causing systemic inflammatory effects. Such responses are recognised as producing HACA (Human Anti-Chimeric) antibody antibodies or HAMA (Human Anti-Mouse) antibodies.

(iv) Others: Monoclonal antibodies have been generated and approved to treat: cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis, and viral infection.

Multiple Choice Questions

1. Regarding anaphylactic (type I) and immune complex (type III) hypersensitivities, which of the following is the MOST accurate?
   A. IgE is involved in both anaphylactic and immune complex hypersensitivibes
   B. Complement is involved in both anaphylactic and immune complex hypersensitivities
   C**. Less antigen is typically needed to trigger and anaphylactic reaction than an immune complex reaction**
   D. Neutrophils play more important role in anaphylactic reactions than in immune complex reactions

2. The principle difference between type II and type III hypersensitivity is:
   A. The class (isotype of antibody)
   B**. Whether the antibody reacts with the antigen on the cell or reacts with antigen before it interacts with the cell**
   C. The participation of complement
   D. The participation of T cells

3. A child stung by a bee experiences respiratory distress within minutes and lapses into unconsciousness. This reaction is probably mediated by:
   A**. IgE antibody**
   B**.** IgG antibody
   C. Sensitized T cells
   D. Complement

4. A patient with severe asthma gets no relief from antihistamines. The symptoms are MOST likely to be caused by:
   A**.** IL-2
   B**. Leukotrienes**

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C. Seotonin
D. Bradykinin

5. A patient with severe asthma gets no relief from antihistamines. The symptoms are MOST likely to be caused by:
A**.** Mediated by IgE antibody

B**.** Mediated by IgG and IgM antibody

C. **Intiated by haptens**
D. Initiated by Th2 cells

6. Majority of antigens are
A**. proteins**

B**.** carbohydrates

C. nucleic acid
D. lipids

7. The bonds involved in antigen antibody interactions are
A**. weak hydrogen bonds and vanderwalls forces**

B**.** strong covalent bonds

C. strong di-sulphide bonds
D. all of these

8. The direct ELISA test requires
A**. known antibody**

B**.** known antigen

C. complement
D. patient antibody

9. In the indirect ELISA test the enzyme-linked antibody will attach to
A**.** the patient antigen

B. the variable region of the patient antibody

C. **the constant region of the patient antibody**

D. the wall of the microtiter well

E. known antibody

10. In the indirect ELISA test the development of color means the patient has the antibody being tested for

A. **True**

B. False

11. The term best associated with the cause of disease is:

A. pathogen

B. pathogenesis

C. **etiology**

D. parasitism

E. infection

12. In the human intestinal tract, *E. coli* produces vitamins beneficial to the host and can inhibit pathogen growth. In turn, the bacterium is supplied with nutrients and an environment for growth. This symbiotic relationship between *E. coli* and its host is an example of:

A. commensalism

B. antagonism

C. **mutualism**

D. parasitism

E. opportunism

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Section –D

Bacterial Diseases & General Account of viral & protozoan diseases

Q.1 Write short notes on:

(a) Mycosis

Ans. Mycosis (pl: *mycoses*) is a condition in which fungi pass the resistance barriers of the human or animal body and establish infections.

Mycoses are classified according to the tissue levels initially colonized:

(i) Superficial mycoses: Superficial mycoses - limited to the outermost layers of the skin and hair. An example of a fungal infection is Tinea versicolor. Tinea versicolor is a fungus infection that commonly affects the skin of young people, especially the chest, back and upper arms and legs. Tinea versicolor is caused by a fungus that lives in the skin of almost all adults. It doesn't usually affect the face. This fungus produces spots that are either lighter than the skin or a reddish-brown. This fungus exists in two forms, one of them causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities. However, almost all people with this very common condition are healthy. The causative agent is lipophilic yeast like fungus *Pityrossporum orbiculare* (Malassezia furfur).

(ii) Cutaneous mycoses: It extend deeper into the epidermis, as well as invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be evoked, resulting in pathologic changes expressed in the deeper layers of the skin. The organisms that cause these diseases are called dermatophytes. The resulting diseases are often called ringworm (even though there is no worm involved) or tinea. Cutaneous mycoses are caused by *Microsporum*, *Trichophyton*, and *Epidermophyton* fungi, which together comprise 41 species.

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(iii) Subcutaneous mycoses: It involve the dermis, subcutaneous tissues, muscle, and fascia. These infections are chronic and can be initiated by piercing trauma to the skin, which allows the fungi to enter. These infections are difficult to treat and may require surgical interventions such as debridement.

(iv) Systemic mycoses due to primary pathogens: Originate primarily in the lungs and may spread to many organ systems. Organisms that cause systemic mycoses are inherently virulent. Generally, primary pathogens that cause systemic mycoses are dimorphic.

(v) Systemic mycoses due to opportunistic pathogens: Infections of patients with immune deficiencies who would otherwise not be infected. Examples of immunocompromised conditions include AIDS, alteration of normal flora by antibiotics, immunosuppressive therapy, and metastatic cancer. Examples of opportunistic mycoses include Candidiasis, Cryptococcosis and Aspergillosis.

Treatment: Antifungal drugs are used to treat mycoses. Depending on the nature of the infection, a topical or systemic agent may be used. Photochemotherapy or photopheresis is a technique used at medical centers for the treatment of mycosis fungoides.

Prevention: Keeping the skin clean and dry, as well as maintaining good hygiene, will help larger topical mycoses. Because fungal infections are contagious, it is important to wash after touching other people or animals. Sports clothing should also be washed after use. Wearing flip-flops if using a community swimming pool or shower will also help prevent topical infections.

Q.1 (b) Hepatitis B

Ans. Hepatitis B is a disease caused by hepatitis B virus (HBV) which infects the liver and causes an inflammation called hepatitis. Originally known as "serum hepatitis", the disease has caused epidemics in parts of Asia and Africa, and it is endemic in China. About a third of the world's population, more than 2 billion people, have been infected with the hepatitis B virus. This includes 350 million chronic carriers of the virus. Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood.

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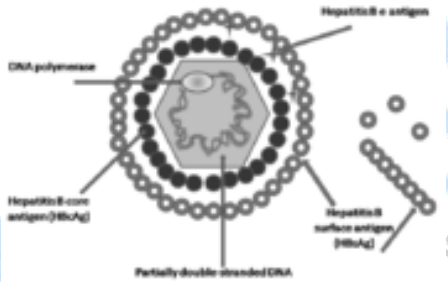
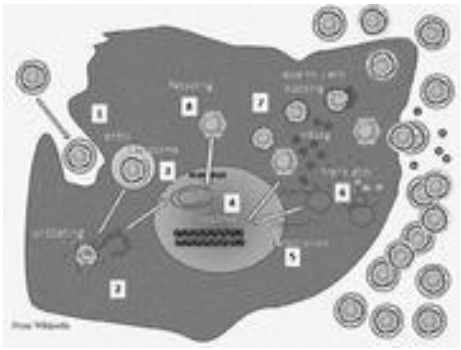
The acute illness causes liver inflammation, vomiting, jaundice and rarely death. Chronic hepatitis B may eventually cause liver cirrhosis and liver cancer, a fatal disease with very poor response to current chemotherapy. The infection is preventable by vaccination.

Hepatitis B virus is an hepadnavirus—*hepa* from *hepatotrophic* and *dna* because it is a DNA virus and it has a circular genome composed of partially double-stranded DNA. The viruses replicate through an RNA intermediate form by reverse transcription and in this respect they are similar to retroviruses. Although replication takes place in the liver, the virus spreads to the blood where virus-specific proteins and their corresponding antibodies are found in infected people. Blood tests for these proteins and antibodies are used to diagnose the infection.



A simplified drawing of the HBV particle and surface antigen.

The virus particle, (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The outer envelope contains embedded proteins which are involved in viral binding and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus.



Hepatitis B virus replication.

Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process. The virus gains entry into the cell by binding to an unknown receptor on the surface of the cell and enters it by endocytosis. Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity.

The hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. By killing infected cells and by producing antiviral cytokines capable of purging HBV from viable hepatocytes, CTLs eliminate the virus. Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen

CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver.

Several vaccines have been developed for the prevention of hepatitis B virus infection. These rely on the use of one of the viral envelope proteins (hepatitis B surface antigen or HBsAg). The vaccine was originally prepared from plasma obtained from patients who had long-standing hepatitis B virus infection. However, currently, these are more often made using recombinant DNA technology, though plasma-derived vaccines continue to be used; the two types of vaccines are equally effective and safe.

Unlike Hepatitis A, Hepatitis B does not generally spread through water and food. Instead, it is transmitted through body fluids, from which prevention is taken to avoid: unprotected sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission during child birth. Infants may be vaccinated at birth.

Acute infection with hepatitis B virus is associated with acute viral hepatitis - an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types.

Q.1 (c) Sexually transmitted diseases

Ans. A sexually transmitted disease (STD) is an illness that has a significant probability of transmission between humans or animals by means of sexual contact, including vaginal intercourse, oral sex, and anal sex. The term sexually transmitted infection (STI) is used, as it has a broader range of meaning. Some STIs can also be transmitted via use of an IV drug needle after its use by an infected person, as well as through childbirth or breastfeeding.

Until the 1990s, STDs were commonly known as *venereal diseases.* Public health officials originally introduced the term *sexually transmitted infection.* "Sometimes the terms STI and STD are used interchangeably. An infected person does not necessarily have any symptoms or signs that the virus or bacteria is actually hurting his or her body; they do not necessarily feel sick. A disease means that the infection is actually causing the infected person to

feel sick. If the infected person has no symptoms — is a much broader term than STD.

In general, an STI is an infection that has a negligible probability of transmission by means other than sexual contact, but has a realistic means of transmission by sexual contact (more sophisticated means — blood transfusion, sharing of hypodermic needles —are not taken into account). Thus, one may presume that, if a person is infected with an STI, e.g., chlamydia, gonorrhea, genital herpes, it was transmitted to him/her by means of sexual contact.

The English language has short words for two of the most common: "pox" (syphilis) and "the clap" (gonorrhea). Depending on the STD, a person may still be able to spread the infection if no signs of disease are present. For example, a person is much more likely to spread herpes infection when blisters are present (STD) than when they are absent (STI). However, a person can spread HIV infection (STI) at any time, even if he/she has not developed symptoms of AIDS (STD).

As may be noted from the name, sexually transmitted diseases are *transmitted* from one person to another by certain sexual activities rather than being actually *caused by* those sexual activities. Bacteria, fungi, protozoa or viruses are still the causative agents. It is not possible to catch any sexually transmitted disease from a sexual activity with a person who is not carrying a disease; conversely, a person who has an STD got it from contact (sexual or otherwise) with someone who had it, or his/her bodily fluids. Some STDs such as HIV can be transmitted from mother to child either during pregnancy or breastfeeding.

Healthcare professionals suggest safer sex, such as the use of condoms, as the most reliable way of decreasing the risk of contracting sexually transmitted diseases during sexual activity, but safer sex should by no means be considered an absolute safeguard. The transfer of and exposure to bodily fluids, such as blood transfusions and other blood products, sharing injection needles, needle-stick injuries (when medical staff are inadvertently jabbed or pricked with needles during medical procedures), sharing tattoo needles, and childbirth are other avenues of transmission. These different means put certain groups, such as medical workers, and haemophiliacs and drug users, particularly at risk.

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Vaccines are available that protect against some viral STIs, such as Hepatitis B and some types of HPV. Vaccination before initiation of sexual contact is advised to assure maximal protection. The most effective way to prevent sexual transmission of STIs is to avoid *contact* of body parts or fluids which can lead to transfer, not necessarily any *sexual activity* with an infected partner. No contact minimizes risk. Not all sexual activities involve contact: cybersex, phonesex or masturbation from a distance are methods of avoiding contact. Proper use of condoms (male or female) reduces contact and risk.

The first effective treatment for a sexually transmitted disease was salvarsan, a treatment for syphilis. With the discovery of antibiotics, a large number of sexually transmitted diseases became easily curable. In the 1980s, first genital herpes and then AIDS emerged into the public consciousness as sexually transmitted diseases that could not be cured by modern medicine. Recognition that AIDS threatened a global pandemic led to public information campaigns and the development of treatments that allow AIDS to be managed by suppressing the replication of HIV for as long as possible. Contact tracing continues to be an important measure, even when diseases are incurable, as it helps to contain infection.

Most of the following diseases are most commonly transmitted sexually. Some are commonly transmitted in other ways as well; for example, HIV/AIDS is also commonly transmitted through the sharing of infected needles by drug users, while SARS, which can be spread through casual contact such as coughing and sneezing, is very often not associated with sexual activity. Various bacterial (Shigella, Campylobacter, or Salmonella), viral (Hepatitis A, Adenoviruses), or parasitic (Giardia or amoeba) pathogens are transmitted by sexual practices that promote anal-oral contamination (fecal-oral). Sharing sex toys without washing or multiple partnered barebacking can promote anal-anal contamination. Although the bacterial pathogens may coexist with or cause proctitis, they usually produce symptoms (diarrhea, fever, bloating, nausea, and abdominal pain) suggesting disease more proximal in the GI tract.

Q.1 (d) Leishmaniasis

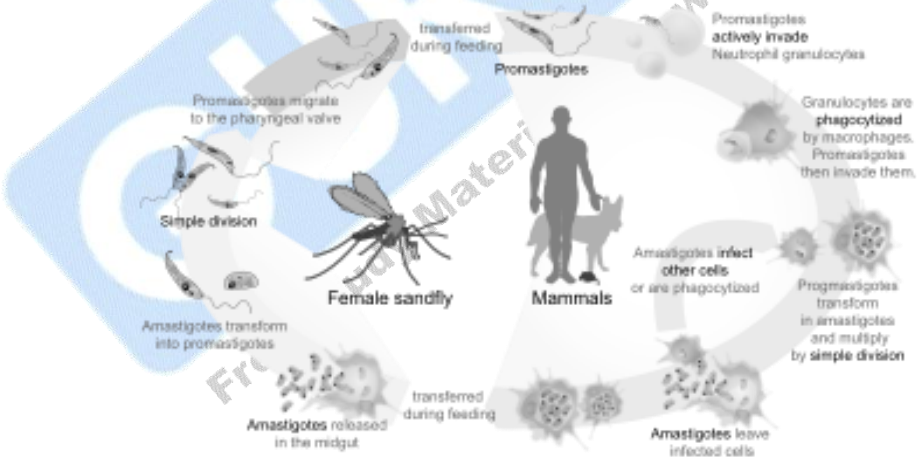
Ans. Leishmaniasis is a disease caused by protozoan parasites that belong to the genus *Leishmania* and is transmitted by the bite of certain species of sand fly

(subfamily Phlebotominae). Two genera transmit *Leishmania* to humans: *Lutzomyia* in the New World and *Phlebotomus* in the Old World.

Human infection is caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with three species (*L. donovani, L. infantum*, and *L. chagasi*); the *L. mexicana* complex with 3 main species (*L. mexicana, L. amazonensis*, and *L. venezuelensis*); *L. tropica; L. major; L. aethiopica*; and the subgenus *Viannia* with four main species (*L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis*, and *L. (V.) peruviana*). Cutaneous leishmaniasis is the most common form of leishmaniasis. Visceral leishmaniasis is a severe form in which the parasites have migrated to the vital organs.

The symptoms of leishmaniasis are skin sores which erupt weeks to months after the person affected is bitten by sand flies. Other consequences, which can become manifest anywhere from a few months to years after infection, include fever, damage to the spleen and liver, and anaemia.

Leishmaniasis can be transmitted in many tropical and sub-tropical countries, and is found in parts of about 88 countries. More than 90 percent of the world's cases of visceral leishmaniasis are in India, Bangladesh, Nepal, Sudan, and Brazil.



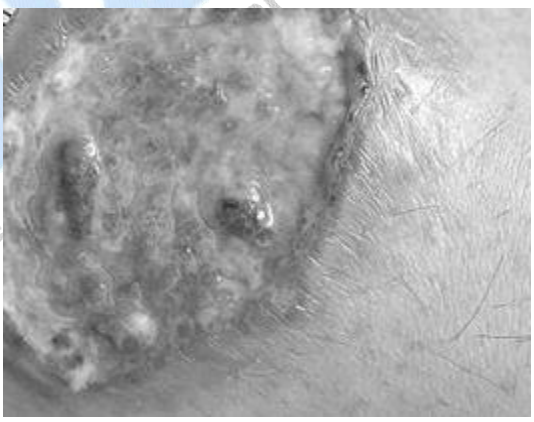
Life cycle of *Leishmania*

Leishmaniasis is transmitted by the bite of female phlebotomine sandflies. The sandflies inject the infective stage, metacyclic promastigotes, during blood meals (1). Metacyclic promastigotes that reach the puncture wound are phagocytized by macrophages (2) and transform into amastigotes (3).

Amastigotes multiply in infected cells and affect different tissues, depending in part on which *Leishmania* species is involved (4). These differing tissue specificities cause the differing clinical manifestations of the various forms of leishmaniasis. Sandflies become infected during blood meals on an infected host when they ingest macrophages infected with amastigotes (5,6). In the sandfly's midgut, the parasites differentiate into promastigotes (7), which multiply, differentiate into metacyclic promastigotes and migrate to the proboscis (8)

In the medical field, leishmaniasis is one of the famous causes of a markedly enlarged spleen, which may become larger even than the liver. There are four main forms of leishmaniasis:

- Visceral leishmaniasis – the most serious form and potentially fatal if untreated.
- Cutaneous leishmaniasis – the most common form which causes a sore at the bite site, which heal in a few months to a year, leaving an unpleasant looking scar. This form can progress to any of the other three forms.
- Diffuse cutaneous leishmaniasis – this form produces widespread skin lesions which resemble leprosy and is particularly difficult to treat.
- Mucocutaneous leishmaniasis – commences with skin ulcers which spread causing tissue damage to (particularly) nose and mouth



Cutaneous leishmaniasis ulcer

There are two common therapies containing antimony (known as pentavalent antimonials), meglumine antimoniate (*Glucantime*) and sodium stibogluconate (*Pentostam*). It is not completely understood how these drugs act against the parasite; they may disrupt its energy production or

trypanothione metabolism. Amphotericin (AmBisome) is now the treatment of choice. Miltefosine (Impavido), is a new drug for visceral and cutaneous leishmaniasis. Paromomycin is said to be an inexpensive and effective treatment for leishmaniasis. Currently there are no vaccines in routine use. However, the genomic sequence of *Leishmania* has provided a rich source of vaccine candidates. Genome-based approaches have been used to screen for novel vaccine candidates.

Q.1 (e) Anthrax

Ans. Anthrax is an acute disease in humans and animals caused by the bacterium *Bacillus anthracis*, which is highly lethal in some forms. There are effective vaccines against anthrax, and some forms of the disease respond well to antibiotic treatment. The name *anthrax* comes from *anthrakitis*, the Greek word for *anthracite* (coal), in reference to the black skin lesions victims develop in a cutaneous skin infection.

Bacillus anthracis is a rod-shaped Gram-positive bacterium. It was shown to cause disease by Robert Koch in 1877. The bacterium normally rests in endospore form in the soil, and can survive for up to decades in this state. Herbivores are often infected whilst grazing or browsing, especially when eating rough, irritant or spiky vegetation: the vegetation has been hypothesized to cause wounds within the gastrointestinal tract permitting entry of the bacterial endo-spores into the tissues, though this has not been proven. Once ingested or placed in an open cut, the bacterium begins multiplying inside the animal or human and typically kills the host within a few days or weeks. The endo-spores germinate at the site of entry into the tissues and then spread via the circulation to the lymphatics, where the bacteria multiply. It is the production of two powerful exo-toxins (edema toxin and lethal toxin) by the bacteria that causes death. Veterinarians can often tell a possible anthrax-induced death by its sudden occurrence, and by the dark, non-clotting blood that oozes from the body orifices. Most anthrax bacteria inside the body after death are out-competed and destroyed by anaerobic bacteria within minutes to hours post-mortem. However, anthrax vegetative bacteria that escape the body via oozing blood or through the opening of the carcass may form hardy spores. One spore forms per one vegetative bacterium. The triggers for spore formation are not yet known,

though oxygen tension and lack of nutrients may play roles. Once formed, these spores are very hard to eradicate.

The infection of herbivores (and occasionally humans) via the inhalational route normally proceeds as follows: once the spores are inhaled, they are transported through the air passages into the tiny air sacs (alveoli) in the lungs. The spores are then picked up by scavenger cells (macrophages) in the lungs and are transported through small vessels (lymphatics) to the lymph nodes in the central chest cavity (mediastinum). Damage caused by the anthrax spores and bacilli to the central chest cavity can cause chest pain and difficulty breathing. Once in the lymph nodes, the spores germinate into active bacilli which multiply and eventually burst the macrophages, releasing many more bacilli into the bloodstream to be transferred to the entire body. Once in the blood stream these bacilli release three substances: lethal factor, oedema factor and protective antigen. Protective antigen combines with these other two factors to form lethal toxin and oedema toxin, respectively. These toxins are the primary agents of tissue destruction, bleeding, and death of the host. If antibiotics are administered too late, even if the antibiotics eradicate the bacteria, some hosts will still die. This is because the toxins produced by the bacilli remain in their system at lethal dose levels.

Anthrax can enter the human body through the intestines (ingestion), lungs (inhalation), or skin (cutaneous) and causes distinct clinical symptoms based on its site of entry. An infected human will generally be quarantined. However, anthrax does not usually spread from an infected human to a noninfected human. But if the disease is fatal the person's body and its mass of anthrax bacilli becomes a potential source of infection to others and special precautions should be used to prevent further contamination. Inhalational anthrax, if left untreated until obvious symptoms occur, will usually result in death, as treatment will have started too late.

Anthrax cannot be spread directly from person to person, but a patient's clothing and body may be contaminated with anthrax spores. Effective decontamination of people can be accomplished by a thorough wash down with anti-microbe effective soap and water. Waste water should be treated with bleach or other anti-microbial agent. Effective decontamination of articles can be accomplished by boiling contaminated articles in water for 30 minutes or longer. Chlorine bleach is ineffective in destroying spores and vegetative cells on surfaces, though formaldehyde is effective. Burning

clothing is very effective in destroying spores. After decontamination, there is no need to immunize, treat or isolate contacts of persons ill with anthrax unless they were also exposed to the same source of infection. Early antibiotic treatment of anthrax is essential—delay seriously lessens chances for survival. Treatment for anthrax infection and other bacterial infections includes large doses of intravenous and oral antibiotics, such as fluoroquinolones, like ciprofloxacin (cipro), doxycycline, erythromycin, vancomycin or penicillin. In possible cases of inhalation anthrax, early antibiotic prophylaxis treatment is crucial to prevent possible death. If death occurs from anthrax the body should be isolated to prevent possible spread of anthrax germs. Burial does not kill anthrax spores.

Q.1 Give an account on the epidemiology, pathogenicity, diagnosis, prevention and control of Tuberculosis.

Ans. **Tuberculosis** (**TB**) is a common and often deadly infectious disease caused by mycobacteria, mainly *Mycobacterium tuberculosis*. Tuberculosis usually attacks the lungs (pulmonary TB) but can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti* also cause tuberculosis, but these species are less common.

The classic symptoms of tuberculosis are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss. Infection of other organs causes a wide range of symptoms. The diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids. Tuberculosis treatment is difficult and requires long courses of multiple antibiotics. Contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in (extensively) multi-drug-resistant tuberculosis. Prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guérin (BCG vaccine).

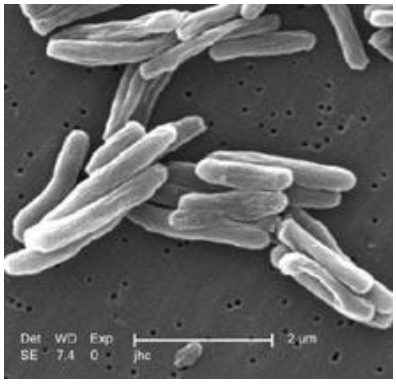
Tuberculosis is spread through the air, when people who have the disease cough, sneeze, or spit. One third of the world's current population have been infected with *M. tuberculosis*, and new infections occur at a rate of one per second.[2] However, most of these cases will not develop the full-blown

disease; asymptomatic, latent infection is most common. About one in ten of these latent infections will eventually progress to active disease, which, if left untreated, kills more than half of its victims. In 2004, mortality and morbidity statistics included 14.6 million chronic active cases, 8.9 million new cases, and 1.6 million deaths, mostly in developing countries. In addition, a rising number of people in the developed world are contracting tuberculosis because their immune systems are compromised by immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe with about 80% of the population in many Asian and African countries testing positive in tuberculin tests, while only 5-10% of the US population testing positive. It is estimated that the US has 25,000 new cases of tuberculosis each year, 40% of which occur in immigrants from countries where tuberculosis is endemic.

Main symptoms of pulmonary tuberculosis :

When the disease becomes active, 75% of the cases are pulmonary TB. Symptoms include chest pain, coughing up blood, and a productive, prolonged cough for more than three weeks. Systemic symptoms include fever, chills, night sweats, appetite loss, weight loss, pallor, and often a tendency to fatigue very easily.

In the other 25% of active cases, the infection moves from the lungs, causing other kinds of TB, collectively denoted extrapulmonary tuberculosis. This occurs more commonly in immunosuppressed persons and young children. Extrapulmonary infection sites include the pleura in tuberculosis pleurisy, the central nervous system in meningitis, the lymphatic system in scrofula of the neck, the genitourinary system in urogenital tuberculosis, and bones and joints in Pott's disease of the spine. An especially serious form is disseminated TB, more commonly known as miliary tuberculosis. Although extrapulmonary TB is not contagious, it may co-exist with pulmonary TB, which *is* contagious.



Mycobacterium tuberculosis

When people suffering from active pulmonary TB cough, sneeze, speak, or spit, they expel infectious aerosol droplets 0.5 to 5 μm in diameter. A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low and the inhalation of just a single bacterium can cause a new infection.

People with prolonged, frequent, or intense contact are at particularly high risk of becoming infected, with an estimated 22% infection rate. A person with active but untreated tuberculosis can infect 10–15 other people per year. Others at risk include people in areas where TB is common, people who inject drugs using unsanitary needles, residents and employees of high-risk congregate settings, medically under-served and low-income populations, high-risk racial or ethnic minority populations, children exposed to adults in high-risk categories, patients immunocompromised by conditions such as HIV/AIDS, people who take immunosuppressant drugs, and health care workers serving these high-risk clients.

Transmission can only occur from people with active — not latent — TB. The probability of transmission from one person to another depends upon the number of infectious droplets expelled by a carrier, the effectiveness of ventilation, the duration of exposure, and the virulence of the *M. tuberculosis* strain. The chain of transmission can, therefore, be broken by isolating patients with active disease and starting effective anti-tuberculous therapy. After two weeks of such treatment, people with non-resistant active TB generally cease to be contagious. If someone does become infected, then it will take at least 21 days, or three to four weeks, before the newly infected person can transmit the disease to others. TB can also be transmitted by eating meat infected with TB. *Mycobacterium bovis* causes TB in cattle. (See details below.)

Tuberculosis is diagnosed definitively by identifying the causative organism (*Mycobacterium tuberculosis*) in a clinical sample (for example, sputum or pus). When this is not possible, a probable diagnosis may be made using imaging (X-rays or scans) and/or a tuberculin skin test.

A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears and cultures. It may also include a tuberculin skin test, a serological test. The interpretation of the tuberculin skin test depends upon the person's risk factors for infection and progression to TB disease, such as exposure to other cases of TB or immunosuppression.

Currently, latent infection is diagnosed in a non-immunized person by a tuberculin skin test, which yields a delayed hypersensitivity type response to an extract made from *M. tuberculosis*. Those immunized for TB or with past-cleared infection will respond with delayed hypersensitivity parallel to those currently in a state of infection, so the test must be used with caution, particularly with regard to persons from countries where TB immunization is common. Tuberculin tests have the disadvantage in that they may produce false negatives, especially when the patient is co-morbid with sarcoidosis, Hodgkins lymphoma, malnutrition, or most notably active tuberculosis disease. New TB tests are being developed that offer the hope of cheap, fast and more accurate TB testing. These include polymerase chain reaction detection of bacterial DNA, and assays to detect the release of interferon gamma in response to mycobacterial proteins such as ESAT-6. These are not affected by immunization or environmental mycobacteria, so generate fewer false positive results. The development of a rapid and inexpensive diagnostic test would be particularly valuable in the developing world.

Treatment for TB uses antibiotics to kill the bacteria. The two antibiotics most commonly used are rifampicin and isoniazid. However, instead of the short course of antibiotics typically used to cure other bacterial infections, TB requires much longer periods of treatment (around 6 to 12 months) to entirely eliminate mycobacteria from the body. Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance. People with latent infections are treated to prevent them from progressing to active TB disease later in life. However, treatment using Rifampicin and Pyrazinamide is not risk-free. The Centers for Disease Control and Prevention (CDC) notified healthcare professionals of revised recommendations against the use of rifampin plus pyrazinamide for treatment of latent tuberculosis infection, due to high rates of hospitalization and death from liver injury associated with the combined use of these drugs.

Drug resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons who are infected with a resistant strain of TB. A patient with fully-susceptible TB develops secondary resistance (acquired resistance) during TB therapy because of inadequate treatment, not taking the prescribed regimen appropriately, or using low quality medication. Drug-resistant TB is a public health issue in many developing countries, as treatment is longer and requires more expensive drugs. Multi-drug resistant TB (MDR-TB) is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-

resistant TB (XDR-TB) is also resistant to three or more of the six classes of second-line drugs. The DOTS (Directly Observed Treatment Short-course) strategy of tuberculosis treatment based on clinical trials done in the 1970s by Tuberculosis Research Centre, Chennai, India, focusing on a neglected area of infectious disease control is now showing promising results in effectively treating all TB cases in the community.

TB prevention and control takes two parallel approaches. In the first, people with TB and their contacts are identified and then treated. Identification of infections often involves testing high-risk groups for TB. In the second approach, children are vaccinated to protect them from TB. Unfortunately, no vaccine is available that provides reliable protection for adults. However, in tropical areas where the levels of other species of mycobacteria are high, exposure to nontuberculous mycobacteria gives some protection against TB.

The World Health Organization (W.H.O.) declared TB a global health emergency in 1993, and the Stop TB Partnership developed a Global Plan to Stop Tuberculosis that aims to save 14 million lives between 2006 and 2015. Since humans are the only host of Mycobacterium tuberculosis, eradication would be possible: a goal that would be helped greatly by an effective vaccine.

Vaccines:

Many countries use Bacillus Calmette-Guérin (BCG) vaccine as part of their TB control programs, especially for infants. According to the W.H.O., this is the most often used vaccine worldwide, with 85% of infants in 172 countries immunized in 1993. This was the first vaccine for TB and developed at the Pasteur Institute in France between 1905 and 1921. However, mass vaccination with BCG did not start until after World War II. The protective efficacy of BCG for preventing serious forms of TB (e.g. meningitis) in children is greater than 80%; its protective efficacy for preventing pulmonary TB in adolescents and adults is variable, ranging from 0 to 80%.

Epidemiology:

According to the World Health Organization, nearly 2 billion people—one third of the world's population—have been exposed to the tuberculosis pathogen. Annually, 8 million people become ill with tuberculosis, and 2 million people die from the

disease worldwide. Tuberculosis is the world's greatest infectious killer of women of reproductive age and the leading cause of death among people with HIV/AIDS.

The rise in HIV infections and the neglect of TB control programs have enabled a resurgence of tuberculosis. The emergence of drug-resistant strains has also contributed to this new epidemic with, from 2000 to 2004, 20% of TB cases being resistant to standard treatments and 2% resistant to second-line drugs. The rate at which new TB cases occur varies widely, even in neighboring countries, apparently because of differences in health care systems.

In 2005, the country with the highest estimated incidence of TB was Swaziland, with 1262 cases per 100,000 people. India has the largest number of infections, with over 1.8 million cases. In developed countries, tuberculosis is less common and is mainly an urban disease. In the United Kingdom, TB incidences range from 40 per 100,000 in London to less than 5 per 100,000 in the rural South West of England; the national average is 13 per 100,000.

The incidence of TB varies with age. In Africa, TB primarily affects adolescents and young adults. However, in countries where TB has gone from high to low incidence, such as the United States, TB is mainly a disease of older people, or of the immunocompromised.

There are a number of known factors that make people more susceptible to TB infection: worldwide the most important of these is HIV. Co-infection with HIV is a particular problem in Sub-Saharan Africa, due to the high incidence of HIV in these countries. Smoking more than 20 cigarettes a day also increases the risk of TB by two to four times. Diabetes mellitus is also an important risk factor that is growing in importance in developing countries. Other disease states that increase the risk of developing tuberculosis are Hodgkin lymphoma, end-stage renal disease, chronic lung disease, malnutrition, and alcoholism.

Diet may also modulate risk. For example, among immigrants in London from the Indian subcontinent, vegetarian Hindu Asians were found to have an 8.5 fold increased risk of tuberculosis, compared to Muslims who ate meat and fish daily. Although a causal link is not proved by this data, this increased risk could be caused by micronutrient deficiencies: possibly iron, vitamin B12 or vitamin D. Further studies have provided more evidence of a link between vitamin D deficiency and an increased risk of contracting tuberculosis. Globally, the severe malnutrition common in parts of the developing world causes a large increase in the risk of developing active tuberculosis, due to its damaging effects on the immune system. Along with

overcrowding, poor nutrition may contribute to the strong link observed between tuberculosis and poverty.

Multiple Choice Questions

SECTION-D

1. A diagnosis of diphtheria is confirmed by:
   A) Microscopic appearance of organisms stained with methylene blue
   B) Isolation of a typical colony on Tinsdale's agar
   C) Isolation of typical organisms from materials such as blood, showing invasiveness
   D) Detection of β phage plagues in cultures o suspicious isolates
   E) **Demonstration of toxin production by a suspicious isolate.**

2. Listeria monocytogenes shows which of the following characteristics?
   A) **It can grow at refrigerator temperatures (4^oC)**
   B) It is an extracellular pathogen
   C) It is catalase-negative
   D) It is a gram-negative coccus
   E) It is strictly a human pathogen

3. Which one of the following is characteristic of N.meningitidis but not N.gonorrhoeae?
   A) Ferments glucose
   B) **Contains a polysaccharide capsule**
   C) Is oxidase-positive
   D) Most isolates show resistance to penicillin
   E) No effective vaccines are available

4. Which of the following is true of Haemophilus influenzae?
   A) **Invasive infections are most commonly associated with encapsulated strains**
   B) Most invasive infections occur in infants during the neonatal period
   C) Most human infections are acquired from domestic pets
   D) The organism can be readily cultured on sheep blood agar in an environment of elevated CO_2

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E) Older adults are rarely at risk for infection with this organism because they typically have a high level of immunity.

5. For which of the following organisms is there no known animal reservoir?
   A) *Francisella tularensis*
   B) *Pasteurella multocida*
   C) ***Bordetella pertussis***
   D) *Brucella melitensis*
   E) *Yersinia pestis*

6. A distinguishing feature of human mycoplasma species is that they:
   A) Stain well with Giemsa, but not by Gram stain
   B) **Contain no bacterial pepidoglycan**
   C) Are not immunogenic because they mimic host cell membrane components
   D) Cannot be cultivated in vitro
   E) Are dependent on host sources of ATP

7. Which one of the following is most characteristic of *Mycoplasma pneumoniae* infection?
   A) Infection results in a fever of sudden onset accompanied by a productive cough
   B) **Infection most commonly occurs in the upper respiratory tract**
   C) Infection is definitively diagnosed by direct microscopic examination of sputum
   D) Re-infection is rare and less severe than primary infection
   E) Infection causes extensive scarring and calcification of affected lung tissue

8. Which of these is not traditionally considered medical parasites?
   A) Helminths
   B) Insects
   C) **Virus**
   D) Protozoa

9. Which of these is not an ectoparasite?
   A) Ticks
   B) Bed bugs

C) Lice
D) **Protozoa**

10. Malaria is an example of
A) **Protozoa**
B) Virus
C) Prion
D) Bacteria

11. Which statement about these helminths is incorrect?
A) **Taenia saginata is an example of a trematode**
B) Trematodes are unsegmented
C) Cestodes are segmented
D) Nematodes have their own digestive tract

12. Which statement about these diplococci is incorrect?
A) Round shape
B) Group in pairs
C) Pneumococcus is an example
D) **Rod shaped**

13. Choose the false statement regarding HIV
A) Can cause auto-immune diseases
B) Higher prevalence in developing countries
C) **Binds to GP120 receptor on T-helper cells**
D) Binds to CCR5 on T-helper cells

14. Creutzfeldt-Jakob Disease is NOT
A) **transmitted via GHRH**
B) caused by prion
C) a rare, fatal neurodegenerative neuropathy
D) similar to bovine spongiform encelopathy

15. Which statement about Cystitis is incorrect?
A) Can cause dysuria
B) Can be caused by *E.coli*
C) **Urination frequency reduced**
D) Lower abdominal pain

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16. Which of these is a cause of endogenous infection transmission?
    A) **Migration**
    B) Injury
    C) Mother-to-baby
    D) Sex

17. Choose the incorrect option regarding cholera
    A) Causes kidney failure, dehydration
    B) Toxin
    C) **Blocks pancreatic function**
    D) Severe rice water diarrhea

18. People taking drugs for stomach ulcers: (choose false option)
    A) **are less susceptible to salmonella**
    B) do so to increase their stomach acid pH
    C) Probably have a H. Pylori infection

19. Along with a blood sample, which other sample would be useful in suspected pneumonia?
    A) **Sputum**
    B) Urine
    C) Faeces
    D) Throat swab

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Key Terms

ctive immunity: usually long-lasting immunity that is acquired through the production of *antibodies* and memory T cells within the organism in response to the presence of *antigens*.

adaptive immune system: also called the acquired immune system, this component of the immune system comprises *white blood cells*, particularly *lymphocytes.* When it is presented with a new microbe or vaccine, it may take days or weeks to respond or adapt, but the resultant improved immune readiness, or "memory," is sustained for long periods (years).

adenosine deaminase (ADA): an enzyme found in mammalian tissues that is capable of catalyzing the process in which adenosine is split into inosine and ammonia. A deficiency can cause problems with metabolic reactions in cells, which leads to the destruction of *B* and *T cells*. ADA deficiency can lead to one form of severe combined immunodeficiency disease.

allergy: a misguided reaction by the immune system to harmless foreign substances.

antibody: a protein on the surface of *B cells* that is also secreted in large amounts into the blood or lymph in response to an *antigen*, a component within an invader such as a bacterium, virus, parasite, or transplanted organ. Antibodies neutralize the antigen, and thereby the invader, by binding to it, often directing it toward a macrophage for destruction. Also called an immunoglobulin.

antigen: a foreign substance (usually a protein or carbohydrate) capable of triggering an immune response in an organism.

antiretroviral drugs: drugs that act against *retroviruses* (such as HIV).

autoimmune disorders: conditions in which the body's own immune system acts against it.

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autoreactive: describes immune cells that mount a response against the body's own cells or tissues.

B cell: a type of *lymphocyte* that produces *antibodies*, which bind to free-floating microbes circulating in the blood so that they cannot infect other cells.

biochemicals: chemicals produced within living organisms. Many coordinate to fight off invasion in an immune response.

biological barriers: the body's first layer of protection against harmful microbes; skin is a prime example.

blood-brain barrier: a tight layer of cells and tissue that separates the brain from the rest of the body; a physical roadblock that normally keeps immune cells outside the brain.

blood-forming stem cells: immature cells in the bone marrow that multiply extensively and produce *red* and *white blood cells* and platelets.

CD4+ helper T cells:*T cells* with CD4 receptors that respond to *antigens* on the surface of specific molecules by secreting a certain type of cytokine that stimulates *B cells* and killer *T cells.* Helper T cells are infected and killed by *HIV;* people who develop *AIDS* have no more than one-fifth the normal number of helper T cells.

central nervous system: the brain and spinal cord, to which sensory impulses are transmitted and from which motor impulses emanate. The central nervous system supervises and coordinates the activity of the entire nervous system and interacts with the immune system.

clones: copies that viruses make of themselves.

cytokine: a class of substance secreted by cells of the immune system to regulate immune cells.

dendritic cell: an *antigen*-presenting immune cell that initiates the immune response by activating *lymphocytes* and stimulating the secretion of *cytokines.*

Dendritic cells also prevent autoimmune reactions by instructing the T lymphocytes to be silent or tolerant to the body itself.

DNA vaccine: vaccines that often use "naked" *DNA* (DNA not associated with a cell or a virus) with instructions for making protective *antigens*. When injected, the DNA is taken in by other cells, which then produce protective antigens.

epidemic: an outbreak of disease that simultaneously affects an atypically large number of individuals within a population, community, or region.

granulocyte: a type of *phagocyte* with cytoplasm that contains grainlike particles.

immune deficiency diseases (IDDs): diseases that result when one or more parts of the immune system are missing or defective.

immunoglobulin E (IgE): a class of *antibodies* that function in allergic reactions.

immunosuppressive: describes a treatment that suppresses natural immune responses—for example, chemotherapy for cancer.

inactivated vaccines:*vaccines* made by growing and purifying large numbers of the target organism in the laboratory and then killing them with heat, radiation, or chemicals. The immune system reacts to the dead microorganisms, producing immunity.

inflammation: a buildup of fluid and cells that occurs as the immune system fights a hostile invader.

innate immune system: component of the immune system that consists of a set of genetically encoded responses to pathogens and does not change or adapt during the lifetime of the organism. Innate immunity involves quickly mobilized defenses triggered by receptors that recognize a broad spectrum of microbes; in contrast to adaptive immunity, it does not acquire memory for an improved response during a second exposure to infection.

killer T cell: a type of *lymphocyte* that directly attacks and kills infected cells or other targets, including tumor cells and even one's own tissues. Killer cells are generated by the coordinated action of *dendritic cells* and *CD4+ helper T cells*.

knockout: term used in genetic engineering when a specific gene is deliberately removed in order to create an organism unable to carry out the functions the gene codes for; knockouts are used by immunologists to determine the functions of specific genes that encode immune proteins.

latency: the state or period in which a virus has invaded a host but is not actively multiplying, and during which symptoms of the infection are not seen. "Microbial latency" means the microbe is not multiplying, as occurs in some cells in HIV infection, while "clinical latency" means that the patient does not have symptoms of disease even though the virus is multiplying and damaging the immune system. In HIV, clinical latency precedes the AIDS stage.

lymph nodes: small, rounded structures in the lymphatic system that contain disease-fighting *white blood cells*, especially *lymphocytes*, and filter out harmful microbes and toxins. Lymph nodes may become enlarged when they are actively fighting infection.

lymphocyte: a type *of white blood cell* involved in the human body's immune system, of which there are two broad categories, *T cells* and *B cells*. Lymphocytes are an integral part of the body's defenses because they are highly specific for antigens associated with microbes, tumor cells, transplants, allergies, and tissues attacked in autoimmune diseases. The immune system comprises clones of lymphocytes, each with a single specificity, and exposure to antigens leads to clonal expansion, the acquisition of helper and killer functions, and formation of immune memory.

lysozyme: an *enzyme* in saliva and tears that destroys bacteria.

macrophages: large *phagocyte* cells that remove harmful microbes from the body.

major histocompatibility complex (MHC) molecules: a group of molecules that help the immune system distinguish between harmful and safe foreign

substances in the body. Recent research suggests some classes of MHC molecules also play an essential role in brain function.

mast cells: large cells, found in connective tissues, that mediate allergic reactions. Mast cells play an important protective role in wound healing and defend against *pathogens*.

memory B and T cells: *B* and *T cells* that remain in the body after the completion of an immune response to ward off future attacks by the same microbe. Memory is imparted by the increased size in the *antigen*-specific B or T cell clone, as well as improved function of individual cells within the clone.

microglia: specialized immune cells, related to macrophages, that protect the *central nervous system.*

molecular mimicry: an occurrence in many *autoimmune disorders* in which a microbe carries *antigens* that resemble those on a particular organ, causing the immune system to attack the body.

monoclonal antibodies: *antibodies* derived from a single cell and used against a specific *antigen* such as a cancer cell. Rituxan and Herceptin are monoclonal antibodies used in the treatment of lymphoma and breast cancer, respectively.

mutation: a process in which a microbe or organism undergoes a permanent change in hereditary material. When viruses or bacteria mutate they are no longer recognized by the immune system and become resistant to previously administered *vaccines* and drugs.

myelin: a white, fatty material that sheathes nerves and enhances the transmission of signals between the brain and the body. In *multiple sclerosis*, an *autoimmune disorder,* immune cells attack myelin, affecting the transmission of nerve signals.

pandemic: an outbreak of disease occurring over a wide geographical area and affecting an exceptionally high proportion of the population.

passive immunity: immunity acquired by the transfer of *antibodies* (as by injection of serum from an individual with active immunity).

pathogen: a specific causative agent of disease, such as a bacterium or a virus.

phagocyte: a cell such as a *white blood cell* that engulfs and consumes foreign material, such as microorganisms.

plasma cell: an *antibody*-producing l*ymphocyte* derived from a *B cell* upon reaction with a specific *antigen*.

protease: an *enzyme* that catalyzes the splitting of proteins into smaller molecules. To treat AIDS, scientists have designed drugs that interfere with protease made by the HIV virus, which is essential to its replication.

red blood cells: any of the hemoglobin-containing cells that carry oxygen to the tissues and are responsible for the red color of vertebrate blood..

regulatory T cells (Treg cells): special *T cells* that regulate or suppress immune responses, preventing autoimmunity for example.

replication: process by which an organism produces a copy of itself—for example, the way microbes reproduce.

retrovirus: a type of *RNA* virus (such as HIV) that reproduces by transcribing itself into *DNA* (using *reverse transcriptase*). The resultant DNA inserts itself into a cell's DNA and is reproduced by the cell.

reverse transcriptase (RT): an *enzyme* that catalyzes the formation of *DNA* using *RNA* as a template.

rotavirus: a *retrovirus* with a double-layer protein shell and a wheel-like appearance. Rotaviruses cause diarrhea, especially in infants.

stem cell transplants: a kind of *passive immune therapy* that transfers cells instead of antibodies. Stem cells have the capacity to give rise to all elements of the immune system, such as many types of *lymphocytes* and *phagocytes*.

subunit vaccines:*vaccines* that contain only a part of the target microorganism.

synapses: specialized junctions at which cells of the nervous system signal to one another and to nonneuronal cells, such as those of muscles and glands.

T cell: a type of lymphocyte that possesses highly specific cell-surface antigen receptors; types include *CD4+ helper T cells, regulatory T cells*, *and killer T cells*.

tolerance: the capacity of the body to become less responsive to a substance or a physiological insult. Tolerance to components of the self prevents or suppresses autoimmunity.

toxoid vaccine: an inactivated and weakened version of the disease-causing toxin a microbe produces; it is still capable of inducing the formation of *antibodies* when injected.

transgenic technology: technology used to deliberately alter the genome of an organism by the transfer of a gene or genes from another species or breed.

two-photon microscopy: an imaging technique using high-powered laser microscopes to examine immune response in the nervous system.

vaccine: killed microorganisms, weakened living organisms, fully virulent living organisms, or subunit proteins of a microbe, administered to produce or artificially increase immunity to a particular disease.

vector vaccines: *vaccines* made by inserting protective *antigen* genes into harmless bacteria or viruses (vectors). As the vectors multiply in the body, they expose the immune system to protective antigens, stimulating *active immunity* against the harmful organism.

white blood cells: any of the blood cells that are colorless, lack hemoglobin, and contain a nucleus. They include the *lymphocytes, dendritic cells*, monocytes, neutrophils, eocinophils, and basophils; also called leukocytes.

cranium: the bones of the skull surrounding the brain, not including the face bones; the bone just above/in front of the ear is the **temporal bone**

mandible: the jaw bone, so the hinge of the jaw is the **temporo-mandibular joint**, and problems with malfunctioning of this joint are known as **TMJ**

vertebrae: bones which make up the spine, which include:

cervical vertebrae -the vertebrae in the neck region

thoracic vertebrae- the vertebrae with ribs attached

lumbar vertebrae- the vertebrae in the lower back

sacrum- five fused vertebrae which are joined to the pelvis

coccyx- four fused vertebrae which comprise the tailbone

ribs - bones protecting the chest cavity

sternum- the breastbone

clavicle- the collar bone

scapula- the shoulder blade

humerus- the top of the arm

ulna- the little finger side of the lower arm which also forms the elbow

radius- the thumb side of the lower arm; the **R**adius **R**otates around

Acetylcholine - A type of chemical (called a neurotransmitter) that transmits messages among nerve cells and muscle cells.

Acquired heart disease - Heart disease that arises after birth, usually from infection or through the build-up of fatty deposits in the arteries that feed the heart muscle.

Alveoli - Air sacs in the lungs where oxygen and carbon dioxide are exchanged.

Amiodarone (Cordarone, Pacerone) - A kind of medicine (called an antiarrhythmic) used to treat irregular heart rhythms such as atrial fibrillation and ventricular tachycardia. It works by regulating nerve impulses in your

heart. Amiodarone is mainly given to patients who have not responded to other antiarrhythmic medicines.

Aneurysm - A sac-like protrusion from a blood vessel or the heart, resulting from a weakening of the vessel wall or heart muscle.

Angina or angina pectoris - Chest pain that occurs when diseased blood vessels restrict blood flow to the heart.

Angiography - An x-ray technique where dye is injected into the chambers of your heart or the arteries that lead to your heart (the coronary arteries). The test lets doctors measure the blood flow and blood pressure in the heart chambers and see if the coronary arteries are blocked.

Angioplasty - A nonsurgical technique for treating diseased arteries by temporarily inflating a tiny balloon inside an artery.

Angiotensin II receptor blocker - A medicine that lowers blood pressure by blocking the action of angiotensin II, a chemical in the body that causes the blood vessels to tighten (constrict).

Annulus - The ring around a heart valve where the valve leaflet merges with the heart muscle.

Antiarrhythmics - Medicines used to treat patients who have irregular heart rhythms.

Anticoagulant - Any medicine that keeps blood from clotting; a blood thinner.

Antihypertensive - Any medicine or other therapy that lowers blood pressure.

Antiplatelet therapy - Medicines that stop blood cells (called platelets) from sticking together and forming a blood clot.

Aorta - The largest artery in the body and the initial vessel to supply blood from the heart.

Aortic valve - The valve that regulates blood flow from the heart into the aorta.

Aphasia - The inability to speak, write, or understand spoken or written language because of brain injury or disease.

Arrhythmia (or dysrhythmia) - An abnormal heartbeat.

Arrhythmogenic right ventricular dysplasia (ARVD) - ARVD is a type of cardiomyopathy with no known cause. It appears to be a genetic condition (passed down through a family's genes). ARVD causes ventricular arrhythmias. The most common symptoms are heart palpitations, fainting or loss of consciousness (syncope), and, sometimes, sudden death.

Arteriography - A test that is combined with cardiac catheterization to visualize an artery or the arterial system after injection of a contrast dye.

Arterioles - Small, muscular branches of arteries. When they contract, they raise resistance to blood flow, and blood pressure in the arteries increases.

Artery - A vessel that carries oxygen-rich blood to the body.

Arteritis - Inflammation of the arteries.

Arteriosclerosis - A disease process, commonly called "hardening of the arteries", which includes a variety of conditions that cause artery walls to thicken and lose elasticity.

Artificial heart - A manmade heart. Also called a total artificial heart (TAH).

Ascending aorta - The first portion of the aorta, emerging from the heart's left ventricle.

Aspirin - Acetylsalicylic acid; a medicine used to relieve pain, reduce inflammation, and prevent blood clots.

Atherectomy - A nonsurgical technique for treating diseased arteries with a rotating device that cuts or shaves away material that is blocking or narrowing an artery.

Atherosclerosis - A disease process that leads to the buildup of a waxy substance, called plaque, inside blood vessels.

Atrium (right and left) - The two upper or holding chambers of the heart (together referred to as atria).

Atrial flutter - A type of arrhythmia where the upper chambers of the heart (the atria) beat very fast, causing the walls of the lower chambers (the ventricles) to beat inefficiently as well.

Atrial tachycardia - A type of arrhythmia that begins in the heart's upper chambers (the atria) and causes a very fast heart rate of 160 to 200 beats a minute. A resting heart rate is normally 60 to 100 beats a minute.

Atrioventricular block - An interruption or disturbance of the electrical signal between the heart's upper two chambers (the atria) and lower two chambers (the ventricles).

Atrioventricular (AV) node - A group of cells in the heart located between the upper two chambers (the atria) and the lower two chambers (the ventricles) that regulates the electrical current that passes through it to the ventricles.

Atrium - Either one of the heart's two upper chambers.

Autologous - Relating to self. For example, autologous stem cells are those taken from the patient's own body.

Autoregulation - When blood flow to an organ stays the same although pressure in the artery that delivers blood to that organ may have changed.

Bacteria - Germs that can lead to disease.

Bacterial endocarditis - A bacterial infection of the lining of the heart's chambers (called the endocardium) or the heart's valves.

Balloon catheter - A long tube-like device with a small balloon on the end that can be threaded through an artery. Used in angioplasty or valvuloplasty.

Balloon valvuloplasty - A procedure to repair a heart valve that is not working properly. A balloon-tipped catheter is threaded through an artery and into the heart. The balloon is inflated to open and separate any narrowed or stiffened flaps (called leaflets) of a valve. The catheter and deflated balloon are removed after the procedure.

Beta-blocker - An antihypertensive medicine that limits the activity of epinephrine, a hormone that increases blood pressure.

Biopsy - The process by which a small sample of tissue is taken for examination.

Blalock-Taussig procedure - A shunt between the subclavian and pulmonary arteries used to increase the supply of oxygen-rich blood in "blue babies" (see below).

Blood clot - A jelly-like mass of blood tissue formed by clotting factors in the blood. Clots stop the flow of blood from an injury. Clots can also form inside an artery when the artery's walls are damaged by atherosclerotic buildup, possibly causing a heart attack or stroke.

Blood pressure - The force or pressure exerted by the heart in pumping blood; the pressure of blood in the arteries.

Blue babies - Babies who have a blue tinge to their skin (cyanosis) resulting from insufficient oxygen in the arterial blood. This condition often indicates a heart defect.

Body mass index (BMI) - A number that doctors use to determine the risk of cardiovascular disease created by a person being overweight. BMI is calculated

using a formula of weight in kilograms divided by height in meters squared (BMI =W [kg]/H [m^2]).

Bridge to transplant - Use of mechanical circulatory support to keep heart failure patients alive until a donor heart becomes available.

Bundle branch block - A condition in which parts of the heart's conduction system are defective and unable to conduct the electrical signal normally, causing an irregular heart rhythm (arrhythmia).

Bypass - Surgery that can improve blood flow to the heart (or other organs and tissues) by providing a new route, or "bypass," around a section of clogged or diseased artery.

Calcium channel blocker (or calcium blocker) - A medicine that lowers blood pressure by regulating calcium-related electrical activity in the heart.

Capillaries - Microscopically small blood vessels between arteries and veins that distribute oxygen-rich blood to the body's tissues.

Cardiac - Pertaining to the heart.

Cardiac amyloidosis - A disorder caused by deposits of an abnormal protein (amyloid) in the heart tissue, which make it hard for the heart to work properly. Also called "stiff heart syndrome."

Cardiac arrest - The stopping of the heartbeat, usually because of interference with the electrical signal (often associated with coronary heart disease).

Cardiac cachexia - A term for the muscle and weight loss caused by severe heart disease. It is often related to the depressed cardiac output associated with end-stage heart failure, but it can also occur with severe coronary artery disease.

Cardiac catheterization - A procedure that involves inserting a fine, hollow tube (catheter) into an artery, usually in the groin area, and passing the tube

into the heart. Often used along with angiography and other procedures, cardiac catheterization has become a primary tool for visualizing the heart and blood vessels and diagnosing and treating heart disease.

Cardiac enzymes - Complex substances capable of speeding up certain biochemical processes in the heart muscle. Abnormal levels of these enzymes signal heart attack.

Cardiac output - The amount of blood the heart pumps through the circulatory system in one minute.

Cardiologist - A doctor who specializes in the study of the heart and its function in health and disease.

Cardiology - The study of the heart and its function in health and disease.

Cardiomegaly - An enlarged heart. It is usually a sign of another underlying problem, such as high blood pressure, heart valve problems, or cardiomyopathy.

Cardiomyopathy - A disease of the heart muscle that leads to generalized deterioration of the muscle and its pumping ability.

Cardiopulmonary bypass - The process by which a machine is used to do the work of the heart and lungs so the heart can be stopped during surgery.

Cardiopulmonary resuscitation (CPR) - An emergency measure that can maintain a person's breathing and heartbeat. The person who performs CPR actually helps the patient's circulatory system by breathing into the patient's mouth to give them oxygen and by giving chest compressions to circulate the patient's blood.

Cardiovascular (CV) - Pertaining to the heart and blood vessels that make up the circulatory system.

Cardiovascular Disease (CVD) - A general term referring to conditions affecting the heart (cardio) and blood vessels (vascular system). May also simply be called heart disease. Examples include coronary artery disease, valve disease, arrhythmia, peripheral vascular disease, congenital heart defects, hypertension, and cardiomyopathy. Refer to specific conditions for detailed explanations.

Cardioversion - A technique of applying an electrical shock to the chest to convert an abnormal heartbeat to a normal rhythm.

Carotid artery - A major artery (right and left) in the neck supplying blood to the brain.

Cerebral embolism - A blood clot formed in one part of the body and then carried by the bloodstream to the brain, where it blocks an artery.

Cerebral hemorrhage - Bleeding within the brain resulting from a ruptured blood vessel, aneurysm, or head injury.

Cerebral thrombosis - Formation of a blood clot in an artery that supplies part of the brain.

Cerebrovascular - Pertaining to the blood vessels of the brain.

Cerebrovascular accident - Also called cerebral vascular accident, apoplexy, or stroke. Blood supply to some part of the brain is slowed or stopped, resulting in injury to brain tissue.

Cerebrovascular occlusion - The blocking or closing of a blood vessel in the brain.

Cholesterol - An oily substance that occurs naturally in the body, in animal fats and in dairy products, and that is transported in the blood. Limited amounts are essential for the normal development of cell membranes.

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Cineangiography - The technique of using moving pictures to show how a special dye passes through blood vessels, allowing doctors to diagnose diseases of the heart and blood vessels.

Circulatory system - Pertaining to the heart, blood vessels, and circulation of blood.

Claudication - A tiredness or pain in the arms and legs caused by an inadequate supply of oxygen to the muscles, usually due to narrowed arteries.

Collateral circulation - Blood flow through small, nearby vessels in response to blockage of a main blood vessel.

Commissurotomy -A procedure used to widen the opening of a heart valve that has been narrowed by scar tissue. First developed to correct rheumatic heart disease.

Computed tomography (CT or CAT scan) - An x-ray technique that uses a computer to create cross-sectional images of the body.

Conduction system - Special muscle fibers that conduct electrical impulses throughout the heart muscle.

Congenital - Refers to conditions existing at birth.

Congenital heart defects - Malformation of the heart or of its major blood vessels present at birth.

Congestive heart failure - A condition in which the heart cannot pump all the blood returning to it, leading to a backup of blood in the vessels and an accumulation of fluid in the body's tissues, including the lungs.

Coronary arteries - Two arteries arising from the aorta that arch down over the top of the heart and divide into branches. They provide blood to the heart muscle.

Coronary artery anomaly (CAA) - A congenital defect in one or more of the coronary arteries of the heart.

Coronary artery bypass (CAB) - Surgical rerouting of blood around a diseased vessel that supplies blood to the heart. Done by grafting either a piece of vein from the leg or the artery from under the breastbone.

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B.Sc./M.Sc. (Part III) Examination, 2011

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

PAPER BT-801

MEDICAL BIOTECHNOLOGY

Year-2011

Time.: 3 Hours ***Max. Marks : 50***

Attempt FIVE questions in all, including Questions No. 1 which is compulsory, selecting ONE question from each Section.

1. Write notes on the following : 5 x 2 = 10
   (a) Neuromuscular system
   (b) Haptens
   (c) NK cells
   (d) Flow cytometry
   (e) Name the pathogen that causes whooping cough and chickenpox.

Section-A

2. Describe anatomy and physiology of heart in detail. 10

or

   (a) Define NMR
   (b) Structure of Nephron 5 x 2 = 10

Section- B

3. Discuss the distinguishing features of five different classes of immunoglobulin's. 10

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or

What do you understand by the term "Cell-mediated immune response? 10

Section-C

4. Explain briefly radioimmunoassay (RIA) and its application. 10

or

Describe hybridoma technology and its application. 10

Section –D

5. Discuss the epidemiology, pathogenicity, diagnosi, prevention and control and leprosy. 10

or

Explain the following: 5+5 =10
(a) Sexually transmitted disease
(b) Malaria

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B.Sc./M.Sc. (Part III) Examination, 2009

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

PAPER BT-801

MEDICAL BIOTECHNOLOGY

Year-2009

Time.: 3 Hours ***Max. Marks : 50***

Attempt FIVE questions in all, including Questions No. 1 which is compulsory, selecting one question from each Section.

1. Answer the following questions : 1x10=10
   (i) How much time a heart takes in a complete cycle?
   (ii) Define transmembrance pressure.
   (iii) What are haptens?
   (iv) Allergy and hypersentivity reactions are the characteristics of which immunoglobin type?
   (v) The ascending limb of the loop of Henle is permeable to which ions?
   (vi) Name the chemical which stop reaction in an ELISAA assay.
   (vii) What is a tracer?
   (viii) Define the reservoir in a disease cycle with a suitable example.
   (ix) How many GP120 molecules are present in an HIV?
   (x) Name the pathogen which causes Gonoorhea.

Section A

2. Describe : 2x5=10

   (i) Electrical problems in the heart and its solution;

   (ii) Biomaterials and implantable sensors.

Or

Give a detailed account on the medical imagining system. 10

Section B

3. Describe structure and functions of antibodies. 10

Or

Write short notes on : 5x2=10
(a) Major histocompatibility complex;
(b) Lymphokines

Section C

4. What are monoclonal antibodies? Give a brief account of different applications of monoclonal antibodies. 2+8=10

Or

Explain the following: 5x2=10
(i) Disease cycle;
(ii) Immunoblotting.

Section D

5. Give an account on the epidemiology, pathogenicity, diagnosis, prevention and control of Anthrax. 5x2=10

Or

Write short notes on : 5x2=10
(i) Mycosis;
(ii) Sexually transmitted diseases.

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Bibliography

1. Text Box of Microbiology R. Ananthanarayanan and C.K. Jayaram Paniker, Orient Longman, 1997.
2. Medical Microbiology , Vol 1 ; Microbial infection : Mackie and MaCartny, Churchil Livingstone, 1996
3. Bailey and Scott's Diagnostic Microbiology : Baron EJ, Peterson LR and Finegold SM Mosby, 1990.
4. Essential immunology (1995) - Roitt, I.M. Blackwell Scientific Publictions Oxford.
5. Fundamental immunology : W.E. Paul 1984, Raven Press, New York.
6. Fundamentals of immunology : R.M. Coleman, M.F. Lombord and R.E. Sicarc (1992), 2nd ed, C. Brown publishers.
7. Immunology : D.M. Weir and J Steward, 7thEd, (1993).
8. Broude A.I. (1981) : Medical " Microbiology" ; and Infectious Diseases, W.B. Saunders & Co. Philadelphia.
9. Immunology : Jaind Kuby.
10. An Introduction to Immunology : Ian R. Tizzard.
11. J.T. Bushberg. J.A. Scibert, E.M. Leidholdt (JR), J.M. Boone : The Essential Physics of medical Imaging, Lippincott Williams and Wilkins, USA, 2002.
12. R.B. Buxton : Introduction to Functional Magnetic Resonance Imagining :Principle & Techniques, Cambridge Univ. Press, UK, 2002.
13. J.Enderle, S. Blancechard & J. Bronzino (Eds) : Introduction to Biomedical Engineering, Academic Press, 2000.
14. John G. webster(Ed): Medical Instrumentation - Application and Design, 3rd Ed, John Wiley & Sons, 1989
15. J.B. West. (Ed) Best and Taylor's Physiological basis of Medical Practice, 11th Ed., Williams and Wilkins, Baltimore 1985
16. Y.C. Fung: Biomechanics, Spring and Verlag, New York, 1981.
17. S. Oka: Cardiovascular H-emorheology, Cambridge University Press, 1981.
18. http://www.mycqs.com/mcqs/Medical/Biochemistry/Infectious+diseases/answers/

19. http://www.ncl.ac.uk/dental/oralbiol/oralenv/mcqs/oralmicro/periodisease1.htm
20. http://www.localhealth.com/article/bacterial-diseases

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